Project title:	Biology and Control of Currant-Lettuce Aphid (*Nasonovia ribisnigri*)
Project number:	CP 067
Project leader:	Rosemary Collier, University of Warwick
Report:	Final report, March 2013
Previous report:	Annual report, October 2011
Key staff:	Gemma Hough (PhD student)
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Date project commenced:	1 October 2009
Date project completed (or expected completion date):	31 March 2013

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The results and conclusions in this report are based on an investigation conducted over a three-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.

Rosemary Collier
Director, Warwick Crop Centre, School of Life Sciences
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GROWER SUMMARY

Headline

- More accurate prediction of population development of currant-lettuce aphid can now be made for use in forecasting models
- Active currant-lettuce aphids (adults and nymphs) can overwinter in the Midlands, on 'alternative' host-plants to lettuce, particularly speedwell

Background

The currant-lettuce aphid (*Nasonovia ribisnigri*) is one of four significant species of pest aphid infesting lettuce, and is the most important due to its preference to feed in the centre of lettuce heads where the infestation is often difficult to control with foliar insecticides, resulting in unmarketable produce and therefore financial losses for growers. Rapid population development of *N. ribisnigri* can also lead to stunted plant growth and affect the palatability of harvested lettuce.

Historically, aphids have been controlled by farmers and growers through the application of pesticides. However, due to recent concerns about potential chemical residues and the imposition of high selective pressures for insecticide resistance, there have been increased demands for farmers and growers to adopt Integrated Pest Management (IPM) practices. For *N. ribisnigri,* resistant lettuce cultivars are also available but now that these are grown widely, the increased selection pressure appears to have resulted in a new resistance-breaking biotype of *N. ribisnigri* which has overcome the resistance provided by these cultivars.

Recent research on *N. ribisnigri* has focused on its development, insecticide resistance and its response to resistant cultivars. Therefore, there is little information available on its basic biology which is vital for creating new and informed control strategies.

The overall aim of this PhD project was to quantify aspects of the life-cycle of both wild type (WT) and host-plant-resistance-breaking (Rb) *N. ribisnigri* to inform the development of a more effective and targeted control strategy. The specific objectives were to:

1) Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.
2) Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching.

3) Investigate alternative host plants (to lettuce) and confirm whether *N. ribisnigri* can use them as overwintering hosts.
4) Investigate the population dynamics of *N. ribisnigri* in response to natural enemies and entomopathogenic fungi.

Summary

One clone and one population of wild type (WT) and two clones and one population of host-plant-resistance-breaking (Rb) *N. ribisnigri* biotypes were used in this research.

1. Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.

The effects of temperature and daylength on the developmental parameters of WT *N. ribisnigri* have been established. This has included establishing optimum temperatures and lower and upper developmental thresholds (agreeing with those determined in a similar study). The study confirmed that temperature is a significant factor affecting development time, development rate, the intrinsic rate of increase, fecundity and the propensity to become winged, of both WT and Rb *N. ribisnigri*.

The data collected in this study described a linear relationship between development rate and temperature, allowing for the estimation of the day-degree requirements for development from nymph to the final adult moult (which was again similar to other work). Daylength did not influence development, and estimates were similar between WT and Rb *N. ribisnigri,* meaning that aphid 'type' does not need to be considered in the development of the forecast.

Prior to this study, the method used for predicting the population development of *N. ribisnigri* in the UK was based on a day-degree model, using an Estimated Lower Developmental Threshold temperature. This forecast can now be refined, using the values determined specifically for *N. ribisnigri*, to provide a more accurate forecast of its activity.

This study raised questions about the effectiveness of aphid-resistant cultivars at lower temperatures, where the control provided in resistant cultivars by the Nr-gene appeared to fail. However, as the ambient temperature fluctuates in the field, and is likely to be above 15ºC for at least some of the period during which lettuce crops are grown, resistance will still be provided against WT *N. ribisnigri*. As a breakdown in resistance was not observed in the field prior to the 'arrival' of the new resistance-breaking biotype, it seems likely that the temperature sensitivity of the Nr-gene is unlikely to threaten the control of WT *N. ribisnigri*.

Despite this, the effects of temperature, particularly fluctuating temperatures, on the performance of new resistant cultivars should be analysed to clarify this.

2. Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching

Nasonovia ribisnigri reproduces throughout the summer months, without mating, on lettuce and other broad leaved hosts. As temperature and daylength decrease in autumn, winged males are produced initially, followed by winged females, which migrate around mid-October to the winter host (currant species). The winged females then produce another type of female, which lays eggs after mating with males found on the winter host. Once the eggs have been deposited, usually in the angle between a stem and a bud, they enter a state of diapause. Experiments showed that rearing conditions of 12°C 13 hours light: 11 hours darkness can induce production of the winged males and females, which leads to the production of diapausing eggs after approximately 49 days.

In the field, diapause ended during mid-late January, but temperatures below the Lower Developmental Threshold (4.6°C) prolonged post-diapause development and hatching until early February. It was estimated that post-diapause development takes just under 50 day-degrees, using a Lower Developmental Threshold of 4.6°C. The eggs hatch to produce female aphids, which develop and begin reproduction, feeding from the nutrient-rich buds of the currant plant. Once winged progeny are produced, migration to lettuce crops occurs. At this stage, currant is no longer accepted as a suitable host for colonisation (no nymphs are produced).

3. Investigate alternative host-plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts.

The data collected in this study confirmed that WT and Rb *N. ribisnigri* can utilise several alternative hosts in the summer. Eight species of plant (mainly wild species) were confirmed as suitable hosts, including *Cichorium intybus, Crepis capillaris, Lapsana communis, Hieracium aurantiacum, Hieracium pilosella, Veronica arvensis, Veronica spicata* and *Veronica officinalis*. Development of WT and Rb *N. ribisnigri* biotypes was similar regardless of the host-plant. A selection of these host-plants also supported overwintering, active stages of *N. ribisnigri* between November and March, confirming that *N. ribisnigri* may overwinter as nymphs/adults, an attribute which could have implications for the timing of their spring migration, as aphids overwintering in the active stages continue development as soon as temperatures exceed the Lower Developmental Threshold. It is likely that such aphids would migrate to lettuce crops ‘sooner’ and develop larger summer populations than

those overwintering as eggs. Removal of potential winter host-plants would remove possible refuges for *N. ribisnigri* but consideration must be given to their 'other' roles, for example, as a nectar source for natural enemies during the summer.

