Project title:	**Biology and control of currant lettuce aphid (*Nasonovia ribisnigri*)**
Project number:	CP 67
Project Leader:	Rosemary Collier Warwick HRI, Wellesbourne, Warwick CV35 9EF
Annual report:	2009/2010
Key workers:	Gemma Hough
Location of project:	Warwick HRI, Wellesbourne, Warwick, CV35 9EF
Project co-ordinators:	David Norman
Date project commenced:	1 October 2009
Date project completion due	31 December 2012
Key words:	*Nasonovia ribisnigri*, lettuce, resistance-breaking, wild type, aphid, treatment.

Signed on behalf of: **Warwick HRI**

Signature: Rosemary Collier ***Date***: 11 October 2010

Name

Dr Rosemary Collier
Acting Head of Site

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

CONTENTS PAGE

GROWER SUMMARY

SCIENCE SECTION

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

Headline

- Host-plant resistance-breaking (Rb) *N. ribisnigri* develop at the same rate as wild type (WT) *N. ribisnigri* at 10, 15, 20 and 25 °C. Five wild host plants support both Rb and WT *N. ribisnigri* populations. The survival of WT *N. ribisnigri* was equally poor on resistant butterhead cultivars from different breeding companies.

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 insecticides are effective for part of the crop's life, in particular the imidacloprid seed treatment (Gaucho) and a new systemic insecticide spirotetramat (Movento), other insecticides applied as foliar sprays to hearted crops often have relatively little impact 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 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 the EU thematic strategy for pesticides.

In recent years, lettuce cultivars with resistance to *N. ribisnigri* have been developed and released commercially but many growers still grow susceptible cultivars. Reliance on insecticides is likely to be important for many years to come. In addition, in continental Europe and more recently in the UK, certain clones of *N. ribisnigri* have overcome this host plant resistance, which is based on a single gene (Nr), suggesting that widespread failure of this asset 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 the project and main conclusions

The following experiments were done at Warwick HRI, 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**

The 6 treatments which included a control are shown in Table A. 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 10, 15, 20 or 25°C (further temperatures will include 5 and 17.5°C).

The aphids were monitored and their development times to adulthood; whether they were winged or wingless; their survival time; fecundity, and positional behaviour were recorded.

Table A **Treatments used in Experiment 1**

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

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 development time. At 10, 15, 20 and 25 °C the average development times to the adult stage for the control treatment were 17.75, 11.38, 7.6 and 6.25 days respectively, and the development times of the other treatments were similar to this. Rb *N. ribisnigri* developed 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 15°C 3 WT aphids developed to adulthood on cv Eluarde and at 10°C, 1 WT *N. ribisnigri* aphid survived to adulthood on cv. Rotary. While such aphids did survive to adulthood they often had a longer development time and also suffered earlier mortality.

Preliminary observations suggest that Rb *N. ribisnigri* are more likely to develop into winged adults compared with WT *N. ribisnigri,* meaning they may have an enhanced dispersal potential.

Experiment 2 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.

Including a control, there were 12 treatments as summarised in Table B. Alternative host plant species were sown at intervals so that all the species reached a pre-determined size, appropriate for aphid inoculation, on 3 August 2010. Plants were raised in a controlled environment room (20°C, 16h light 8h dark light regime). Five new born nymphs were then inoculated per plant. Plants were assessed on 10, 12, and 19 August. The number of surviving aphids and the occurrence of reproduction were recorded.

Six potential host plants were assessed for capacity to support both Rb and WT *N. ribisnigri* population development and reproduction. These were wall speedwell (*Veronica arvensis*), smooth hawksbeard (*Crepis capillaries*), chicory (*Chichorium intybus*), spiked speedwell (*Veronica spicata*) and orange hawkweed (*Hieracium aurantiancum*) and prickly sowthistle

(*Sonchus asper*). Survival of aphids on these species would indicate that they could be potential overwintering hosts.

When comparing the numbers of surviving aphids on the control (chicory) to the other plant hosts, the numbers on smooth hawksbeard, spiked speedwell, and wall speedwell were not significantly different. These three were the best hosts, whilst orange hawkweed and prickly sowthistle were the least successful in supporting populations. Both Rb and WT *N. ribisnigri* inoculated onto prickly sowthistle were dead by the second assessment date.

