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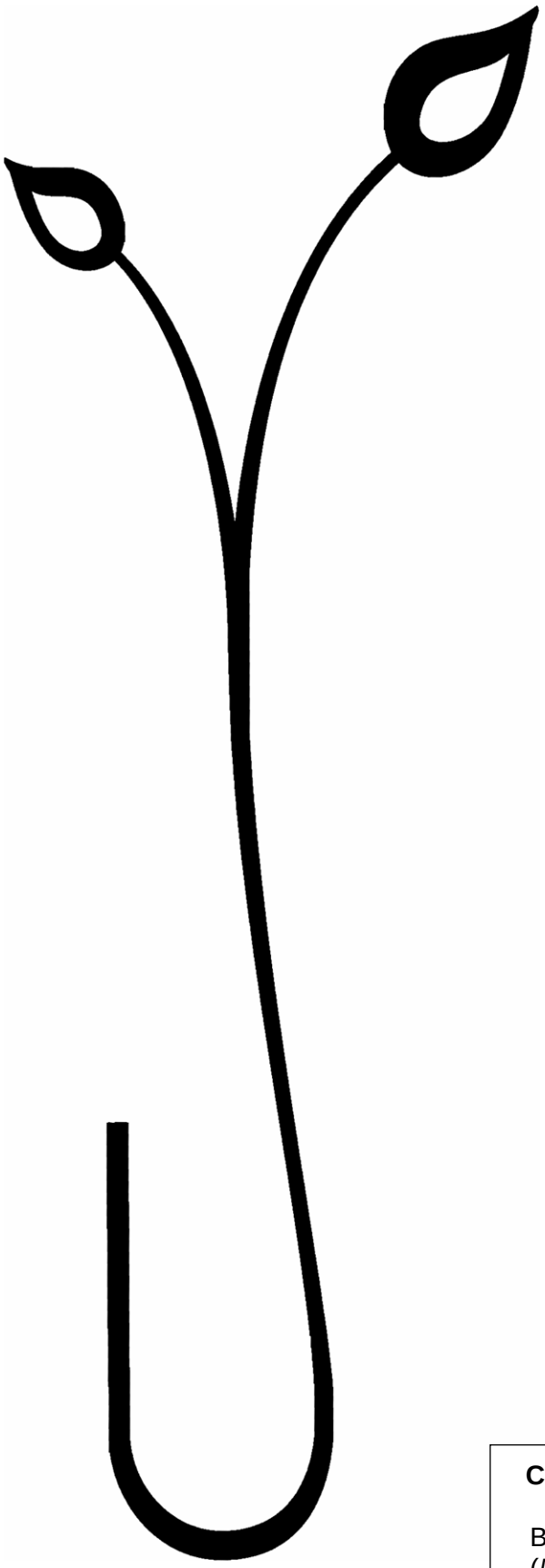

Research **Report**

CP67

Biology and control of currant-lettuce aphid (*Nasonovia ribisnigri)*

Annual Report 2011

Project title:	Biology and control of currant lettuce aphid (*Nasonovia ribisnigri*)
Project number:	CP67
Project leader:	Dr Rosemary Collier
Report:	Annual, 2010/2011
Previous report:	n/a
Key staff:	Gemma Hough
Location of project:	Warwick Crop Centre School of Life Sciences, Wellesbourne Campus Wellesbourne Warwickshire CV35 9EF
Industry Representative:	David Norman
Date project commenced:	1 October 2009
Date project completed:	31 December 2012
Key words:	*Nasonovia ribisnigri*, lettuce, resistance-breaking, wild type, aphid, treatment.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Authentication
We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Rosemary Collier
Director, Warwick Crop Centre
School of Life Sciences
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Signature ..... Date .....

Report authorised by:

Professor Brian Thomas
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Contents

Grower Summary

Headlines

- Host-plant resistance-breaking (Rb) *N. ribinsigri* develop at the same rate as wild type (WT) *N. ribisnigri* at 5, 10, 15, 20 and 25 °C. Results suggest that Rb *N. ribisnigri* may produce more winged offspring, which would be important in terms of their potential to disperse.
- Nine species of wild host plant support both Rb and WT *N. ribisnigri* populations. These include Wall Speedwell, Smooth Hawksbeard, Chicory, Spiked speedwell, Orange hawkweed, Nipplewort, Smooth Sowthistle, Common Speedwell and Mouse-ear Hawkweed.

Background and expected deliverables

UK lettuce crops are infested commonly by four species of aphid. Of these, the currant-lettuce aphid, *Nasonovia ribisnigri*, is of greatest economic importance, being difficult to control, particularly on crops that are close to maturity. While some systemic insecticides remain effective for part of the crop's life, other insecticides often have relatively little effect because the aphids are hidden within the foliage. In addition, there is evidence that some populations of *N. ribisnigri* have reduced sensitivity to pirimicarb or to pyrethroid insecticides.

Although, in recent years, lettuce cultivars with resistance to *N. ribisnigri* have been developed and released commercially, many growers still grow susceptible cultivars and reliance on insecticides is likely to be important for many years to come. In addition, in continental Europe and the UK, certain clones of *N. ribisnigri* have overcome the resistance, which is based on a single gene (Nr gene), suggesting that widespread failure of host plant resistance could soon be possible. Therefore, it is important to continue to develop an integrated control strategy for this pest.

The expected deliverables from this work include:

- Quantification of the life-cycle of the currant-lettuce aphid and, in particular, its overwintering biology.
- A forecast of the timing of key events in the life-cycle/population development of the currant-lettuce aphid

- Information on currant-lettuce aphid biology (e.g. the mid-summer crash, important natural enemies, alternative hosts) that can be used to improve the control strategy for this pest.

Summary of results and conclusions

The following experiments were done at Warwick Crop Centre, Wellesbourne:

Experiment 1. Quantifying the temperature requirements for summer development of wild type (WT) and resistance-breaking (*Rb*) *N. ribisnigri* on susceptible and resistant (*Nr*) lettuce cultivars

Including a control, there were 6 treatments. WT and Rb *N. ribisnigri* were reared on three cultivars of lettuce (cvs Saladin (susceptible), Eluarde (resistant), Rotary (resistant)) with each treatment consisting of 10 lettuce plants. Each lettuce plant was inoculated with one aphid and the treatments were kept at 5, 10, 15, 20 or 25°C. The aphids were monitored and their development time to adult, whether they were winged/wingless, survival time, fecundity and behaviour were recorded.

Temperature had a significant impact on the development of both WT and Rb *N. ribisnigri*. Higher temperatures resulted in a shorter development time while lower temperatures increased the development time. At 5, 10, 15, 20 and 25 °C the average development times to adult for the control treatments were 42.5, 16.5, 11.48, 8 and 6.25 days respectively, and the development times on the other treatments were similar to this. Therefore, Rb *N. ribisnigri* develops at the same rate as WT *N. ribisnigri* at each temperature.

It was expected that WT *N. ribisnigri* would suffer 100% mortality on the resistant lettuce cultivars (Rotary and Eluarde) but, unexpectedly, at 5, 10 and 15°C some WT aphids developed to adulthood but their survival and reproduction was often compromised.

Observations suggest that Rb *N. ribisnigri* are more likely to develop into winged adults compared with WT *N. ribisnigri.* Another experiment is being carried out confirm this.

Experiment 2. Determine whether day length has an effect on WT and Rb *N. ribisnigri* development

The aim of this experiment was to determine whether different day lengths have an effect on WT and Rb *N. ribisnigri* development to see whether day length is a factor which needs to be included in temperature-based forecasts.

There were 12 treatments including 1 WT clone (4850a), 1 WT population (WTKent10), 3 Rb *N. ribisnigri* clones (KentC1, UCK31C1 and GermC1) and 1 Rb population (Kentpop) which were reared on two cultivars of lettuce (cvs Saladin (susceptible) and Rotary (resistant)). Each treatment consisted of 6 replications. These 12 treatments were kept at different photoperiods of 16L 8D (June conditions), 14L 10D (August conditions), and 10L 14D (October conditions) at 20°C in controlled environment room 8 in the insect rearing unit at Wellesbourne. Data are still being collected.

Experiment 3. Identifying wild plant species that might serve as overwintering hosts

The aim of this experiment was to determine which alternative hosts *N. ribisnigri* could potentially use to overwinter as adult aphids during mild winters. The method has been revised since the 2010 report. Host plants were screened in batches of 6 including a control (Chicory). There were 12 treatments in each batch as both WT and Rb *N. ribisnigri* were screened. There were 5 replicates for each treatment. The various host plant species were sown at intervals so that all the species reached a pre-determined size, appropriate for aphid inoculation, on 17 May 2011 for batch 1 and 4 August 2011 for batch 2. Four new born nymphs were inoculated per plant. Plants were then assessed after 3 weeks and the numbers of winged and non-winged aphids were counted.

Alternative host plant species screened so far include Chicory, Field Sowthistle, Smooth Sowthistle, Common Speedwell, Nipplewort, Mouse-ear Hawkweed, Wall speedwell, Smooth Hawksbeard, Spiked Speedwell, Prickly Sowthistle and Orange Hawkweed.