Finally, this study confirmed that temperature and host-plant location were the key factors determining aphid survival during the winter, with a combination of sheltered plants and mild winters resulting in enhanced survival and potentially larger spring populations.

4. Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi

The monitoring of *N. ribisnigri* populations in field trials during 2010 and 2011 recorded the occurrence of the mid-summer crash, which has been described for various aphid species. In this study, in both years, high natural enemy numbers were observed prior to the decline, suggesting that this was one of the most important regulating factors for *N. ribisnigri* populations. Entomopathogenic fungi, hover fly larvae and parasitoids were present in the highest numbers during these trials and future work should focus on determining the effects of individual predator species.

Emigration was also determined to be an important factor regulating aphid populations as the percentage of winged aphids was observed to increase prior to the mid-summer crash in both field trial years. As this study only analysed the potential for emigration to occur, future work should implement methods to monitor 'real time' emigration to confirm its role in the mid-summer crash.

Like various other studies, this study has failed to identify a single factor which resulted in the mid-summer crash, but it has identified significant factors involved. Due to its complex nature it is uncertain whether the mid-summer crash will ever be understood fully, but achieving this would allow researchers to predict when aphids will decline naturally, therefore avoiding unnecessary insecticide applications. Idealistically, identifying the factors responsible could facilitate the re-creation of these conditions in the field to induce an aphid decline when required.

Life cycle

Using the information collected in this study a more detailed life-cycle of *N. ribisnigri* can be provided:

- Female *N. ribisnigri* reproduce without mating throughout the summer months, feeding on lettuce and several species of broad-leaved weed. Development occurs at temperatures above 4.6°C, where development from nymph to the final adult moult takes approximately 121 day-degrees. Temperatures exceeding 26°C are deleterious to development.

- As temperature and daylength decrease in autumn, winged males are produced initially, followed by winged females and these migrate around mid-October to the winter host (currant species). The females then produce another form of female, which lays eggs after mating with males found on the winter host.

- Once the eggs have been deposited, usually in the angle between a stem and a bud, they enter a state of diapause, which terminates naturally in the field between late-January and early-February. However, the preponderance of temperatures below the Lower Developmental Threshold for egg development delays hatching until late February.

- Female aphids hatch from the eggs, develop and begin reproduction, feeding from the buds of the primary host-plant. Once winged females are produced, migration to lettuce (and weed hosts) occurs. At this stage, currant is no longer accepted as a suitable host for colonisation.

- It has also been confirmed that *N. ribisnigri* can overwinter as active aphids (adults and nymphs) in the Midlands, on 'alternative' host-plants to lettuce, particularly *Veronica arvensis* (speedwell).

Financial Benefits

As this is a PhD project, there are no direct immediate financial benefits, but in the longer term this work will support the improved accuracy of pest forecasting and hence grower management programmes.

Action Points

- There are no action points for growers.

SCIENCE SECTION

Introduction

Nasonovia ribisnigri is one of four significant species of aphid infesting lettuce, and is the most important due to its preference to feed in the centre of lettuce heads where the infestation is often difficult to control with foliar insecticides, resulting in unmarketable produce and therefore financial losses for growers. Rapid population development of *N. ribisnigri* can also lead to stunted plant growth and affect the palatability of harvested lettuce. *N. ribisnigri* is known to transmit gooseberry vein-banding virus on its winter host *Ribes* species and the mosaic diseases of cauliflower and cucumber. However, *N. ribisnigri* appears to be unable to transmit lettuce mosaic virus.

Historically, aphids have been controlled by farmers and growers through the application of pesticides. However, due to recent concerns about potential chemical residues and the imposition of high selective pressures for insecticide resistance, there have been increased demands for farmers and growers to adopt Integrated Pest Management (IPM) practices. For *N. ribisnigri,* resistant lettuce cultivars are available, but now that these are grown widely, the increased selection pressure appears to have resulted in a new resistance-breaking biotype of *N. ribisnigri*.

Recent research on *N. ribisnigri* has focused on its development, insecticide resistance and response to resistant cultivars. Therefore, there is little information available on its basic biology which is vital for creating new and informed control strategies.

The overall aim of this PhD project was to quantify aspects of the life-cycle of both wild type (WT) and host-plant-resistance-breaking (Rb) *N. ribisnigri* to inform the development of a more effective and targeted control strategy. The specific objectives are to:

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3) Investigate alternative host-plants (to lettuce) and confirm whether *N. ribisnigri* can use them as overwintering hosts.

4) Investigate the population dynamics of *N. ribisnigri* in response to natural enemies and entomopathogenic fungi.

Materials and methods

Objective 1. Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.

Three clones and two populations of either wild type (WT) or host-plant-resistance-breaking (Rb) *N. ribisnigri* biotypes were used throughout this research.

Table 1. Aphid biotype name, lineage and known history.

Biotype name	Lineage	History
WT4850a	WT Clone	Collected in September 2003 from a lettuce field in Lincolnshire.
WTKent10Pop	WT Population	Received on 12 November 2010 from an infested lettuce field in Kent. Maintained as a population.
RbKentPop	Rb Population	Received on 16 October 2009 from an infested lettuce field in Kent on resistant (Nr) cultivars. Maintained as a population.
RbKent	Rb Clone	A clonal line established in the laboratory from a single founding mother taken from RbKentPop.
RbUK631	Rb Clone	Received on 3 December 2009. UK geographical location unknown. Clonal line established in the laboratory from a single founding mother.

Each of the aphid biotypes described in Table 1 were reared as continuous cultures in the Insect Rearing Unit at Warwick Crop Centre to ensure a regular supply of *N. ribisnigri* of all life stages.