Except for orange hawkweed, there were no survival differences between WT and Rb *N. ribisnigri* on the same host plant species. The differences seen when comparing orange hawkweed were probably due to the inconsistent growth of this plant species, which led to the use of a range of plant sizes in the experiment, with the larger plants being the better hosts.

Table B Treatments used in Experiment 2

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

Experiment 3 Determining the role of predators and entomopathogenic fungi in regulating populations of N . ribisnigri

Including an untreated control, there were 9 treatments which had various fungicide (Nativo- strobilurin + triazole), insecticide (Decis - pyrethroid) and netting regimes. Table C summarises the treatments used. There were 2 replicates of each treatment (18 plots in

total) and the experiment was repeated on three occasions to allow continuous observations over the summer.

The fine mesh netting was used to exclude natural enemies from entering particular plots, (thereby reducing their impact on the aphid population), and to stop the movement of aphids in and out of the plots. Fungicide and insecticide treatments were used to attempt to reduce the numbers of entomopathogenic fungi and natural enemies respectively.

Table C Summary of treatments used in Experiment 3

Treatment Number	Aphid Inoculation	Insect proof netting	Fungicide treatment	Insecticide treatment
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

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. The plants were raised in a greenhouse.

Data were collected between June and October through the destructive sampling of 3 lettuce plants per plot each week (54 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, predators and entomopathogenic fungi were counted and identified on each plant. The insects recovered were stored in 70% ethanol for further examination and classification.

Data collection (from a field experiment) to determine the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri* is still ongoing. Once the complete data set has been collected and summarised, comparisons between treatments should help explain the reasons for changes in aphid numbers, particularly during the mid-summer aphid crash where aphid populations remain low for 6-8 weeks.

Experiment 4 Preliminary comparison of resistant and susceptible butterhead lettuce cultivars collected from different plant breeding companies

The aim of this experiment was to determine whether varying Nr gene introgression backgrounds used by different plant breeding companies have an impact on the level of resistance in their cultivars.

Including a control, there were 12 treatments as summarised in Table D. The seed (cvs Clarion, Charles, Aljeiva, Malfalda, Skyphos and Rotary) was sown on 5 April in vermiculite before being transplanted on 12 April into pots. Plants were grown in a controlled environment room for a further 2 weeks. On 27 April each plant was inoculated with 8 aphids per plant.

Beginning on 29 April the numbers of surviving aphids were assessed each day for a period of 9 days.

Table D Treatments used in Experiment 4

Treatment	Aphid type	Butterhead cultivar*	Breeder	Replication
1	8x WT *N. ribisnigri*	Clarion (Sus outdoor)	Enza Zaden	5 plants
2	8x WT *N. ribisnigri*	Charles (Sus greenhouse)	Nunhems	5 plants
3	8x WT *N. ribisnigri*	Aljeva (Nr outdoor)	Enza Zaden	5 plants
4	8x WT *N. ribisnigri*	Malfalda (Nr outdoor)	Nunhems	5 plants
5	8x WT *N. ribisnigri*	Skyphos (Nr red, organic)	Rijk Zwann	5 plants
6	8x WT *N. ribisnigri*	Rotary (Nr outdoor)	Elsoms	5 plants
7	8x Rb *N.ribisnigri*	Clarion	Enza Zaden	5 plants
8	8x Rb *N. ribisnigri*	Charles	Nunhems	5 plants
9	8x Rb *N. ribisnigri*	Aljeva	Enza Zaden	5 plants
10	8x Rb *N. ribisnigri*	Malfalda	Nunhems	5 plants
11	8x Rb *N. ribisnigri*	Skyphos	Rijk Zwann	5 plants
12	8x Rb *N. ribisnigri*	Rotary	Elsoms	5 plants

*sus- susceptible butterhead cultivar, *Nasonovia ribisnigri* (Nr) resistant butterhead cultivar)

When comparing the effectiveness of the resistant cultivars in controlling WT *N. ribisnigri*, there were no significant differences between the cultivars, suggesting that there is no effect of genetic background on the control of WT *N. ribisnigri*.

Rb *N. ribisnigri* had relatively high survival on all the butterhead cultivars indicating that Rb *N. ribisnigri* can survive on both the resistant and susceptible lettuce cultivars equally well.