All host plants supported WT and Rb *N. ribisnigri* development and reproduction, except for Prickly Sowthistle and Field Sowthistle. There was variation between the host plants suggesting some were better host plants than others. Rb and WT *N. ribisnigri* performed just as well on each host plant except Chicory where Rb *N. ribisnigri* performed better than

the WT. The survival of aphids on these species suggests that they could be potential overwintering hosts.

Experiment 4. Preliminary work to determine the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2010)

Including an untreated control, there were 9 treatments which had various fungicide (Nativo-Strobilurin + triazole), insecticide (Decis - pyrethroid) and netting regimes. Table A summarises the treatments used. There were 2 replicates of each treatment (18 plots in total) and the experiment was repeated on three occasions. The seed (cv. Saladin Supreme) was sown on 19 May, 16 June, 20 July, and transplanted into the field on 9 June, 19 July, and 31 August respectively. Fine mesh netting was used to exclude natural enemies from entering particular plots, reducing their impact on the aphid population, whilst also stopping the movement of aphids in and out of the plots. The fungicide and insecticide treatments were used to attempt to reduce the numbers of entomopathogenic fungi and natural enemies respectively.

Table A: Summary of treatments used in Experiment 4

Treatment Num.	Aphid Inoculation	Insect netting	Fungicide	Insecticide
1	5 WT *N. ribisnigri*	Yes	No	No
2	5 WT *N. ribisnigri*	No	No	No
3 Control	No	No	No	No
4	5 WT *N. ribisnigri*	Yes	Yes	No
5	5 WT *N. ribisnigri*	No	Yes	No
6	5 WT *N. ribisnigri*	Yes	No	Yes
7	5 WT *N. ribisnigri*	No	No	Yes
8	5 WT *N. ribisnigri*	No	Yes	Yes
9	5 WT *N. ribisnigri*	Yes	Yes	Yes

Data were collected between June and October through the destructive sampling of 4 lettuce plants per plot each week (72 plants) over a period of 5-6 weeks. Once the lettuce plants had been cut they were kept in a cold store (5°C) until they were destructively sampled. Aphids and predators were counted and identified on each plant.

There were significant differences between netted and un-netted plots when looking at numbers of winged, non-winged and total *N. ribisnigri,* with more aphids found in netted plots compared to un-netted plots. Sampling date also interacted with this indicating that the longer plots were netted, the more aphids were found. There was no effect of insecticide and fungicide treatments.

Unfortunately, the mid-summer crash was not identified in the field trial as the plots did not overlap sufficiently. Increases in aphid numbers were identified in June, July and August but no decreases were observed. Aphid populations remained low throughout September.

While the numbers of natural enemies were low for some insects, the trial did successfully document the seasonal occurrence of natural enemies throughout the summer.

Experiment 5. Determining the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2011)

There were 9 treatments combining various fungicide (Amistar- azoxystrobin), insecticide (Decis - pyrethroid) and netting regimes (see Table B) which were used for the same reasons as Experiment 4. There were 2 replicates of each treatment (18 plots in total) in each experiment which were repeated in May, June, July, August and September. At 4 weeks of growth, Saladin plants were transplanted into the field in 18 beds/plots of 20 plants in a randomised design. The method of aphid inoculation was the same as in Experiment 4 but 10 predetermined plants were inoculated.

One week later, 6 of the netted plots (treatments 4-6 for both replications) were uncovered and protected with bird netting. One week following this, 10 plants were taken from each plot and were assessed in the same way as in Experiment 4 (but including the assessment of aphids infected with entomopathogenic fungi). In addition to the treatments, a monitoring plot was established in a different area of the field where two new plots of 40 plants were planted every 3 weeks with 10 plants inoculated with 5 aphids. Every week, 4 plants were sampled from each plot up to a period of 5 weeks.

Table B: Summary of treatments used in Experiment 5

Treat. Num.	**Period netted for**	**Fungicide**	**Insecticide**
1 (Control)	Never	No	No
2	Never	Yes	No
3	Never	No	Yes
4	1 week	No	No
5	1 week	Yes	No
6	1 week	No	Yes
7	2 weeks	No	No
8	2 weeks	Yes	No
9	2 weeks	No	Yes

Data are still being collected but the mid-summer crash has been monitored successfully, occurring in mid-July.

Experiment 6. Stimulating the production of sexual male and female aphids in the laboratory to obtain eggs and in the field

The aim of this experiment was to obtain *N. ribisnigri* eggs to investigate their development under different temperatures and photoperiods to predict their hatching in spring. In this experiment aphids were subjected to autumn conditions in incubators, including 13L: 11D, 12L: 12D, 11L: 13D and 10L: 14D at 15°C, to stimulate male and female aphid production.

At 13L: 11D and 11L: 13D (photoperiods evaluated so far) winged male production was stimulated from day 10 of reproduction until the end of reproductive life, with non-winged and winged aphids being produced at the same time. Currently production of sexual females has not been stimulated and infested lettuce have been placed with currant cuttings to see if this stimulates female production and egg laying

Conclusions

- Rb *N. ribisnigri* have the same development rates as WT *N. ribisnigri* at 5, 10, 15, 20 and 25°C. Some WT *N. ribisnrigri* can develop to adulthood on resistant lettuce cultivars, but their survival and reproduction is often affected adversely.
- Observations indicate that Rb *N. ribisnigri* are more likely to develop into winged adults on both resistant and susceptible lettuce cultivars compared to the WT *N. ribisnigri* on susceptible lettuce cultivars.
- The midsummer aphid crash was not identified in the 2010 field trial but has been observed in the 2011 field trial. Treatments of fungicide and pesticide had no effect in 2010 but natural enemy seasonality was recorded.
- Both WT and Rb *N. ribisnigri* can develop and reproduce equally well on Wall Speedwell, Smooth Hawksbeard, Chicory, Spiked speedwell, Common Speedwell and Mouse-ear Hawkweed, Nipplewort, Smooth Sowthistle and Orange Hawkweed.
- Male *N. ribisnigri* were produced at 13L: 11D and 11L: 13D at 15°C from day 10 of an adult's reproductive life. No females have been produced yet.

Financial benefits

Currently there are no direct financial benefits from this work

Action points for growers

Currently there are no action points for growers

Science Section

Introduction

UK lettuce crops are infested commonly by four species of aphid. Of these, the currant-lettuce aphid, *Nasonovia ribisnigri*, is of greatest economic importance, being difficult to control, particularly on crops that are close to maturity.

While some insecticides do remain effective for part of the crop's life, for example, an imidacloprid seed treatment (Gaucho) and a new systemic insecticide, spirotetramat (Movento), other insecticides applied as foliar sprays to hearted crops often have relatively little effect because the aphids are hidden within the foliage. There is evidence that some populations of *N. ribisnigri* have reduced sensitivity to pirimicarb or to pyrethroid insecticides.

Several new insecticides may soon become available to lettuce growers through full or off-label approvals. Some of these appear to be more effective against *N. ribisnigri* than older active ingredients, but may still not give complete control on maturing crops. In addition, there is concern that some insecticides may be withdrawn in the future as a result of changes in approvals and legislation.

Although, in recent years, lettuce cultivars with resistance to *N. ribisnigri* have been developed and released commercially, many growers still grow susceptible cultivars and reliance on insecticides is likely to be important for many years to come. In addition, reports have been confirmed that, in continental Europe and the UK, certain clones of *N. ribisnigri* have overcome the resistance, which is based on a single gene (Nr gene), suggesting that widespread failure of host plant resistance could soon be possible. Therefore, it is important to continue to develop an integrated control strategy for this pest

The experiments that have been carried out between October 2010 and September 2011 are as follows:

Experiment 1 - Quantifying the temperature requirements for summer development of wild type (WT) and resistance-breaking (*Rb*) *N. ribisnigri* on susceptible and resistant (*Nr*) lettuce cultivars.

Experiment 2 - Determining whether day length has an effect on WT and Rb *N. ribisnigri* development

Experiment 3 - Identifying wild plant species that might serve as overwintering hosts

Experiment 4 - Preliminary work to determine the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2010)

Experiment 5 - Determining the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2011)

Experiment 6- Stimulating the production of sexual male and female aphids in the laboratory to obtain eggs

Experiment 1 - Quantifying the temperature requirements for summer development of wild type and resistance-breaking *N. ribisnigri* on susceptible and resistant (*Nr*) lettuce cultivars

Materials and methods

The experiments were done in various controlled environment (CE) rooms in the Insect Rearing Unit at Warwick Crop Centre, Wellesbourne.

There were 6 treatments (Table 1.1). WT and Rb *N. ribisnigri* were reared on three cultivars of lettuce (cvs Saladin (susceptible), Eluarde (resistant), Rotary (resistant)) with each treatment consisting of 10 lettuce plants. Lettuce seeds were sown in vermiculite and transplanted to pots 1 week later, where they were then grown for a further 2 weeks.
The WT aphids used were a clone maintained at Warwick Crop Centre (clone 4850a) and the Rb aphids were from a population obtained in October 2009 (from Kent).