The effects of temperature (mean incubator temperatures of 5.5, 12.5, 15.9, 21.4 and 26.4°C) and photoperiod (14L:10D and 16L:8D) on the developmental parameters of *N. ribisnigri* were determined by rearing aphids under different treatment regimes in the laboratory using controlled environment rooms and cooling incubators. WT and Rb *N. ribisnigri* were reared on three cultivars of lettuce (cvs Saladin (susceptible), Eluarde (resistant), Rotary (resistant)) at different temperatures, while several WT and Rb *N. ribisnigri* biotypes were reared on two cultivars (cvs Saladin (susceptible) and Rotary

(resistant)) at different photoperiod. WT *N. ribisnigri* reared on cv. Saladin was used as a control. Aphid biotypes WT 4850a and RbKent were screened at different temperatures and aphid biotypes WT 4850a, WTKent10Pop, RbKent, RbUK631 and RbKentPop were screened at different photoperiods.

Objective 2. Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching

Obtaining and monitoring eggs from the field

Year 2010

To enable monitoring and collection of *N. ribisnigri* eggs laid under natural field conditions, blackcurrant bushes and infested lettuce plants were caged together, using one of two types of enclosure. During July 2010, seven enclosures were erected at Warwick Crop Centre, Wellesbourne in a field known as Long Meadow Centre. Each enclosure consisted of four metal frames (each 40-50cm wide x 70-80cm high) which were covered with insect-proof netting (Enviromesh® 1.35mm mesh size to exclude aphids) as shown in Figure 1. Three blackcurrant bushes (*Ribes nigrum*) were then planted within the enclosure and MyPex® was laid over the soil to suppress weed growth.

At the same time, two walk-in cages (3m long x 2m wide x 2m high) were also placed in Long Meadow Centre. These contained three blackcurrant bushes as shown in Figure 1b.

a)  b) 

c) 

Figure 1. a) Seven enclosures covered with insect proof netting **b)** walk-in cages **c)** plastic planting troughs containing lettuce plants, all used to monitor and collect *N. ribisnigri* eggs laid under natural field conditions.

On 18 August 2010, 80 seeds of lettuce cv. Saladin were sown in vermiculite and were transplanted individually into 400ml square plant pots one week later, where they were then grown for a further three weeks.

On 15 September 2010, the lettuce plants were infested with *N. ribisnigri* (clone WT4850a) by inoculating each plant with 20 aphids, consisting of a mixture of developmental stages. These were transplanted into the enclosures. Six lettuce plants were planted through the MyPex® in each of the seven enclosures and the remainder was divided between the two large cages. Fresh infested lettuce plants were added to the enclosures as required and irrigation was applied using a sprinkler system.

Once planting had taken place, the blackcurrant bushes in each enclosure were monitored weekly for the arrival of sexual morphs and the deposition of eggs. The small enclosures were numbered 1-7 and each of the three small blackcurrant bushes was monitored. In the two larger walk-in cages, six branches of each of the three blackcurrant bushes in each cage were tagged and these were checked each week. Sampling commenced on 1 November 2010 and continued until 6 December 2010. When necessary, specimens of the aphids were collected and taken to the laboratory for identification. Weekly monitoring for egg hatch

commenced on 6 January 2011 and continued until fundatrices (mature wingless female aphids which hatch from over-wintering eggs) emerged.

Year 2011

In 2011, the method used in 2010 was repeated but the use of the large cages was abandoned as during monitoring in 2010, *Hyperomyza lactucae* was found depositing eggs on the blackcurrants inside the cages, indicating that they were not excluding other aphid species. Furthermore, once eggs had been laid it was impossible to identify them until they had hatched in the following spring.

The plant raising and infestation process was repeated for the seven enclosures as per 2010. Plants were sown on 13 June 2011 and were infested with *N. ribisnigri* and planted on 13 July 2011. However, instead of planting infested lettuce plants through the MyPex®, six lettuce plants were planted into plastic trough planters, which were then placed inside the enclosures as shown in Figure 1c. New troughs were added as required and irrigation was applied using sprinklers.

Weekly monitoring for sexual morphs and egg deposition ran from 5 September 2011 until 17 December 2011. Weekly monitoring for egg hatch commenced on 6 January 2012 until fundatrices emerged. The small enclosures were numbered 1-7 and each of the three small blackcurrant bushes was monitored. When necessary, specimens of the aphids were collected and taken to the laboratory for identification.

Producing sexual morphs

To induce sexual morph production, the natural conditions that *N. ribisnigri* would experience in September were determined. Using the 2009 records from the University of Warwick, Wellesbourne meteorological station, the mean maximum and minimum temperatures for September were calculated, which gave a mean maximum temperature of 19.3°C and a minimum of 10°C. These values gave an average of 14.7°C, which was the temperature used in the experiment. The ‘typical’ photoperiod for September (13L:11D) was also determined.

The effect on sexual morph production of a lower temperature (12°C) and longer photoperiods (14L:10D, 16L:8D) was also investigated. Longer photoperiods were included to determine which day lengths in excess of 13 hours were suitable for inducing sexual morph production following confirmation that 13L:11D induced sexual morphs. The photoperiods screened included 12°C 16L:ID, 15°C 14L:10D, 15°C 13L:11D, 12°C 13L:11D and 12 14L:10D.

Initially ten third instar nymphs of WT *N. ribisnigri* (clone 4850a) were obtained from the stock culture. In later experiments, 15 aphids were used to compensate for any mortality and the reduced reproduction at the lower treatment temperature. The aphids were then grown on lettuce plants (cv. Saladin) under one of the five treatment regimes until they began to reproduce (G_0), which provided pre-natal conditioning of the embryos.

The G_0 aphids were then moved to new lettuce plants and left for either 24 hours or 48 hours under the same treatment regime to provide G_1 nymphs of a similar age. Aphids kept at 15°C were left for 24 hours and those at 12°C for 48 hours because reproduction was slower at the lower temperature. Aphids were divided between several plants to avoid the development of alate parthenogenetic aphids as a result of crowding.

The G_0 aphids were then discarded and the G_1 nymphs were left to develop to adulthood under the treatment regime used to provide post-natal conditioning. The nymphs were divided between two lettuce plants (cv. Saladin) to avoid a crowding stimulus. Once the G_1 nymphs reached adulthood they were transferred to individual lettuce plants and kept under the same treatment regime, where they began to produce G_2 offspring.