Conclusions

- Rb *N. ribisnigri* has the same development rates as WT *N. ribisnigri* at 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.
- Preliminary 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.
- Different resistant butterhead lettuce cultivars do not show any variation in their ability to control WT *N. ribisnigri*.
- Both WT and Rb *N. ribisnigri* can develop and reproduce equally well on wall speedwell, smooth hawksbeard, chicory, spiked speedwell and orange hawkweed.

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, in particular an imidacloprid seed treatment (Gaucho, Bayer UK) and a new systemic insecticide spirotetramat (Movento, Bayer UK), 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 the EU thematic strategy for pesticides.

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, 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 2009 and September 2010 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 Identifying wild plant species that might serve as overwintering hosts

Experiment 3 Carrying out preliminary work to determine the role of predators and entomopathogenic fungi in regulating populations of *N. ribisnigri*.

Experiment 4 Preliminary comparison of resistant and susceptible Butterhead cultivars from different plant breeding companies

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 conducted in controlled environment rooms in the Insect Rearing Unit at Warwick HRI, 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 HRI (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 lettuces 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 microperforated plastic 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 10 (2 repeats), 15 (2 repeats), 20 (2 repeats), and 25°C (3 repeats), with further experiments to be carried out at 5 and 17.5°C.

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. Adulthood was initially determined by a combination of counting the number of moults and by identification of adult characteristics.
- 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. Figure 1.3 indicates how many aphids the results were based on. Interpretations of the data were made with treatment means.

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. 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 and 10°C.

Individually, aphid type and plant host also had significant effects on development time. When considered together there were significant differences depending on which aphid type was on which plant. This can be attributed to the unexpected survival of WT *N. ribisnigri* on the resistant lettuce cultivars where the type of aphid and host plant had a significant effect on development.

There were no significant differences in development time between WT and Rb aphid types within each temperature. Similarly there were no significant differences in development time between host plant types within each temperature. Therefore, development time was the same within each temperature, regardless of host plant or aphid type.