New born nymphs were used in the experiment. These were obtained by inoculating 45 winged adults into a cage containing 3 lettuce plants cv. Saladin, where they were left for 24 hours to produce nymphs (repeated for *Rb* and WT). After 24 hours the new born nymphs were removed and transferred to each treatment plant. One new born nymph was placed on each lettuce plant using a fine paint brush. The 60 inoculated plants were then covered individually with bread bags, secured with an elastic band, and arranged in a randomised design with 4 rows and 15 columns on a single shelf in one of the controlled environment rooms (Figure 1.2).

Treatments so far have been exposed to 5 (2 repeats), 10 (3 repeats), 15 (3 repeats), 20 (3 repeats), and 25°C (3 repeats).

Table 1.1: Summary of treatments used in Experiment 1

Treatment Number	**Aphid type**	**Lettuce cultivar**	**Replication**
1 Control	1 WT *N. ribisnigri*	Saladin	10
2	1 WT *N. ribisnigri*	Rotary (*Nr*)	10
3	1 WT *N. ribisnigri*	Eluarde(*Nr*)	10
4	1 *Rb N. ribisnigri*	Saladin	10
5	1 *Rb N. ribisnigri*	Rotary (*Nr*)	10
6	1 *Rb N. ribisnigri*	Eluarde (*Nr*)	10

Figure 1.2: One of the development experiments in a controlled environment room

Assessments

Aphids were assessed daily when it was estimated that they were approaching adulthood. This was determined by using the development times recorded in a similar study carried out in Spain (Diaz and Fereres, 2005). The following data were recorded:

- Development time from nymph to adult.
- Fecundity of each individual for the same period as the development time to calculate the intrinsic rate of increase (a measure of the rate of growth).
- Total fecundity (to death)
- Mortality
- Adult morph (winged or wingless)
- Position of the aphid on the plant when assessed

Results

Statistical analysis

All analyses were performed using a restricted (or residual) maximum likelihood (REML). There were a varying number of replicates for each treatment due to mortality and the numbers of times the experiments had been repeated to date. A significance level of 0.05% was used.

Developmental time to adult

REML analyses carried out on the data collected so far indicate that there is a highly significant effect of temperature on development time (p=<0.001). Higher temperatures resulted in a shorter development time while lower temperatures increased the development time. As illustrated in Figure 1.3, WT and Rb aphids at 25°C developed to adult in the least number of days, followed by those kept at 20, 15, 10 and 5°C. At 5, 10, 15, 20 and 25 °C the average development times to adult for the control treatment were 42.5, 16.5, 11.48, 8 and 6.25 days respectively, and the development times of the other treatments were similar.

Individually, aphid type (WT or Rb) and plant host (Saladin, Rotary and Eluarde) did not have an effect on development, meaning that the type of aphid or lettuce has no impact on the developmental time. However, significant interactions were seen between temperature and aphid (P= 0.030) which can be attributed to the unexpected survival of WT *N. ribisnigri* surviving on the resistant lettuce cultivars at 5, 10 and 15°C.

Little variation was seen between replications for each treatment and temperature, except for those treatments where WT *N. ribisnigri* should have displayed 100% mortality. The most variation was seen in treatments at 5°C.

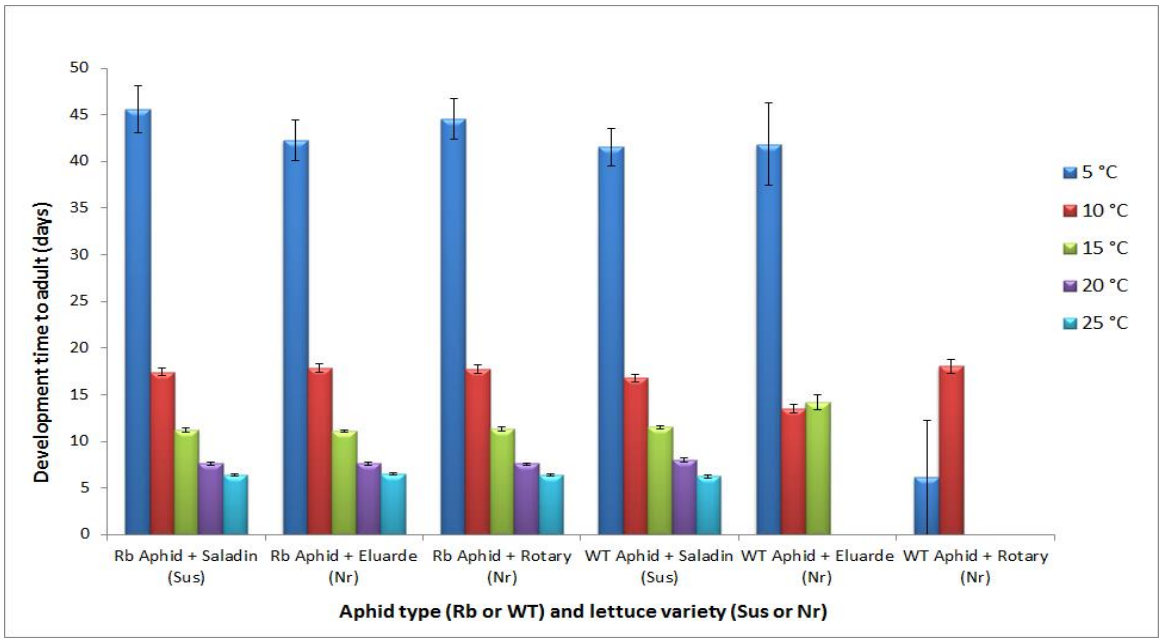


Figure 1.3 Mean number of days for development to adulthood for WT and Rb *N. ribisnigri* on resistant and susceptible lettuce cultivars at 5, 10, 15, 20 and 25°C.

Morph production

Using the data for adults who reached adulthood, the proportions of winged aphids were calculated. Preliminary results presented in Figure 1.4 indicate that Rb *N. ribisnigri* were more likely to develop into winged adults when compared with the control treatment (WT *N. ribisnigri* + Saladin). Experiments are underway to confirm this observation.

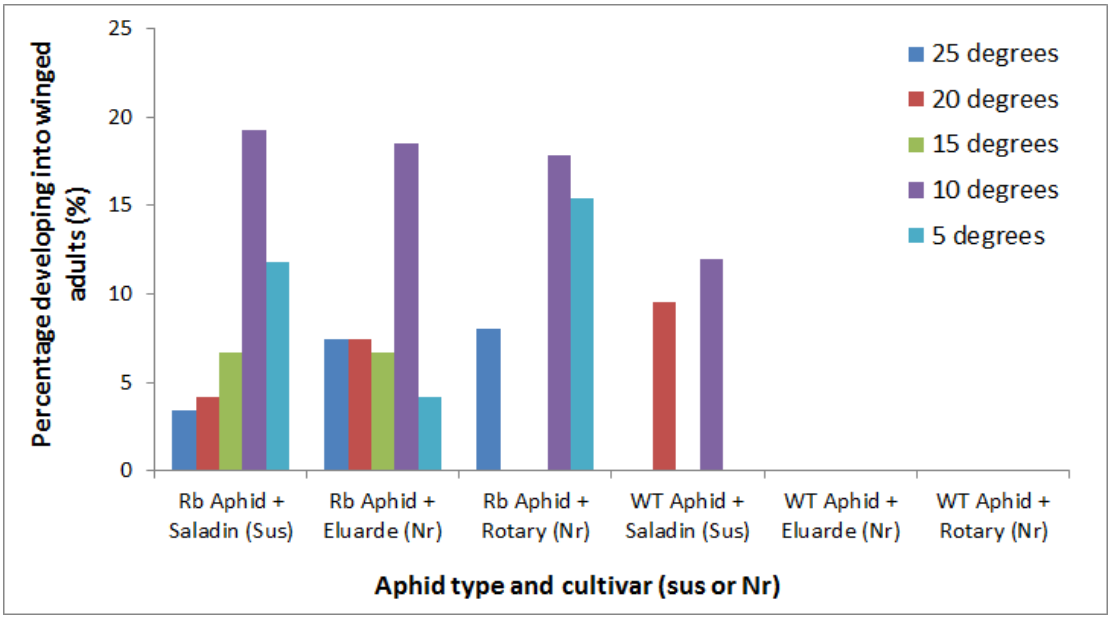


Figure 1.4 Mean proportion of WT and Rb *N. ribisnigri* developing into winged adults on resistant and susceptible lettuce cultivars at 5, 10, 15, 20 and 25°C

Experiment 2 - Determine whether day length has an effect on development of WT and Rb *N. ribisnigri*

Materials and methods

The aim of this experiment was to determine whether different day lengths have an effect on development of WT and Rb *N. ribisnigri*, to see whether day length is a factor which needs to be included in future temperature-based forecasts.

There were 12 treatments as summarised in Table 2.1 which included 1 WT clone (4850a),1 WT population (WTKent10), 3 Rb *N. ribisnigri clones* (KentC1, UCK31C1 and GermC1) and 1 Rb population (Kentpop) which were reared on two cultivars of lettuce ((cvs Saladin (susceptible) and Rotary (resistant)). Each treatment consisted of 6 replicates.