At 15°C, on days 2 and 4, and every three days thereafter, the G_1 adults were moved to a new plant. The G_2 nymphs were allowed to remain on the natal plant to develop to adulthood. The type of adult morph was then recorded. At 12°C, G_1 adults were moved to a new plant every four days as their development and reproduction were slower. This method provided batches of offspring from the reproductive sequence.

The morphs produced were apterous parthenogenetic females, alate parthenogenetic females, males or gynoparae. Males were easily identified by their genitalia as shown in Figure 2, but gynoparae and alate parthenogenetic females were very similar in appearance. Therefore, to distinguish between these two morphs, the alates were kept on a lettuce plant and if nymphs were produced they were identified as parthenogenetic females and if no nymphs were produced they were identified as gynoparae. This approach made the assumption that gynoparae were only able to produce oviparae on blackcurrant bushes.

When a treatment regime resulted in production of sexual morphs, a further experiment was undertaken to confirm the type of sexual morph produced, as the production of eggs would confirm the production of males and gynoparae.

Figure 2. Male *N. ribisnigri* (left) and alate parthenogenetic female (right).

Obtaining eggs in the laboratory

Main experiment

Following successful production of eggs from a preliminary experiment, four blackcurrant bushes were prepared (Figure 3). Aphids were kept at 15°C with 13L:11D for 56 days and then the temperature was reduced to 12°C. The production of eggs was also screened at 15°C 13L:11D, only for a longer period of time (63 days).

To determine whether the temperature change from 15 to 12°C was necessary, or whether being kept continuously 12°C could induce egg production, a new blackcurrant bush was prepared and placed in an incubator at 12°C with 13L:11D. Other regimes which were screened for induction of egg production included 15°C with 14L:10D and 12°C 14L:10D.

a)  **b)** 

Figure 3. **a)** Blackcurrant cuttings paired with infested lettuce **b)** small blackcurrant bushes paired with infested lettuce to obtain eggs under conditions inducing production of sexual morphs.

Host preference and development of fundatrices

A preliminary experiment was carried out to investigate the development time and host preference of the fundatrix. Fundatrices were observed hatching from the eggs on 13 February 2012. On 8 March 2012, several of the fundatrices were collected and placed on leafy blackcurrant cuttings at 12 and 16°C 16L:8D to determine how long it took them to reach adulthood.

On 13 March 2012 five more fundatrices were placed on lettuce plants (cv. Pinokio) which produced alate offspring. Some of the alate offspring were transferred to a blackcurrant bush where they were confined on a leaf using micro-perforated polypropylene bag (200mm x 500mm). Eight alate/apterous parthenogenetic *N. ribisnigri* from the WT4850a cultures were also confined to a blackcurrant leaf.

Diapause termination

Preliminary experiment

A preliminary experiment was conducted using the small number of eggs (approximately 60 eggs) obtained in the field during 2010. Commencing on 6 January 2011, approximately ten eggs were sampled by taking cuttings from several of the blackcurrant bushes contained in enclosure 1-7 located in Long Meadow Centre. Samples were also taken on 20 January, 1, 17 and 25 February and 4 March 2011. Eggs were only sampled from the seven small enclosures due to the occurrence of *H. lactucae* in the large cages during the period of egg deposition.

Sampled cuttings were stood in a piece of domestic foamed plastic polymer sponge in a lidded container (15 x 30cm) and transferred to an incubator at 16°C 16L:8D. A preliminary trial on *N. ribisnigri* showed that these conditions induced egg hatch (Collier, 2007). The eggs were checked at approximately 2-day intervals and percentage hatch was recorded. The cuttings were watered twice a week by adding water to the container, which the sponge absorbed. A closed system ensured high humidity to avoid egg desiccation. TinyTag® loggers were placed inside the container to record humidity and temperature.

Main experiment

Eggs produced during 2011 under natural field conditions were used. Eggs were also obtained from the laboratory, by transferring the sexual morphs to four newly-prepared blackcurrants on 2 November 2011. On 8 November 2011, the four blackcurrant plants supporting the sexual morphs (which had commenced depositing eggs) were moved outside

to expose them to natural conditions comparable to those experienced by the field-produced eggs. The blackcurrant plants were covered with micro-perforated polypropylene bags to protect the sexual morphs from predators and were left outside for more eggs to be deposited.

Once the eggs had been obtained, sampling of eggs from the seven field enclosures, and of eggs produced in the laboratory, began on 26 November 2011 with approximately 30 eggs being removed from each location. Samples were also taken on 9 and 16 December 2011, 6, 20 and 30 January and 13 and 24 February 2012.

Sampled cuttings were stood in Oasis® floral foam which was then placed into a container (7.5 x 15 cm) and transferred to an incubator at 16°C 16L:8D. On average, eggs were checked three times a week to see if they had hatched. Eggs which were infected with fungus or had desiccated were removed. The cuttings were watered twice a week by adding water to the container, which the Oasis® absorbed. The container could not be sealed with a lid as the cuttings were too tall.

Egg chilling requirements and thermal requirements for hatching

On 6 March 2012, four blackcurrant bushes were prepared to acquire eggs in an incubator at 16°C 16L:8D. On 8 June 2012, once the number of the eggs had increased, the blackcurrant bushes were removed from the incubator and the branches of the bushes were cut to provide twelve cuttings supporting various numbers of eggs. All the leaves were removed, together with the sexual morphs.

Six cuttings, supporting 303 eggs, were placed in an incubator at 0ºC in continuous darkness, while the remaining cuttings, supporting 297 eggs, were placed in an incubator at -5ºC, also in continuous darkness. Each cutting was placed in a re-sealable zipped plastic bag to maintain high humidity. A Tiny Tag® logger was placed in one of the bags at each temperature to monitor the temperature and humidity

On 28 June 2012, egg sampling began and this continued until egg hatch occurred. Each week, approximately 25 eggs were removed from each incubator by taking cuttings. The cuttings were stood in Oasis® floral foam, labeled with the sampling date and temperature, and placed in a container which was transferred to an incubator at 16°C 16L:8D as shown in Figure 4.6.1b. A Tiny Tag® logger was placed in the incubator to monitor the temperature and humidity.