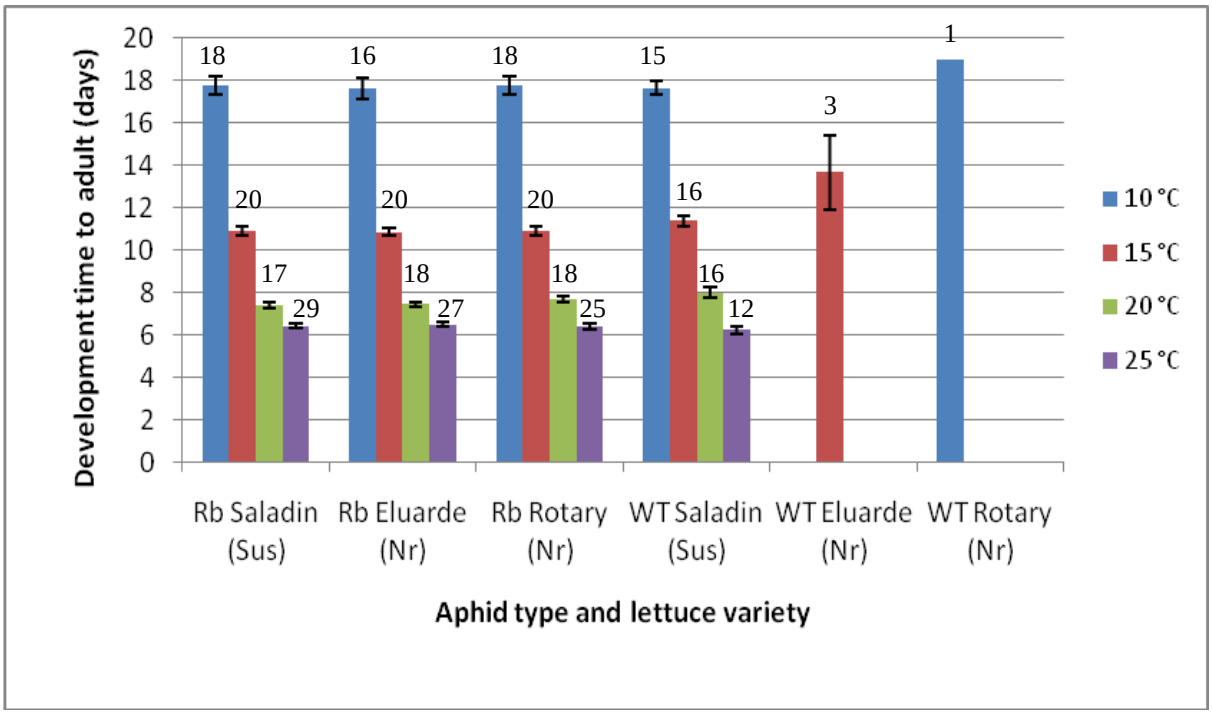

Figure 1.3: Mean number of days for development to adulthood for WT and Rb *N. ribisnigri* on resistant and susceptible lettuce cultivars at 10, 15, 20 and 25°C. The numbers above each bar indicate the number of aphids the mean is based on

Morph production

Using the data for adults which reached adulthood, the proportion of winged aphids was 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). More records are to be collected before the data can be analysed together with information on fecundity and survival.

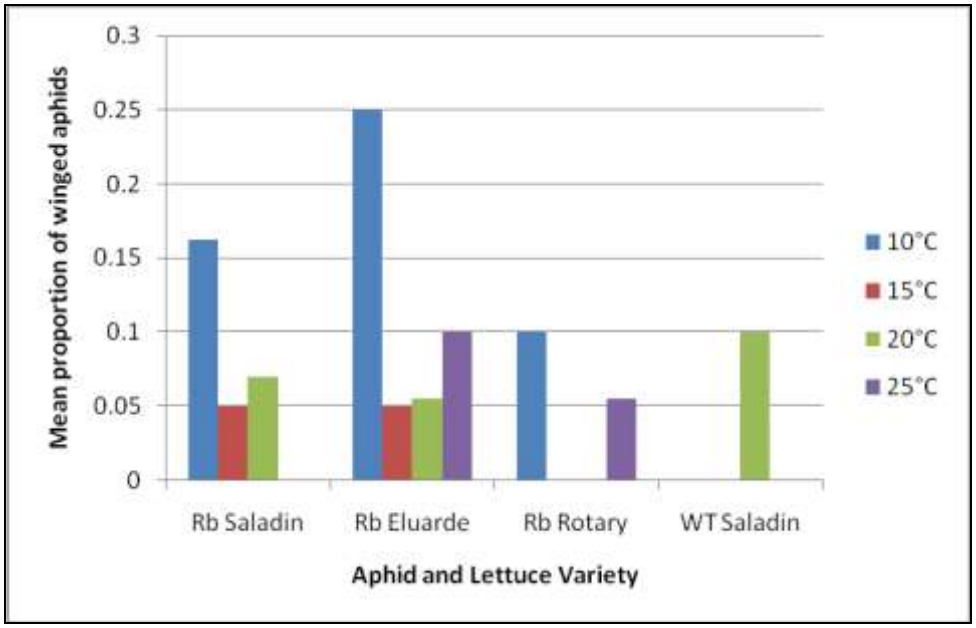

Figure 1.4: Mean proportion of WT and Rb *N. ribisnigri* developing into winged adults on resistant and susceptible lettuce cultivars at 10, 15, 20 and 25°C

Experiment 2 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 experiment was carried out in controlled environment room No. 6 in the Insect Rearing Unit at Warwick HRI, Wellesbourne.

There were 12 treatments. WT and Rb *N. ribisnigri* were reared on 6 alternative hosts (See Table 2.1). The alternative hosts were identified through a literature search. Preliminary work determined the optimum germinating conditions for each species and the appropriate growth stage for inoculation with aphids (ranging from 27-45 days). Each treatment had 5 replicates.

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

Table 2.1: Summary of treatments used in Experiment 2

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

New born nymphs were used in the experiment. These were obtained by inoculating 60 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 5 were placed onto each host plant using a fine paint brush. The 60 inoculated plants were each covered with a bread bag, which was secured with an elastic band, and arranged on a single shelf in controlled environment room 6 (20°C 18L:6D) (Figure 2.1). There were 5 replicates of each treatment which were arranged in a randomised design with 4 rows and 15 columns.

Figure 2.2: The alternative host experiment

Assessments

Plants were assessed on 10, 12, and 19 August. The numbers of surviving nymphs were recorded together with their intra-plant distribution. On reaching adulthood, the morph (winged or wingless) and whether reproduction had occurred were recorded. The aphids were then left to determine whether they could establish a population.