New born nymphs were used in the experiment. These were obtained by inoculating 30 winged adults into a cage containing 2 lettuce plants (cv. Saladin), where they were left for 24 hours to produce nymphs (repeated for all clones). After 24 hours the new born nymphs were removed and transferred to each treatment plant. One new born nymph was placed on each lettuce plant using a fine paint brush.

The 72 inoculated plants were then covered individually with bread bags, secured with an elastic band, and arranged in a randomised design with 4 rows and 18 columns on a single shelf in CE room 8 (20°C) at either 16L 8D (June conditions), 14L 10D (August conditions), or 10L 14D (October conditions).

Table 2.1: Summary of treatments used in Experiment 2

Treatment Num.	**Aphid type**	**Lettuce cultivar**	**Replication**
1 Control	1 WT *4850a*	Saladin	6
2	1 WT Kent10	Saladin	6
3	1 Rb KenctC1	Saladin	6
4	1 *Rb UK631C1*	Saladin	6
5	1 *Rb GermC1*	Saladin	6
6	1 *Rb Kentpop*	Saladin	6
7	1 WT 4850a	Rotary	6
8	1 WT KentC1	Rotary	6
9	1 Rb KenctC1	Rotary	6
10	1 *Rb UK631C1*	Rotary	6
11	1 *Rb GermC1*	Rotary	6
12	1 *Rb Kentpop*	Rotary	6

Aphids were assessed daily when it was estimated that they were approaching adulthood as in Experiment 1. Similarly, the following data were recorded:

- Development time from nymph to adult.
- Fecundity of each individual for the same period as the development time to calculate the intrinsic rate of increase (a measure of the rate of growth).
- Adult morph (winged or wingless)
- Position of the aphid on the plant when assessed

Results

Data are still being collected.

Experiment 3 - Identifying wild plant species that might serve as overwintering hosts

Materials and methods

The aim of this experiment was to determine which alternative hosts *N. ribisnigri* could potentially use to overwinter as adult aphids. The method has been revised since the 2010 report.

The experiment was carried out in CE rooms No. 6 for Batch 2 and No. 3 for Batch 1 in the Insect Rearing Unit at Warwick Crop Centre, Wellesbourne.

Host plants were screened in batches of 6 including a control (depending on germination). There were 12 treatments in each batch and 5 replicates as summarised in Table 3.1. Alternative host plant species were sown at intervals so that all the species reached a pre-determined size, appropriate for aphid inoculation, on 17 May 2011 for batch 1 and 4 August 2011 for batch 2. Plants were raised in a controlled environment room (20°C, 16h light 8h dark light regime).

New born nymphs were used in the experiment. These were obtained by inoculating 75 winged adults into a cage containing 5 lettuces cv. Pinokio, where they were left for 24 hours to produce nymphs (repeated for *Rb* and WT). After 24 hours the new born nymphs were removed and transferred to each treatment plant. Four new born nymphs were placed on each treatment using a fine paint brush. The inoculated host plants were each covered with a bread bag, which was secured with an elastic band, and arranged on a single shelf in CE room 3 for Batch 1 and room 6 for Batch 2 (20°C 18L:6D) (Figure 3.2). There were 5 replicates of each treatment which were arranged in a randomised design with 4 rows and 15 columns.

The wild type aphids were a clone maintained at Warwick Crop Centre (clone 4850a) and the resistance-breaking clone (KentC1) was obtained in October 2009 (from Kent).

Table 3.1: Summary of treatments used in Experiment 3

Batch 1

Treatment Number	Aphid type	Alternative host specie	Replication
1 Control	4x WT *N. ribisnigri*	Chicory (*Chichorium intybus*)	5 plants
2	4x WT *N. ribisnigri*	Field Sowthistle (*Sonchus arvensis)*	5 plants
3	4x WT *N. ribisnigri*	Smooth Sowthistle (*Sonchus Oleraceus)*	5 plants
4	4x WT *N. ribisnigri*	Common Speedwell (*Veronica officinalis)*	5 plants
5	4x WT *N. ribisnigri*	Nipplewort (*Lapsana communis)*	5 plants
6	4x WT *N. ribisnigri*	Mouse-ear Hawkweed (*Hieracium pilosella)*	5 plants
7	*4x Rb N. ribisnigri*	Chicory	5 plants
8	*4x Rb N. ribisnigri*	Field Sowthistle	5 plants
9	*4x Rb N. ribisnigri*	Smooth Sowthistle	5 plants
10	*4x Rb N. ribisnigri*	Common Speedwell	5 plants
11	*4x Rb N. ribisnigri*	Nipplewort	5 plants
12	*4x Rb N. ribisnigri*	Mouse-ear Hawkweed	5 plants

Batch 2

Treatment Number	Aphid type	Alternative host specie	Replication
1 Control	4x WT *N. ribisnigri*	Chicory (*Chichorium intybus*)	5 plants
2	4x WT *N. ribisnigri*	Wall speedwell (*Veronica arvensis*)	5 plants
3	4x WT *N. ribisnigri*	Smooth Hawksbeard (*Crepis capillaris*)	5 plants
4	4x WT *N. ribisnigri*	Spiked Speedwell (*Veronica spicata*)	5 plants
5	4x WT *N. ribisnigri*	Prickly Sowthistle (*Sonchus asper*)	5 plants
6	4x WT *N. ribisnigri*	Orange Hawkweed (*Hieracium aurantiacum*)	5 plants
7	*4x Rb N. ribisnigri*	Chicory	5 plants
8	*4x Rb N. ribisnigri*	Wall speedwell	5 plants
9	*4x Rb N. ribisnigri*	Smooth Hawksbeard	5 plants
10	*4x Rb N. ribisnigri*	Spiked Speedwell	5 plants
11	*4x Rb N. ribisnigri*	Prickly Sowthistle	5 plants
12	*4x Rb N. ribisnigri*	Orange Hawkweed	5 plants

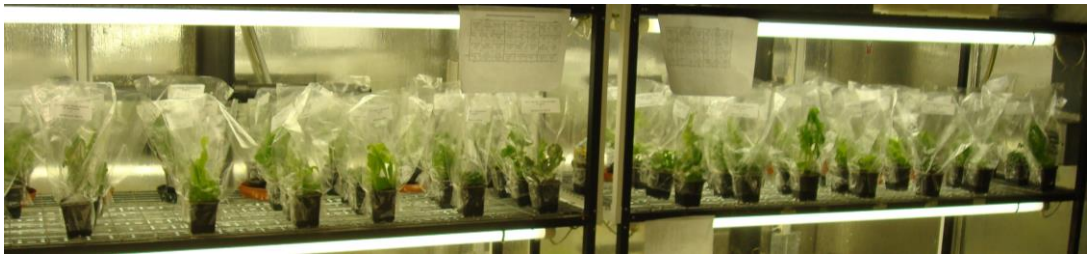


Figure 3.2: The experiment to evaluate alternative host plants

Assessments

Plants were assessed after 3 weeks and the numbers of winged and non-winged aphids were counted.

Results

Analyses were performed using analysis of variance (ANOVA). Square root transformations were used on the winged, non-winged and total *N, ribisnigri* numbers to maintain homogeneity between the data. Interpretations of the data were made using treatment means and 5% LSD values.

WT and Rb *N. ribisinigri* survived on all host plants except Prickly Sowthistle and Field Sowthistle. *N. ribisnigri* numbers were also very low on Spiked Speedwell. There were significant effects of host plant on the number of winged, non- winged and total *N. ribisnigri* after 3 weeks. Chicory was the best host plant.

As shown in Figures 3.3a and b, the first and second experiments (Batches 1 and 2) resulted in significantly different maximum counts of aphids, with Batch 1 reaching 400 aphids while Batch 2 reached 4000 aphids. The experiments were carried out at 20°C but in different CE rooms.

When compared to the control (Chicory) all other hosts were significantly different when considering numbers of winged, non-winged and total *N. ribisnigri*. Furthermore, there were no effects of aphid type (WT or Rb) on the counts of winged, non-winged and total *N. ribisnigri* except on Chicory. This suggests WT and Rb *N. ribisnigri* performed similarly on the same host plant.