Each week, the eggs were checked to see if they had hatched and eggs which were infected with fungus or had desiccated were removed. The cuttings were too large to use a closed

system to maintain high humidity, so a large tray of water was placed in the incubator and refilled regularly to try and raise the humidity.

Objective 3. Investigate alternative host plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts.

Plants to be screened were selected firstly by focusing on broad leaved weeds which are considered very important in field vegetable crops and could potentially provide refuges for *N. ribisnigri* (HDC, 2009). Other plants were chosen by using published information on aphid-host associations and confirming that these plants inhabit areas close to agricultural environments. Preliminary work was carried out to determine the best method to germinate each host-plant and the plant age most suitable for the screening experiments. The most suitable plant age was determined by visually estimating the plant size that would support an aphid colony.

Sixteen host plants germinated successfully and were used in the screens. Host plants were screened in three batches. Each batch consisted of six potential host-plant species (including the control *C. intybus*) which were screened with both WT (4850a) and Rb *N. ribisnigri* (RbKent) aphids. Plants were divided into batches to make data collection manageable and *C. intybus* was used as the control due to it being a relative of lettuce and a confirmed alternative host for *N. ribisnigri.*

The plants were infested with new born nymphs of both WT and Rb *N. ribisnigri*. The plants were then covered with micro-perforated polypropylene bags secured with an elastic band and kept at 20°C 16L:8D. The plants were left for three weeks, after which they were sampled destructively, and the numbers of *N. ribisnigri* were recorded, being separated into the number of alate aphids and the number of other aphids (apterous adults and nymphs).

Cichorium intybus, C. capillaris, V. arvensis and *L. communis* were planted outside on 29th and 30th November 2011 and inoculated with WT *N. ribisnigri* (clone 4850a) to confirm whether the plants could survive the winter and whether *N. ribisnigri* could overwinter on them. These plant species were selected as they were suitable host plants for *N. ribisnigri*, and provided a good representation of the morphological variation present in the family of *Asteraceae* and *Scrophulariaceae* (*V. officinalis* representing the family *Plantaginaceae* was not used as it had a poor germination rate).

On 29 November 2011, 52 of each of these plants were transplanted in a split plot design into an unsheltered soil bed of a Dutch Light at The University of Warwick, Wellesbourne campus (Figure 4a). Twenty six plants each of *C. intybus* and *C. capillaris* were also planted in two sheltered cages (2m x 3m) to determine the effects of shelter on aphid overwintering (Figure 4b).

A total of 260 Eppendorf tubes were each filled with eight 4th instar nymphs and apterous adults, which were inoculated onto each of the host plants following transplanting.
Every week, four plants of each species were destructively sampled from the unsheltered sites and two of each species from the sheltered site, over a 13 week period beginning on 6 December 2011 (there was a two-week sampling interval between 20 December 2011 and 12 January 2012) and ending on 8 March 2012. Sampling was random, as directed by the design, and the numbers of *N. ribisnigri* were recorded, noting the numbers of alates, apterous adults and nymphs.

a)  **b)** 

Figure 4. **a)** Unsheltered soil bed **b)** sheltered cage.

Tiny Tag© loggers were used to record the temperature in both the sheltered and unsheltered sites to determine whether there were any differences in conditions, due to the presence of the cages, which could influence aphid development and survival. Readings were taken every 30 minutes from 30 November 2011 to 7 March 2012.

Objective 4. Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi.

Field trial 2010

This preliminary experiment took place between June and October 2010 at Warwick Crop Centre, Wellesbourne in a field known as Sheep Pens. The purpose of this experiment was to develop techniques to determine the effects of entomopathogenic fungi and arthropod predators on the development of *N. ribisnigri* populations in the field, with a particular focus on the mid-summer crash.

The field trial consisted of nine treatments which combined various fungicide, insecticide and netting regimes (see Table 2). There were two replicates of each treatment (18 plots in total) in each trial and the trial was repeated on three occasions during the summer to allow continuous observation of *N. ribisnigri* populations.

The fungicide, insecticide and netting treatments were used in combination or individually to reduce/exclude aphid natural enemies and/or entomopathogenic fungi. Netted treatments (Enviromesh® 1.35mm) were used to restrict predator access. A broad spectrum pyrethroid insecticide with contact and residual activity was applied at 0.3L/ha (Decis®- deltamethrin) to reduce the occurrence of aphid natural enemies. This was reported to have low toxicity to predatory ground beetles, lacewings, parasitized aphids, low residual toxicity to parasitic wasps, moderate toxicity to ladybirds, and high toxicity to hoverfly larvae. A broad spectrum fungicide was applied at 0.4kg/ha (Nativo®- trifloxystrobin + tebuconazole) to reduce the occurrence of entomopathogenic fungi. These active ingredients were selected following a literature review of fungicides which negatively affected entomophthorales.

Table 2 Nine treatments included in the 2010 field trial with various fungicide, insecticide and netting regimes (F = fungicide; I = insecticide).

Treat. num.	Treat. name	Netting	F treatment	I treatment	Infested artificially
1	Netted	Yes	No	No	Yes
2	Open	No	No	No	Yes
3 Control	Control	No	No	No	No
4	Netted+F	Yes	Yes	No	Yes
5	Open+F	No	Yes	No	Yes
6	Netted+I	Yes	No	Yes	Yes
7	Open+I	No	No	Yes	Yes
8	Open+F+I	No	Yes	Yes	Yes
9	Netted+F+I	Yes	Yes	Yes	Yes

Eight hundred seeds (cv. Saladin Supreme (untreated)) were sown in peat blocks on 11 May, 16 June and 20 July. The lettuce plants were grown in a glasshouse and transplanted after approximately four weeks of growth. One week before transplanting, plants were transferred to a cold frame to harden off. The lettuce plants were scheduled to be transplanted on 9 June, 15 July and 18 August respectively into plots (one bed= 1.83 x 3.5m) containing 40 plants (4 x 10 @ 35cm spacing). However, the transplanting scheduled for 15 July took place on 19 July because high winds prevented spraying, and the transplanting due on 18 August took place on 31 August following a significant period of rainfall, which made conditions too wet for ground preparation. These two batches of lettuce plants were transferred to lower temperatures prior to transplanting to delay growth.