Results

Statistical analysis

Analyses were performed using analysis of variance (ANOVA). Angular transformations were used on the percentage of surviving aphids (out of 5) on day 7 of development to ensure homogeneity between treatments. Interpretations of the data were made using treatment means and 5% LSD values.

Assessment of the number of aphids surviving on day 7 of their development

WT and Rb *N. ribisinigri* survived on all host plants except Prickly Sowthistle (*S. asper*), where WT and Rb *N. ribisnigri* both suffered 100% mortality by the second assessment date. When compared to the control, both *S. asper* treatments were significantly different. The control was also significantly different from the WT *N. ribisnigri* + *H. aurantiacum* treatment.

C. capillaries, V. spicata, C. intybus and *V. arvensis* were amongst the best hosts, with no significant differences between percentage aphid survival when compared with the control and each other. When comparing the survival of Rb and WT *N. ribisnigri* on the same host plant, the only significant difference was seen between WT and Rb *N. ribisnigri* + *H. aurantiacum*.

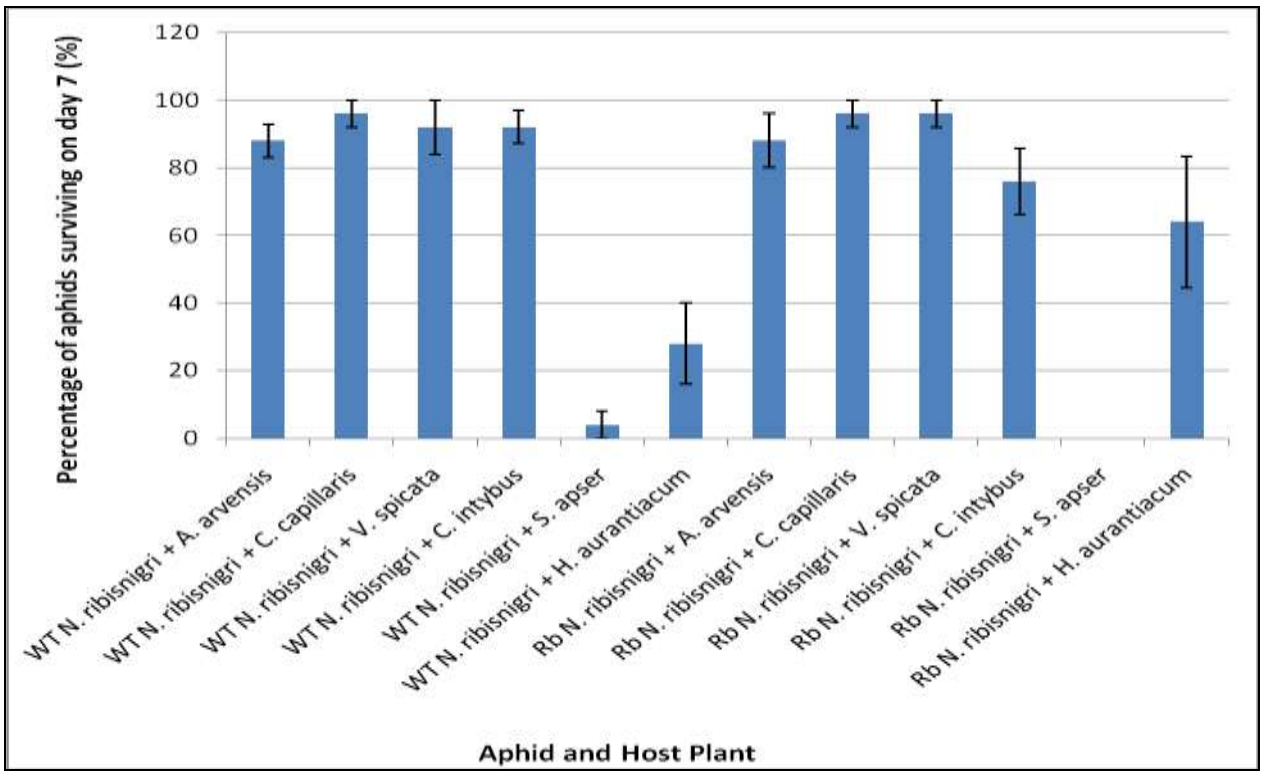

Figure 2.2: Mean percentage survival of WT and *Rb N. ribisnigri* on 6 alternative host plants on day 7 at 20°C (18L:6D)

Experiment 3 Determining the role of predators and entomopath-ogenic fungi in regulating field populations of *N. ribisnigri*

Materials and methods

This preliminary experiment took place between July-October 2010 in the field known as Sheep Pens at Warwick HRI, 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 is in preparation for a larger field experiment during summer 2011.