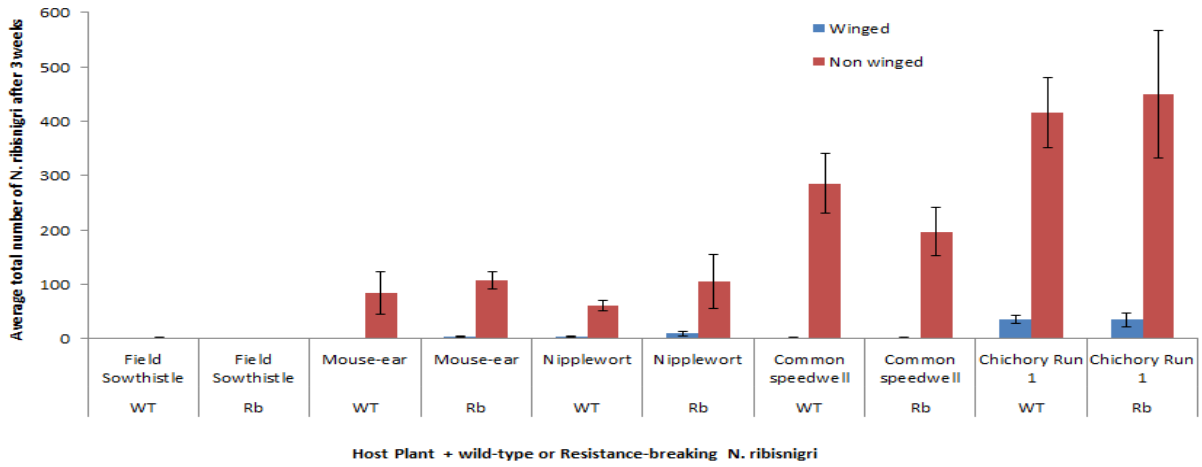


Figure 3.3a: Average total number of winged and non-winged, WT and *Rb N. ribisnigri* on the 4 alternative host plants after 3 weeks at 20°C (16L:8D) in CE room 3. Lacking one host plant due to failed germination (Batch 1).

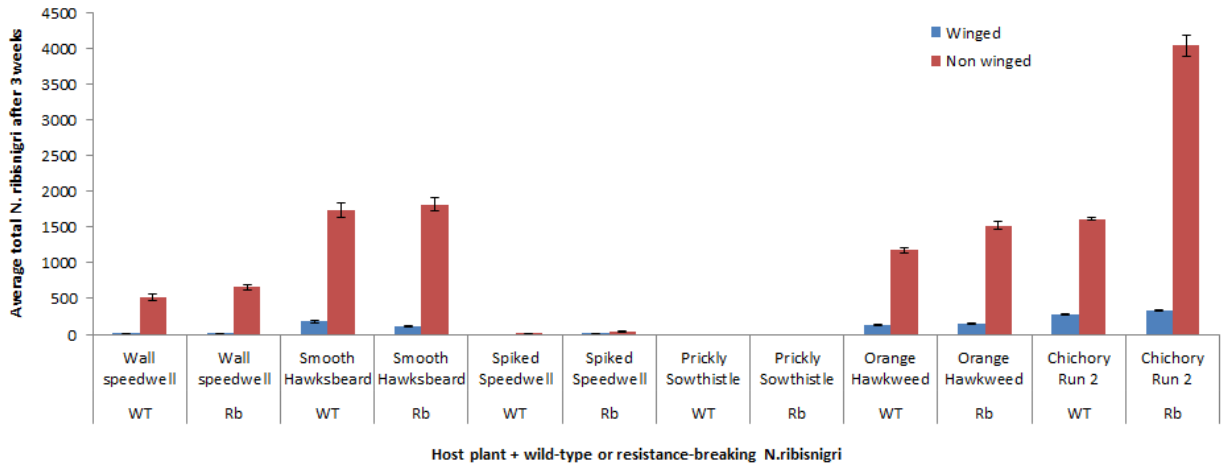


Figure 3.3b Average total number of winged and non-winged, WT and *Rb N. ribisnigri* on the 5 alternative host plants after 3 weeks at 20°C (16L:8D) in CE room 6 (Batch 2).

Experiment 4 - Preliminary work to determine the role of predators and entomopathogenic fungi in regulating populations of N. ribisnigri (Summer 2010)

Materials and methods

This preliminary experiment took place between July-October 2010 in the field known as Sheep Pens at Warwick Crop Centre, Wellesbourne.

The purpose of this experiment was to develop techniques to determine the effects of entomopathogenic fungi and predators on the development of *N. ribisnigri* infestations in the field. This was in preparation for a larger field experiment during summer 2011.

There were 9 treatments combining various fungicide (Nativo-Strobilurin + triazole), insecticide (Decis - pyrethroid) and netting regimes (see Table 4.1).

Fine mesh netting was used to exclude natural enemies from entering particular plots, which reduced their impact on the aphid population while also stopping the movement of aphids in and out of the plots. The fungicide and insecticide treatments were used to attempt to reduce the numbers of entomopathogenic fungi and natural enemies respectively.

There were 2 replicates of each treatment (18 plots in total) in each experiment and the experiment was repeated on two more occasions to allow continuous observations over the summer. 800 seeds (cv. Saladin Supreme) were sown in peat blocks on 19 May, 16 June, 20 July, and transplanted into the field on 9 June, 19 July and 31 August respectively. The plants were raised in a greenhouse and were transplanted into beds 1.83m x 3.5m containing 40 plants with 35cm spacing. Fungicide and insecticide treatments were sprayed on the same day as the plants were transplanted. The treatments were arranged in a 3 x 3 randomised block design which was different for each occasion. Figure 4.2 shows the field experiment.

The day after transplanting, 15 plants in each plot (except the control treatments) were inoculated with 5 wingless adult aphids (or 4th instar), which had been placed previously into Eppendorf tubes.

Table 4.1: Summary of treatments used in Experiment 5

Treatment Number	**Plots**	**Aphid Inoculation**	**Insect proof netting**	**Fungicide treatment**	**Insecticide treatment**
1	2x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	Yes	No	No
2	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	No	No	No
3 Control	2 x (1 bed x 3.5m)	No	No	No	No
4	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	Yes	Yes	No
5	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	No	Yes	No
6	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	Yes	No	Yes
7	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	No	No	Yes
8	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	No	Yes	Yes
9	2 x (1 bed x 3.5m)	5 Wild type *N. ribisnigri*	Yes	Yes	Yes

Figure 5.2: Image of one of the field experiments

Assessments

Aphid numbers were assessed weekly. Four plants were removed from each plot per week over a period of 5-6 weeks. Plants were sampled from the plot ends, alternate ends were sampled each week to maintain plot integrity. Plants were stored in paper bags in a cold store (5°C) where 4 lettuces from each plot were then sampled destructively. Whole plants were sampled until they grew to an unmanageable size and then only half of each plant was sampled. Both halves were weighed and the heaviest half was sampled.

Aphids were counted and identified, together with the predators. Due to the work load of this field trial it was not possible to assess entomopathgenic fungi in addition to natural enemies. All insects were stored in 70% ethanol for further identification.

Results

ANOVA was carried out on the LOG transformed number of aphids or natural enemies per number of lettuce plants sampled. A significance level of 0.05 % was used for all statistical tests. Netting, fungicide, pesticide and sampling week were used as treatments so interactions could be identified.

There was a significant difference between netted and un-netted plots when considering numbers of winged (p= <.001), non-winged (p= <.001) and total *N. ribisnigri* (p= <.001), with more aphids in netted plots. Sampling date also interacted with this (p= <.001) indicating that the longer plots were netted, the more aphids were found. There was no effect of insecticide or fungicide treatments.

Unfortunately, as illustrated in Figure 4.3, the mid-summer crash was not identified in the field trial as the field plots did not overlap sufficiently. Increases in aphid numbers were identified in June, July and August but no decreases were observed. Populations remained low through September.

While the numbers of natural enemies were low for some species, the trial did successfully document the seasonal occurrence of natural enemies throughout the summer. Sampling date interacted significantly with natural enemy numbers indicating these natural enemies were only present on some sampling dates and not others ((syrphid larvae (p = <.001), parasitized aphids (p= <.001), anthocoridae (p= 0.001) and lacewings (p= <.001)).

As shown in Figure 4.4 a-d, parasitized aphids were found throughout the summer, peaking in early July and August, but in lower numbers in the September trial period. Lacewings occurred in late August, flowerbugs occurred in mid-July, peaking in mid-August, while

syrphid larvae occurred throughout the summer, but with significantly more in July. There was also a significant effect of netting on selected natural enemy numbers compared to the control with less syrphid larvae (p = <.001), parasitized aphids (p= 0.003), anthocoridae (p= <.001) and lacewings (p= <.001) in the netted plots.