Fungicide, insecticide and netting (Enviromesh® 1.35mm) treatments were applied on the same day as transplanting. Treatments with no netting were protected from birds using wider mesh netting. The treatments were arranged in a 3 x 3 randomised split plot design which was different on each of the three field trial occasions. The control was always situated in the centre of the design, with the open treatments in each corner, to limit movement of *N. ribisnigri* from untreated plots into the control. Figure 5 shows the layout of the field trials.

Figure 5. A 2010 field trial (June occasion).

The day after transplanting, 15 plants in each plot (except the control treatments) were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a. The aphids had been placed in Eppendorf® tubes over the two preceding days and were stored in a refrigerator to prolong their survival. Although it would have been preferable to monitor a natural infestation of *N. ribisnigri* in all of the field trials, their occurrence could not be relied upon.

Each week, over a period of five-six weeks, four plants were sampled from each bed/treatment (72 plants per week). Samples were removed from alternate ends of each bed each week to maintain bed integrity and were stored in labelled paper bags in a cold store at 5°C in continuous darkness. Initially whole plants were sampled and examined, though sample size was reduced to half of each lettuce plant once lettuce plants grew to an unmanageable size, approximately 2-3 weeks after transplanting.

Weekly sampling dates varied depending on weather conditions. Plants from occasion one were sampled on 18, 25 June, 5, 12, 19 and 27 July. Plants from occasion two were sampled on 28 July, 3, 11, 18, 24 and 31 August. Plants from occasion three were sampled on 8, 15, 21, 28 September and 7 October.

Plants were sampled destructively and the numbers of aphids and natural enemies were recorded including Coccinellidae, Araneae, Anthocoridae, Neuroptera, syrphid larvae and parasitized aphids. All insects were identified to family, and where possible to species except for syrphid larvae. The level of parasitism was a measure of the total number of parasitized aphids (all species of aphid), rather than specifically just the percentage of parasitized *N. ribisnigri.* Due to the amount of data collection involved in this experiment, entomopathogenic fungi were not assessed. All insects were stored in 70% ethanol in case further identification was required. After the trial plants were destroyed and composted.

Field trial 2011

Experimental plot

This experiment took place during two weeks of each month from May-September 2011 (five occasions) at Warwick Crop Centre, Wellesbourne in a field known as Big Cherry. As outlined in Table 3, the field trial consisted of nine treatments, which included fungicide or insecticide treatments combined with three netting regimes. There were two replicates of each treatment (18 plots in total).

As with the field trial in 2010, fungicide and insecticide applications were used to reduce the numbers of natural enemies and/or entomopathogenic fungi. However, the treatment where insecticide and fungicide were combined was removed, as no effect was seen during the trial in 2010. Instead, the following three netting regimes were introduced:

- Open - beds open for the entire two week experimental period allowing the movement of natural enemies in and out of the plots.
- Permanently netted - beds permanently netted for entire two week experimental period to exclude natural enemies.
- Temporarily netted - beds netted for the first week of experimental period and then uncovered to allow natural enemies to move into the plots.

It was assumed that, after one week, the populations of *N. ribisnigri* in the permanently netted plots would be equal to those in the temporarily netted plots, since both had been covered for one week. By uncovering the temporarily netted beds after one week, the effect of introducing natural enemies could be determined by comparing the numbers of *N. ribisnigri* at the end of the two week experimental period with the numbers present in the permanently netted and open plots.

Table 3. Nine treatments included in the 2011 field trial including fungicide or insecticide application combined with different periods of netting (F= fungicide and I= insecticide).

Treat. num.	Treatment name	Period netted for	F application	I application
1 (Control)	Open	Never	No	No
2	Open+F	Never	Yes	No
3	Open+I	Never	No	Yes
4	Temp netted	1 week	No	No
5	Temp netted+F	1 week	Yes	No
6	Temp netted+I	1 week	No	Yes
7	Perm netted	2 weeks	No	No
8	Perm netted+F	2 weeks	Yes	No
9	Perm netted+I	2 weeks	No	Yes

When compared with the field trial in 2010, shortening the trial period to two weeks provided a snap shot of the effect of natural enemies in each month and also ensured that the insecticide and fungicide treatments remained more effective over the two week period. Furthermore, shorter sampling periods made data collection more manageable since the plants were smaller.

During the 2010 trial, there were no observed effects of either the Decis® or Nativo® applications. As a result in 2011, the broad spectrum fungicide Amistar® was applied (1L/ha) instead of Nativo. This is a systemic, translaminar and protectant strobilurin fungicide (Azoxystrobin). Decis was used again (0.3L/ha.), since a greater effect might be observed by having a shorter trial period.

Four hundred seeds (cv. Saladin Supreme (untreated)) were sown in peat blocks on 12 April, 16 May, 13 June, 18 July and 15 August and these were scheduled for transplanting on 9 May, 14 June, 12 July, 15 August and 12 September respectively. However, the September transplanting was delayed until 15 September to fit around other experimental commitments.

The plants were grown in a glasshouse and transplanted after approximately four weeks of growth into 18 plots (one plot= 1.83 x 3.5m) of 20 plants (4 x 5 @ 35cm spacing). One week before transplanting, plants were moved to a cold frame to harden off. Fungicide, insecticide and netting treatments were applied on the same day as transplanting. The treatments were arranged in a 3 x 3 block design which was randomised for each occasion. Treatments

which were open were protected from bird damage with wide mesh netting. The experiment was located close to hedgerows containing wild flowers to increase the proximity of natural enemies. Figure 6 shows the trial planted on 9 May.

Figure 6. Field trial in 2011 (planted 9 May 2011).

The day after transplanting, ten plants in each bed were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a. These were placed in Eppendorf® tubes during the preceding two days and stored in a refrigerator to prolong their survival.