There were 9 treatments combining various fungicide (Nativo – trifloxystrobin + tebuconazole), insecticide (Decis – deltamethrin) and netting regimes (see Table 3.1).

The 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 were used to attempt to reduce the numbers of aphid 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 3.2 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 previously placed into Eppendorf tubes.

Table 3.1: Summary of treatments used in Experiment 3

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 3.2: 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 which were alternated each week to maintain plot integrity. Plants were stored in paper bags in a cold store (5°C) where 3 of the 4 lettuces from each plot were then destructively sampled. Whole plants were sampled until they grew to an unmanageable size and then only half of each plant was sampled.

Aphids were counted and identified, together with the predators and the number of aphids infected by entomopathogenic fungi was recorded. All insects were stored in 70% ethanol for further identification.

Results

Data are still being collected**.**

Experiment 4 Preliminary comparison of resistant and susceptible butterhead lettuce cultivars from different plant breeding companies

Materials and methods

The aim of this experiment was to determine whether the varying Nr gene introgression backgrounds used by different plant breeding companies have an impact on the level of resistance in their cultivars.

The experiment was done in controlled environment room No. 6 in the Insect Rearing Unit at Warwick HRI, Wellesbourne.

There were 12 treatments (Table 4.1). WT and Rb *N. ribisnigri* were reared on 6 different susceptible and resistant butterhead lettuce cultivars from different plant breeding companies. Each treatment consisted of 5 plants.

The WT aphids used were a clone maintained at Warwick HRI (clone 4850a) and the Rb aphids were from a population obtained from Kent in October 2009.

New born nymphs were used in the experiment. These were obtained by inoculating 180 winged adults into a cage containing 6 lettuces 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. Eight new born nymphs were placed onto each lettuce plant using a fine paint brush. The 60 inoculated plants were each covered with a bread bag, which was secured with an elastic band, and arranged on a single shelf in controlled environment room 6 (20°C 18L:6D). The numbers of surviving aphids per plant were assessed. There were 5 replicates of each treatment which were arranged in a randomised design with 4 rows and 15 columns.

Table 4.1: Summary of treatments used in Experiment 4

Treatment	Aphid type	Butterhead cultivar*	Breeder	Replication
1	8x WT *N. ribisnigri*	Clarion (Sus outdoor)	Enza Zaden	5 plants
2	8x WT *N. ribisnigri*	Charles (Sus greenhouse)	Nunhems	5 plants
3	8x WT *N. ribisnigri*	Aljeva (Nr outdoor)	Enza Zaden	5 plants
4	8x WT *N. ribisnigri*	Malfalda (Nr outdoor)	Nunhems	5 plants
5	8x WT *N. ribisnigri*	Skyphos (Nr red, organic)	Rijk Zwann	5 plants
6	8x WT *N. ribisnigri*	Rotary (Nr outdoor)	Elsoms	5 plants
7	8x Rb *N.ribisnigri*	Clarion	Enza Zaden	5 plants
8	8x Rb *N. ribisnigri*	Charles	Nunhems	5 plants
9	8x Rb *N. ribisnigri*	Aljeva	Enza Zaden	5 plants
10	8x Rb *N. ribisnigri*	Malfalda	Nunhems	5 plants
11	8x Rb *N. ribisnigri*	Skyphos	Rijk Zwann	5 plants
12	8x Rb *N. ribisnigri*	Rotary	Elsoms	5 plants

Results

Statistical analysis

Analyses were performed using analysis of variance (ANOVA). Angular transformations were used on the percentage of surviving aphids (out of 8) on day 9 of development to ensure homogeneity between treatments. Interpretations of the data were made using treatment means and 5% LSD values.