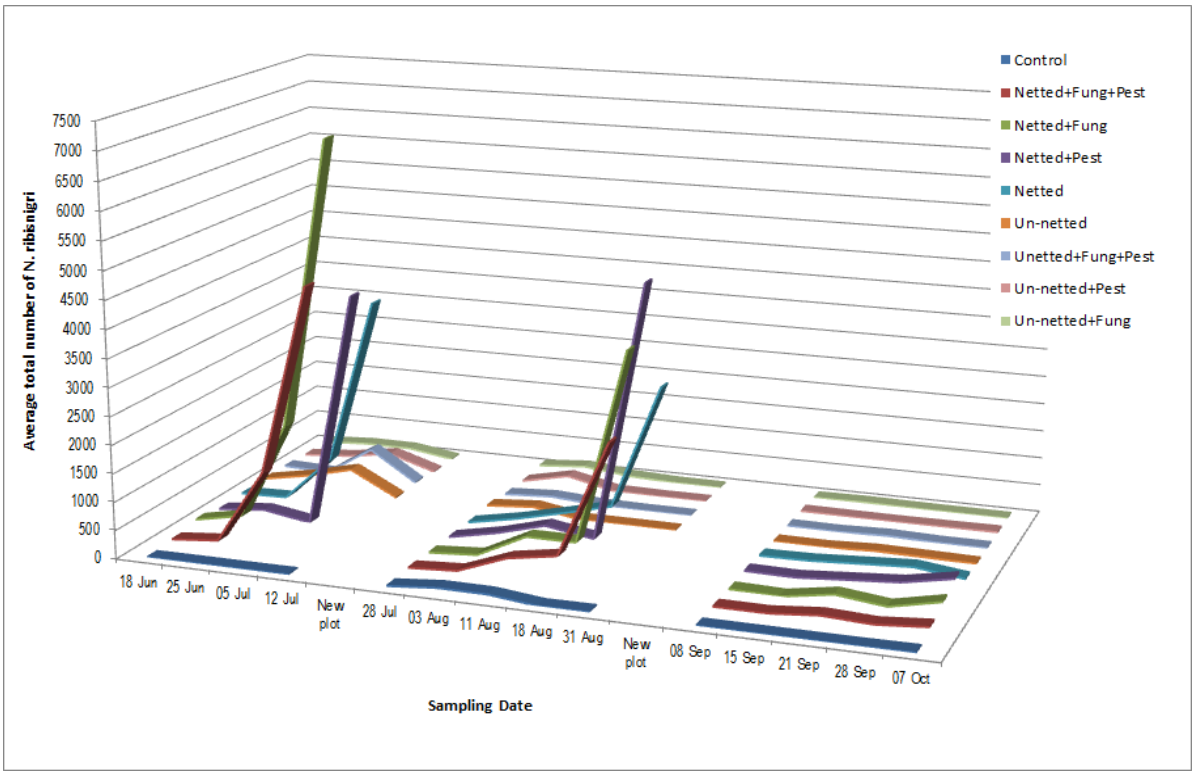
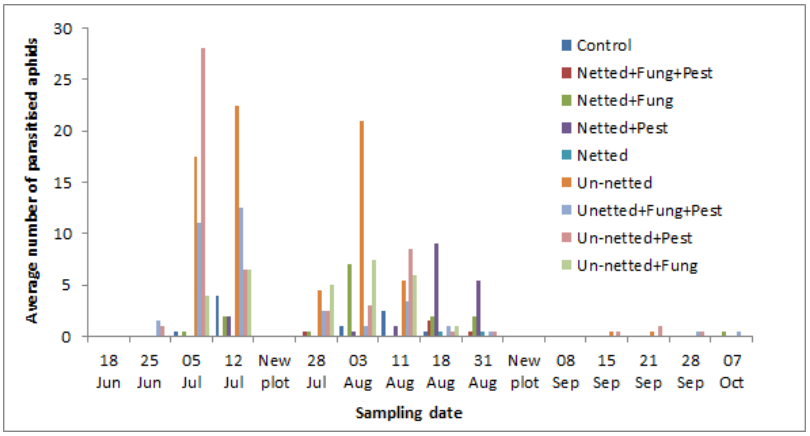
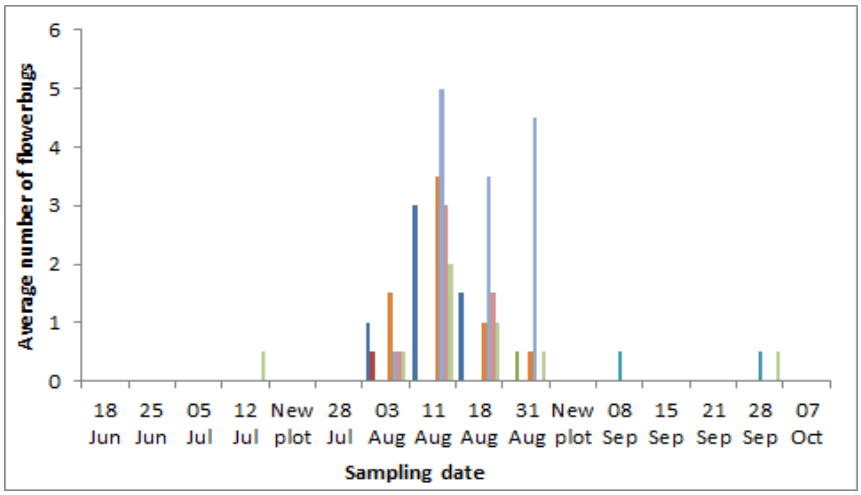


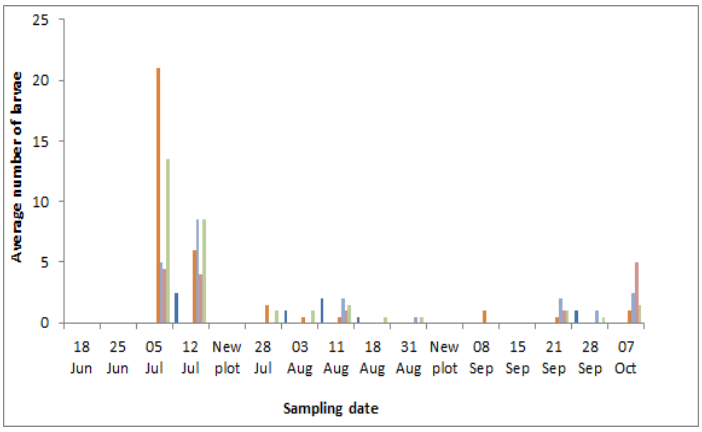
Figure 5.3 Average total number of *N. ribisnigri* counted on lettuce destructively sampled at each sampling date for the 9 treatments.



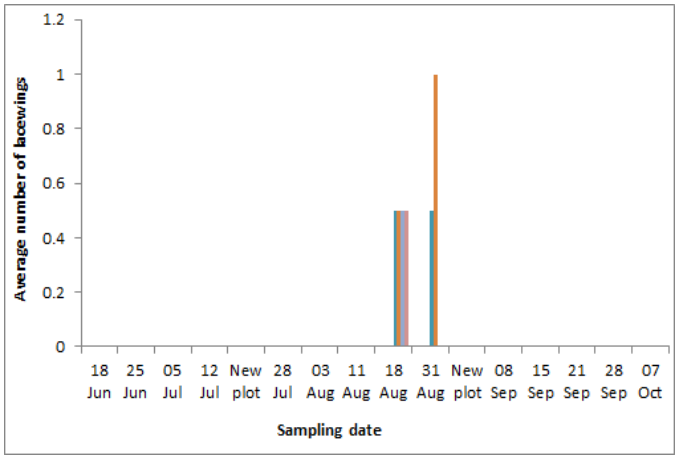
a) Parasitised aphids



b) Anthocoridae



c) Syrphid larvae



d) Lacewing

Figure 5.3a-d Average total number per plot of a) parasitized aphids b) anthocoridae c) syrphid larvae and d) lacewings counted on lettuce sampled destructively at each sampling date - 9 treatments.

Experiment 5 - Determining the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2011)

Materials and methods

There were 9 treatments combining various fungicide (Amistar- azoxystrobin), insecticide (Decis - pyrethroid) and netting regimes. Table 5.1 summarises the treatments used.

The fine mesh netting, fungicide and insecticide treatments were used for the same purposes as described in Experiment 4.

Table 5.1: Summary of treatments used in Experiment 5

Treat. Num.	Period netted for	Fungicide application	Insecticide application
1 (Control)	Never	No	No
2	Never	Yes	No
3	Never	No	Yes
4	1 week	No	No
5	1 week	Yes	No
6	1 week	No	Yes
7	2 weeks	No	No
8	2 weeks	Yes	No
9	2 weeks	No	Yes

There were 2 replicates of each treatment (18 plots in total) in each experiment and the experiment was repeated in May, June, July, August and September. At 4 weeks of growth, Saladin were transplanted into the field in 18 beds/plots (1 bed=1.83 x 3.5m) of 20 plants (4x10 -35cm spacing) in a randomised design. Those plants not covered by netting were covered with bird netting. A Tiny-tag® temperature and humidity recorder was placed in both an un-netted and a netted plot to measure the effect of netting on temperature.

The following day after transplanting 10 predetermined plants were inoculated with 5 wingless adult aphids (or 4th instar), which had been previously placed into Eppendorf tubes (Clone number 4850a). 1 week later, 6 of the netted plots (treatments 4-6 for both replications) were uncovered and protected with bird netting. One week following this, 10 plants were taken from each plot. The lettuce plants were kept in a cold store (5°C) until they were destructively sampled. Aphids, predators and aphids infected with entomopathogenic fungi were identified and counted on each plant. Figure 5.2 shows one of the field experiments.

Figure 5.2 Image of 2011 Summer field trial

In addition to the treatments, a monitoring plot war established a different area of the field. Starting in May, 2 new plots (1 bed= 1.83 x 3.5m) of 40 plants (4x10 -35cm spacing)) were planted every 3 weeks and covered with bird netting. The day after transplanting, 10 randomly-selected plants were inoculated with 5 adult WT *N. ribisnigri* apterae (Clone number 4850a). Every week, 4 plants were sampled from each plot for up to 5 weeks. Water traps were present in both the monitoring plots and at the treatment site. Figure 5.3 shows one of the monitoring plots.