One week later, the netting was removed from six of the temporarily netted plots and the plots were protected with bird netting. This was done on 17 May, 22 June, 19 July, 23 August and 23 September. One week later, eight plants were sampled from each plot. Sampling took place on 24 May, 29 June+1 July, 26 July, 30 August and 29-30 September, which was usually over a period of one day, weather permitting.

Whole plants were sampled destructively and aphids and natural enemies were identified, counted and recorded. Natural enemies were recorded and stored as in 2010. Aphids infected with entomopathogenic fungi (Figure 7) were also counted, and recorded as either early infection (fungal mycelia/branching emerging from the aphid body) or late infection (swelling and discoloration of the body (often described as ‘creamy’ and ‘snotty’)). The species of aphid infected by entomopathogenic fungi were not determined.

Figure 7. Middle (left) and late (right) stage entomopathogenic fungal infections of *N. ribisnigri.*

Tinytag© loggers were used to record the ambient temperature and humidity every 30 minutes. A logger was placed in the centre of a permanently netted and an open plot to determine the effect of netting. Yellow water traps were placed in the empty beds between plots for the two week trial period to sample aerial insects. These were emptied approximately once a week. After the trial, plants were destroyed and composted.

Monitoring plot

In addition to the monthly trials, a monitoring plot was established in a separate area of the same field to allow the build-up of a *N. ribisnigri* infestation (infested artificially) where the development of an infestation and the timing of the mid-summer crash could be monitored. One hundred seeds (cv. Saladin Supreme (untreated)) were sown in peat blocks on 12 April, 5 May, 23 May, 13 June, 4 July, 25 July and 15 August and transplanted on 10 May, 31 May, 20 June, 12 July, 1 August, 22 August, 15 September respectively. Plants were grown in the glasshouse and after approximately four weeks of growth, were transplanted into the field in two plots (one plot= 1.83 x 3.5m) containing 40 plants (4 x 10 @ 35cm spacing). Every three weeks, a further pair of plots was added behind the existing plots until 12 September, to provide 'temporally overlapping' plots containing plants of a range of ages. The monitoring plot was surrounded with an electric fence. Figure 8 shows the monitoring plot planted on 9 May.

The day after transplanting, 15 plants in each plot were inoculated with five wingless adult (or 4th instar) aphids of clone WT4850a. These had been placed in Eppendorf® tubes over the preceding two days and stored in a refrigerator to prolong survival. Four plants were sampled from each bed on 25 May, 8, 14, 20 June, 9, 14, 22, 29 July, 16, 23, 30 August, 5, 15, 23 September, 4, 12, 19 and 26 October. Each bed was sampled for up to five weeks,

depending on plant quality, and samples were removed from alternate ends of each bed to maintain bed integrity. Sampled lettuce plants were stored in labeled paper bags in a cold store at 5°C in constant darkness. Whole plants were sampled destructively and the numbers of aphids, natural enemies and aphids infected were identified, recorded and stored as per the previous field trials. Yellow water traps were placed in between the plots and sampled every week.

Figure 8. Monitoring plot in 2011 (9 May 2011).

Statistical analysis

Field trial 2010

The total number of aphid stages and natural enemies recorded on the lettuces sampled from each plot was determined. A mean per plant was then calculated by dividing the totals by the number of lettuce plants sampled.

An ANOVA was performed on the numbers of alate, non-alate (including nymphs) and all stages of *N. ribisnigri* and natural enemies including; Coccinellidae, Araneae, syrphid larvae, parasitized aphids, Anthocoridae and Neuroptera. As natural enemy numbers were low, the counts of larvae and adults were combined for Coccinellidae and Neuroptera (Chrysopidae and Hemerobiidae). Data on some of the aphid-specific natural enemies were also combined to analyse the effect of treatments on total predator numbers (including: Anthocoridae, Coccinellidae, Neuroptera, parasitized aphids and syrphid larvae).

The ANOVA was performed using the blocking structure: occasion/replication/sampling_week, where occasion represented the three field trials (planting date), replication represented the two replications of each of the treatments and sampling week represented the five sampling weeks on each trial. The treatment structure

used included: sampling_week*(netting*fungicide*insecticide) which contrasted the means for the netting, fungicide and insecticide treatments for each sampling week. The control treatment was not included in the ANOVA as these aphids were from a natural infestation making this treatment unsuitable for comparison with those treatments which were inoculated. Therefore, when interpreting the results, all treatments were compared to the open plot with no insecticide/fungicide treatment, which was essentially the inoculated control.

Due to the large number of zero values present in the dataset, a value of one was added to all data and LOG values were used to normalise the data. The LOG value was then divided by the number of plants sampled from that treatment, to provide a proportion of the data variable per plant. This was necessary as different numbers of plants were sampled from each treatment with sometimes only half a plant being destructively sampled.
In addition to the ANOVA, the relationships between the recorded variables (natural enemies, *N. ribisnigri* (alate, apterous and nymphs) were determined using scatter plots. Where linear relationships were observed, Pearson R correlations and linear regressions were also performed for each sampling date during the mid-summer crash with the data grouped into netted and open (i.e. combining fungicide, insecticide treatment data) to determine any relationship which might explain the aphid decline.

Field trial 2011

Only data collected from plants which were infested artificially with *N. ribisnigri* were included in the analysis as plants that were not infested artificially had significantly lower numbers of aphids. Only data from the experimental plots were analysed with the ANOVA (not the monitoring plot).

An ANOVA was performed on the numbers of alate and all stages of *N. ribisnigri,* parasitized aphids and aphids infected with entomopathogenic fungi. Coccinellidae, Anthocoridae and syrphid larvae were present in very low numbers (not exceeding a mean of one), resulting in zero values for the majority of the treatments and no Neuroptera were recorded. Therefore, ANOVAs were not performed on these variables due to the lack of data. However, Anthocoridae, Coccinellidae, parasitized aphids and syrphid larvae were summed together to analyse the total number of natural enemies.