Assessment of the percentage number of surviving aphids on day 9

The mean percentage of aphids surviving on day 9 of development is presented in Figure 4.1. As expected WT *N. ribisnigri* on resistant lettuce cultivars suffered nearly 100% mortality by day 9, which was significantly different from WT *N. ribisnigri* on the susceptible lettuce cultivars (including the control WT + Clarion) and all Rb *N. ribisnigri* treatments.

There were no significant differences in the percentage survival of the WT *N. ribisnigri* between the resistant butterhead cultivars.

Rb *N. ribisnigri* had relatively high survival on all the butterhead cultivars. Within the Rb *N. ribisnigri* treatments, marginally significant differences were observed between cv. Skyphos and cvs. Clarion, Malfalda and Rotary, suggesting that Skyphos was a better host for Rb N. ribisnigri when compared to these hosts.

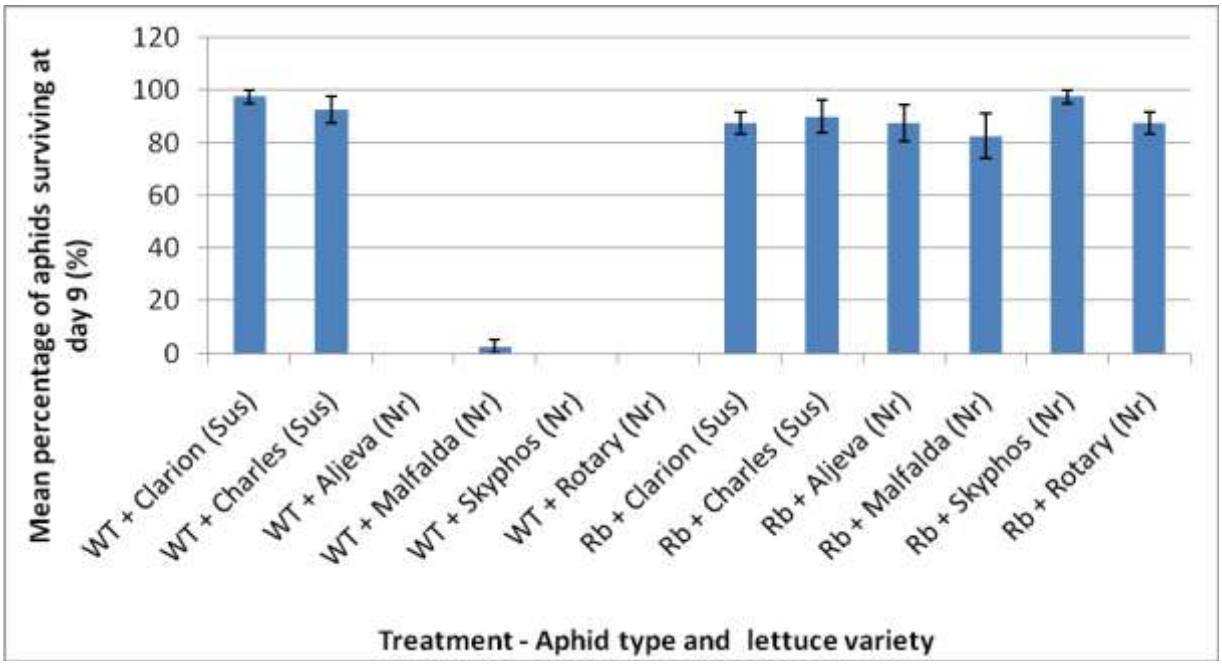

Figure 4.1: Mean percentage of aphids surviving on day 9 of their development

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

In this experiment, temperature, aphid type or host plant type had the potential to affect development time. When analysing these factors individually, temperature had a significant impact on both the development of WT and Rb *N. ribisnigri.* Higher temperatures resulted in shorter development times, while lower temperatures lengthened the development time. Unexpectedly at 15°C, 3 WT aphids developed to adult on cv. Eluarde and at 10°C 1 WT aphid survived to adulthood on cv. Rotary. However, their development time was often lengthened compared with other treatments, and they also suffered earlier mortality.

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. However, it might be possible that within a clonal line certain individuals have pre-existing varying levels of durability to resistant cultivars, supporting the theory that aphid clones may not be genetically identical (Loxdale, 2008).