Figure 6.3 Image of one of the monitoring plots during the 2011 summer field trial

This method provides a 'snapshot' of aphid and natural enemy numbers each month. The use of temporary netting allows comparison between netted and un-netted plots to determine how letting natural enemies enter a plot can impact an aphid numbers. Use of a monitoring plot allows the midsummer crash to be observed. Its occurrence might be expected to coincide with the month when the largest numbers of natural enemies occurred and when the largest decrease in aphid numbers occurred in the plots netted temporarily.

Results

Data are still being collected but the monitoring plot showed that the mid-summer crash occurred during mid-July as shown in Figure 5.4. Interestingly, numbers of winged *N. ribisnigri* also increased prior to the crash, as shown in Figure 5.5.

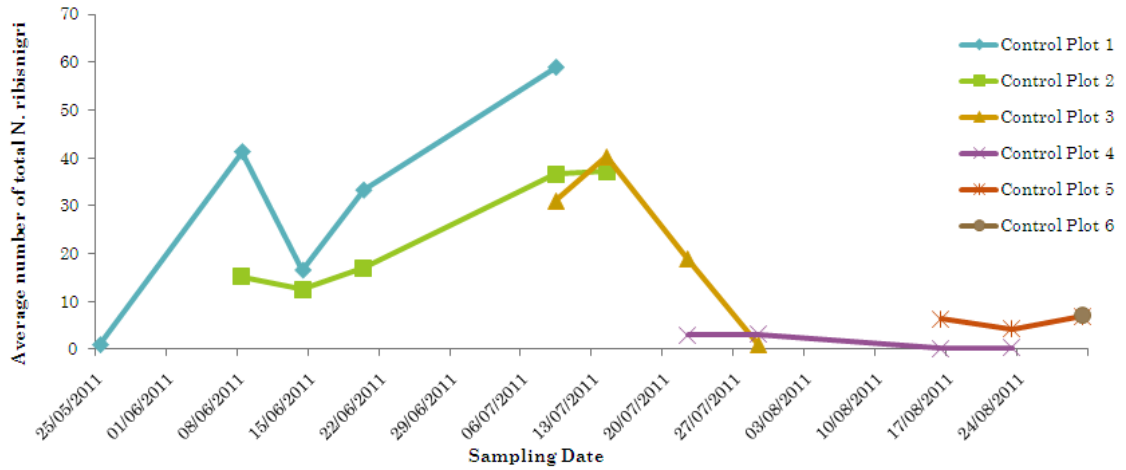


Figure 5.4 Average number of total N. *ribisnigri* per plot recorded on the monitoring plots over several sampling dates

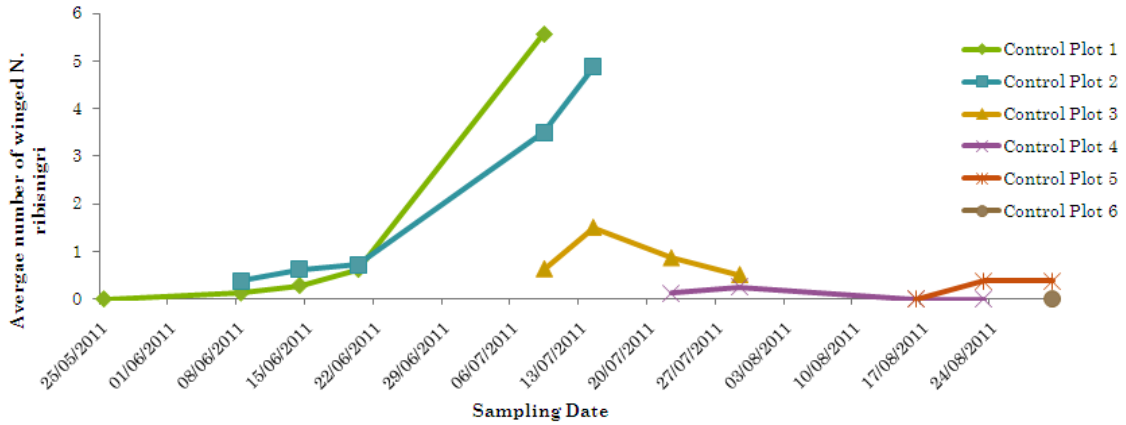


Figure 5.5 Average number of winged *N. ribisnigri* counted on the monitoring plots over several sampling dates

Experiment 6 - Stimulating the production of sexual male and female aphids in the laboratory to obtain eggs

Materials and methods

Aphids were subjected to autumn conditions in incubators to stimulate the production of male and female aphids. If this is achieved, males and females can then be used to produce overwintering eggs where egg development with be investigated. The following method was adapted from Lamb (1972) and Mackay (1987).

1. 10 WT *N. ribisnigri* (clone 4850a) third instar nymphs were obtained from the stock culture and grown to adult (**G_0**) at 15°C on a lettuce plant (cv. Saladin) under treatment conditions until reproduction began. This provided pre-natal conditioning of the embryos.
2. **G_0** were then moved to new lettuce plants and left for 24 hours under treatment conditions to provide **G_1** nymphs of similar age.
3. **G_0** were then discarded and **G_1** nymphs were left to develop to adult under treatment conditions to provide post-natal treatment conditioning. The nymphs were divided between two lettuce plants (cv. Saladin) to avoid a crowding stimulus.
4. 9-14 days later **G_1** nymphs reached adulthood and the morphs were recorded. Adults were then transferred to individual lettuce plants (cv. Saladin) under treatment conditions where they began to produce **G_2** offspring.
5. Following this every 2 days for 4 days and every three days thereafter the **G_2** nymphs were collected:
    a. The **G_1** adults were moved to a new plant and the **G_2** nymphs were left on the plant to develop to adult where the morph is then determined. (See Figure 6.1 for diagram of method).

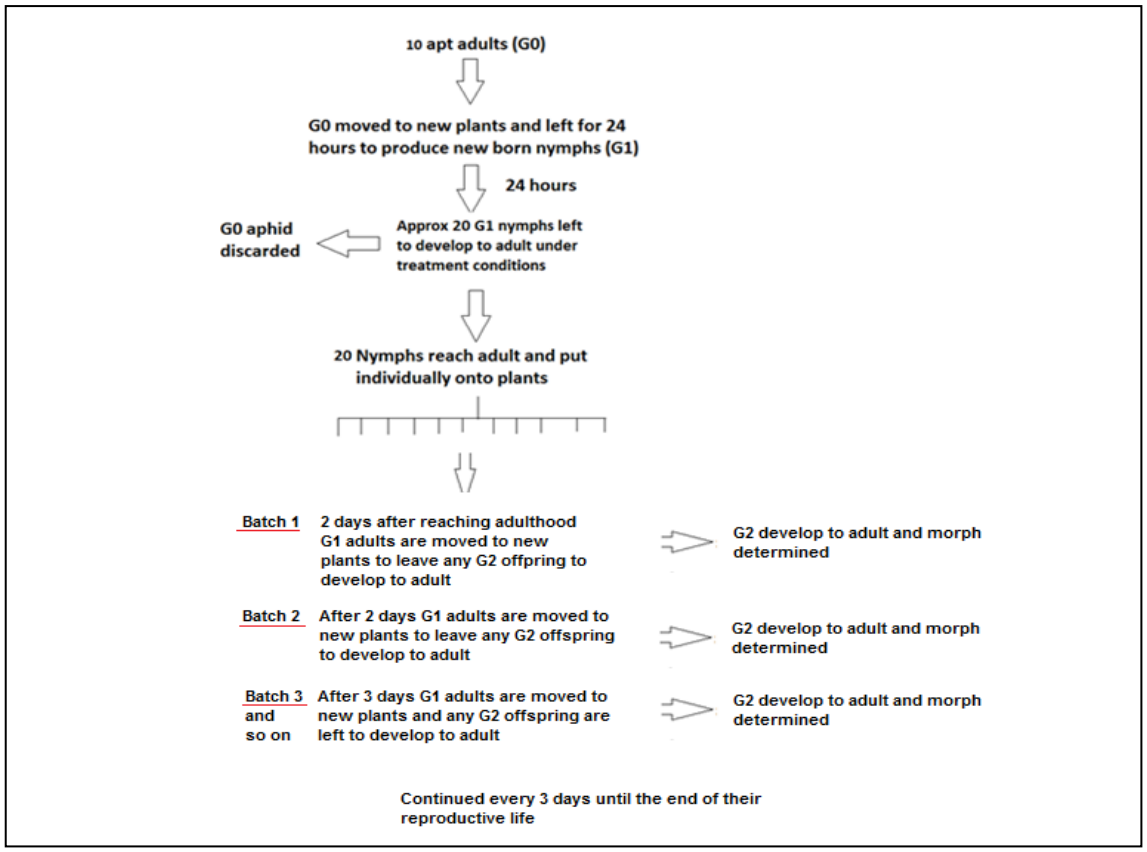


Figure 6.1 Diagram illustrating the method of producing sexual males and females.

Results

The type of aphid produced throughout the reproductive sequence is presented in Table 6.2. At 11L: 13D and 13L: 11D production of winged males was stimulated from day 10 of reproduction until the end of reproductive life, with non-winged and winged aphids being produced at the same time.

Table 6.2: Number of apterous (non-winged), alate (winged) and males produced over the reproductive life of several *N. ribisnigri* adults.

13L:11D 15°C	day 2	day 4	day 7	day 10	day 13	day 16	day 19
Apterous	28	27	30	13	4	1	1
Alate	6	11	17	3	3	0	0
Males	0	0	0	4	14	19	8

11L:13D 15°C	day 2	day 4	day 7	day 10	day 13	day 16	day 19
Apterous	56	78	67	59	17	3	10
Alate	3	0	1	11	2	0	1
Males	0	0	0	6	17	7	8

Figure 6.3 illustrates the difference between winged males and winged asexual females. More photoperiods are to be tested using different temperatures.

Figure 6.3 Winged asexual female on the left and winged sexual male on the right

Discussion

Experiment 1 - Quantifying the temperature requirements for summer development of wild type and resistance breaking N. ribisnigri on susceptible and resistant (Nr) lettuce cultivars

Higher temperatures resulted in shorter development times, while lower temperatures lengthened the development time which is characteristic of a poikilothermic insect. At 5°C, the longest developmental time occurred and this is where the largest variation in development time was seen between replications, which can be attributed to the frequency distribution of development times being skewed towards those aphids which develop the slowest (Phelps et al, 1993).

Unexpectedly, at 5, 10 and 15°C, WT aphids developed to adult on cv. Eluarde and Rotary. However, their development time was often lengthened compared with other treatments, and they also suffered earlier mortality. This suggests that at lower temperatures the Nr gene in resistant varieties fails to provide resistance and thus could be temperature sensitive. It has been suggested that this observation is similar to the way genes that control plant-virus interactions behave, where low temperatures dramatically affect defences due to low temperatures inhibiting RNA silencing-mediated defence (Szittya et al, 2003).

If the cultures had been cross-contaminated one would have expected the resilient WT aphids to have comparable developmental characteristics to their Rb counterparts in other

treatments, which they do not. Other theories could include the possibility that within a clonal line, certain individuals have pre-existing varying levels of durability to resistant cultivars, supporting the theory that clones may not be genetically identical (Loxdale, 2008).

Development time was similar regardless of the aphid type or host plant, meaning that Rb and WT *N. ribisnigri can* develop similarly on acceptable hosts at each temperature. Therefore, being resistant has no negative implications on development. It might be possible however, that other traits such as overwintering may be negatively impacted. For example, it is known that insecticide resistant *Myzus persicae* clones are more susceptible to parasitism due to a reduced response to aphid alarm pheromone (Foster, 2007)

Preliminary results suggest that Rb *N. ribisnigri* have a higher propensity to become winged than WT *N. ribisnigri,* which means that they may have better dispersal potential, which could lead to rapid spread of this new biotype. Further experiments are underway to confirm this.

Experiment 2 - Determine whether day length has an effect on WT and Rb *N. ribisnigri* development

Data are still to be collected

Experiment 3 - Identifying wild plant species that might serve as overwintering hosts

Out of all the alternative host plants screened so far, only Prickly Sowthistle and Field Sowthistle were unsuitable hosts. All other plants supported development and reproduction of both WT and Rb *N. ribisnigri* at some level.

There was large variation in the number of aphids between species of host plant, suggesting *N. ribisnigri* performs better on some host plants than others. There was also considerable variation in aphid numbers between the 1st and 2nd batches which could possibly be due to an effect of the CE room. Batch 1 was screened in Room 3 while Batch 2 was screened in Room 6. While both of these CE rooms are set at 20°C, different rooms and different areas in a room can produce a different microclimate. To investigate this, an experiment with Batch 1 will be repeated in the same room as Batch 2. Due to the large variation in the maximum number of aphids between the two batches, it was difficult to make comparisons between host plants between the batches.

When comparing the survival of Rb and WT *N. ribisnigri* on each host plant the results indicated that, except for Chicory, they had similar numbers of winged, non-winged and total *N. ribisnigri,* suggesting there is no difference in their abilities to use alternative hosts. Resistance-breaking aphids were able to perform better on Chicory compared to the WT aphid.

Experiment 4 - Preliminary work to determine the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2010)

As expected there were significantly more aphids and less predators in netted plots due to the restrictions on the movement of aphids and predators. It is also known that netting has an impact on the microclimate when compared to an un-netted plot, which can have a large effect on the development of poikilothermic insects (Jervis, 2005). Therefore, measuring the differences in temperature and humidity should be implemented in future to determine the significance of this effect.

The observed interaction between sampling week and netting treatment on aphid populations is likely to be because at later sampling dates the treatments had been netted for longer, resulting in a larger numbers of aphids as they have built up over time. However, it is clear from the data that this length of netting period results in un-natural *N. ribisnigri* numbers in netted plots as has been seen in other studies (Gardiner et al, 2009) Methods to reduce this effect have been considered for the field trial in 2011.The ineffectiveness of insecticide and fungicide treatments on the numbers of aphids and natural enemies could be because the treatments did not remain effective over the 4-5 weeks of each trial. The pyrethroid was selected as it is a broad spectrum insecticide and the fungicide was selected using a literature review (Wells et al, 2000: Koch et al, 2010 and Latteur and Jansen, 2002) to identify active ingredients which kill entomopathogenic fungi affecting aphids. New chemicals will be considered for the 2011 trial.

While natural enemies were identified, the numbers were low and trapping should also be implemented to sample insects, particularly winged insects, which are not present during destructive sampling of the lettuce.

Experiment 5 - Determining the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* (Summer 2011)

Once the complete data set has been collected, comparisons between treatments should suggest reasons for changes in aphid numbers, particularly during the mid-summer crash.

Experiment 6 - Stimulating the production of sexual male and female aphids in the laboratory to obtain eggs

Male sexual aphids have been stimulated by exposure to autumn conditions. This method will be used at different photoperiods and temperatures to stimulate sexual female production. The results so far suggest that photoperiod is the main factor which influences sexual aphid production and that it stimulates a change in the reproductive sequence.

Conclusions

- Rb *N. ribisnigri* have the same development rates as WT *N. ribisnigri* at 5, 10, 15, 20 and 25°C.
- Some WT *N. ribisnrigri* can develop to adulthood on resistant lettuce cultivars, but their survival and reproduction is often negatively affected.
- Observations indicate that Rb *N. ribisnigri* are more likely to develop into winged adults on both resistant and susceptible lettuce cultivars compared to the WT *N. ribisnigri* on susceptible lettuce cultivars. Therefore they may have an enhanced dispersal potential. An experiment is being carried out to confirm this.
- The midsummer crash in aphid numbers was not identified in the 2010 field trial but has been observed successfully in the 2011 field trial. Treatments of fungicide and pesticide had no effect in 2010, but natural enemy seasonality was recorded.
- Both WT and Rb *N. ribisnigri* can develop and reproduce equally well on Wall Speedwell, Smooth Hawksbeard, Chicory, Spiked speedwell, Common Speedwell and Mouse-ear Hawkweed, Nipplewort, Smooth Sowthistle and Orange Hawkweed. Resistance-breaking aphids were able to perform better on Chicory compared with the WT aphid.
- Male *N. ribisnigri* were produced at 13L 11D and 11L 13D at 15°C from day 10 of an adult's reproductive life. No females have been produced yet.

Knowledge and Technology Transfer

February 2010	Poster presented at HDC Studentship Conference
19 February 2010	Seminar presentation at Warwick Crop Centre
7 May 2010	Poster presented at Warwick Crop Centre, Student Symposium
21 June 2010	Grower visit - Madestein Chichester
20 August 2010	Grower visit - Intercrop Kent
22 September 2010	Poster presented at RES Aphid Special Interest Group, Syngenta
5 October 2010	Presentation at the British Leafy Salad Association Meeting, Farmers Club, Whitehall.
2-3 February 2011	Poster presented at Royal Entomological Society, Postgraduate Forum Meeting, The Royal Hotel, Hull.
5 April 2011	Poster presented at 2nd meeting of the Vegetable Genetic Improvement Network (VeGIN), Warwick Crop Centre, Wellesbourne
26-27 May 2010	Poster presented at School of Life Sciences Student Symposium
30 June 2011	Presentation at the Open Day - Protecting your Field Veg Crop. STC, Yorkshire
5-6 July 2011	Presented at the HDC 2nd Studentship Conference, East Malling Research, Kent
22 August 2011	Shadowing David Norman (Fresh Produce Consultancy Ltd) during salad crop walks for G's marketing and Shropshire's, Cambridge.
25-28 Sept 2011	Presented at the IOBC working group Integrated Protection of Field Vegetables meeting. Southern Sweden

Acknowledgements

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