When analysing the interaction between the factors it was found that there were no significant differences in development time due to a temperature and aphid type or host plant type interaction. Therefore, development time was similar regardless of the aphid type or host plant meaning Rb and WT *N. ribisnigri can* develop can similarly on acceptable hosts at each temperature.

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 a spread of this new biotype.

Experiment 2 Identifying wild plant species that might serve as overwintering hosts

Out of all the alternative host plants tested, only *S. asper* was an unsuitable host, all other plants supported development and reproduction of both WT and Rb *N. ribisnigri*.

C. capillaries, V. spicata, C. intybus and *V. arvensis* were the best host plants with no significant differences when compared with the control or with each other. *H. aurantiacum* and *S. asper* were the poorer hosts when compared with the control, with *S. asper* unable to sustain a population.

When comparing the survival of Rb and WT *N. ribisnigri,* the results indicated that they had similar survival on all but one host plant. Therefore, for Rb *N. ribisnigri,* being able to overcome host plant resistance appears to have no fitness costs associated with survival on alternative hosts to lettuce. Furthermore, on *H. aurantiacum*, the Rb *N. ribisnigri* outperformed WT. *N. ribisnigri,* but this could be attributed to the differences in plant size, as the *H. aurantiacum* did not have uniform germination and a mixture of plants were used, where the small plants were observed to be poorer hosts.

Experiment 3 Determining the role of predators and entomopathogenic fungi in regulating population of *N. ribisnigri*

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 4 Preliminary comparison of resistant and susceptible butterhead cultivars from different plant breeding companies

Rb *N. ribisnigri* had relatively high survival on all the butterhead cultivars, and are not substantially affected by the resistant/susceptible status of lettuce cultivars.

Within the Rb *N. ribisnigri* treatments, marginally significant differences were observed between the best performing cv. Skyphos when compared to cvs. Clarion, Malfalda and Rotary, suggesting that Skyphos is a more susceptible host for Rb *N. ribisnigri* than several other 'resistant' cultivars.

When comparing the effectiveness of the resistant cultivars in controlling WT *N. ribisnigri*, there were no significant differences between the cultivars. Therefore, the varying Nr gene introgression backgrounds used by different plant breeding companies have no impact on the level of resistance in the cultivar.

CONCLUSIONS

- Resistance-breaking *N. ribisnigri* have the same development rates as WT *N. ribisnigri* at 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.
- Preliminary results indicate that resistance-breaking *N. ribisnigri* have a higher propensity to become winged adults on resistant or susceptible lettuce cultivars compared with WT N. ribisnigri on susceptible lettuce cultivars. Therefore they may have an enhanced dispersal potential.
- Different Nr lettuce cultivars do not show differences in their ability to control WT *N. ribisnigri*. This suggests that the varying Nr gene introgression backgrounds used by

different plant breeding companies does not result in cultivars with different levels of resistance.

- Both WT and Rb *N. ribsinigri* can survive and reproduce on *Veronica arvensis, Crepis capillaries, Veronica spicata, Chichorium intybus,* and *Hieracium aurantiacum*, but not *Sonchus asper. Crepis capillaries, V. spicata, C. intybus* and *V. arvensis* were the best hosts.
- Both WT and Rb *N. ribisnigri* can develop and reproduce equally well on Wall speedwell, Smooth Hawksbeard, Chicory, Spiked Speedwell and Orange Hawkweed.

TECHNOLOGY TRANSFER

February 2010	Poster presented at HDC Studentship Conference
19 February 2010	Seminar presentation at Warwick HRI
7 May 2010	Poster presented at Warwick HRI, 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.

ACKNOWLEDGEMENTS

We thank the HDC for funding this work, Elsoms Limited, Rijk Zwaan UK Ltd, Nunhems and Enza Zaden for providing much of the seed. Chris Wallwork, UAP, for providing the resistance-breaking *N. ribisinigri.* Richard Jackson and Julie Jones for providing experimental designs and statistical analyses.

REFERENCES

DIAZ, B. M. & FERERES, A. 2005. Life table and population parameters of Nasonovia ribisnigri (Homoptera : aphididae) at different constant temperatures. *Environmental Entomology,* 34**,** 527-534.

LOXDALE, H. D. 2008. The nature and reality of the aphid clone: genetic variation, adaptation and evolution. *Agricultural and Forest Entomology,* 10**,** 81-90.