The ANOVA took into account the blocking structure: field trial/replication, where field trial represented the five field trial occasions (planting date) and replication represented the two replications of each of the treatments. The treatment structure included planting

date*netting*treatment. Due to the numbers of zero values present in the dataset, a value of one was added to all data and a LOG transformation was performed to normalise the data.

Relationships between the recorded variables were determined using scatter plots. As described for Field Trial 2010, Pearson R correlations and linear regressions were performed when linear relationships were observed. This process was performed on the data from the artificially infested plants only, for each individual field trial, with the data grouped by netting treatment.

Results

Objective 1. Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.

The study confirmed that temperature is a significant factor affecting development time (Figure 9) and thereby development rate (1/development time), the intrinsic rate of increase (Figure 10), fecundity (Figure 11), and the propensity to become alate, of both WT and Rb *N. ribisnigri*. Between 5.5 and 26.4°C, development time decreased with increasing temperature and only at 5.5°C did variation in development times occur between treatments. Interestingly, at lower temperatures, some aphids from the WT4850a biotype survived on *Nr* cultivars, although their longevity, fecundity and development time were compromised.

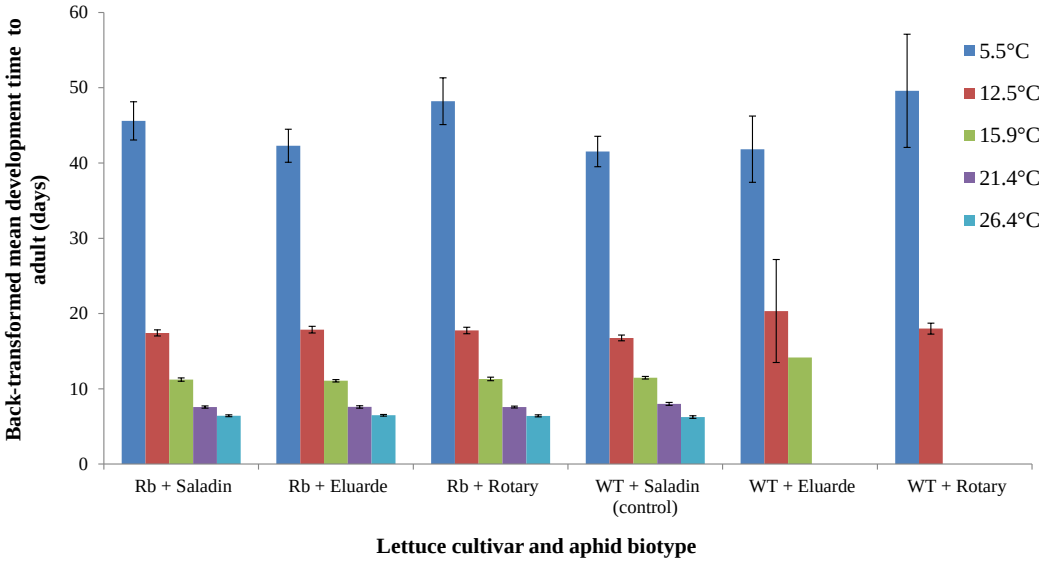


Figure 9. Development time (days) to adult for each treatment at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) including the standard error (SE). Photoperiod (16L:8D).

The intrinsic rate of increase (r_m) was calculated. The r_m = (ln *Md* x *0.738*) /*D,* where ln is the natural logarithm, *D* is the pre-reproductive time (nymph to final adult moult) in days and *Md* is the reproductive output of an individual aphid following the adult moult for a number of days equal to *D* (Awmack and Leather, 2007). The r_m increased with increasing temperature up to 26.4°C (Figure 10). The WT4850a and RbKent biotypes had similar development times and r_m *values*.

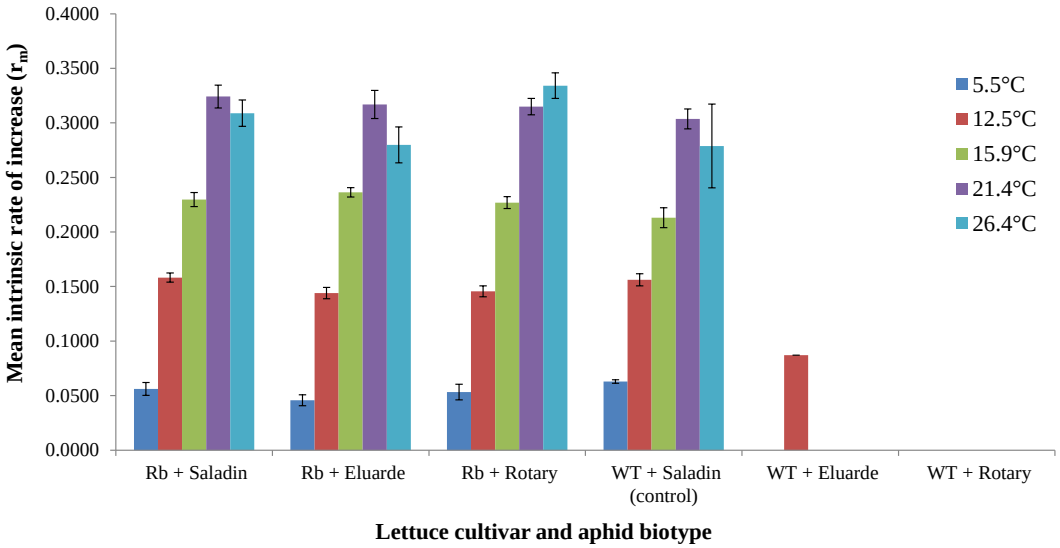


Figure 10. Mean intrinsic rate of increase (r_m) for each treatment at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) with SE. Photoperiod (16L:8D).

The control treatment (WT4850a on cv, Saladin) exhibited poor longevity when compared to the Rb biotype, demonstrating poor pre-reproductive survival and continued poor survival during the reproductive phase, particularly at 5.5 and 26.4°C. The Rb biotype exhibited comparable poor longevity only at 5.5°C. The optimum temperature for longevity was 15°C.

The control treatment exhibited consistent poor fecundity and the RbKent biotype had significantly higher fecundity than the control between 12.5 and 21.4°C (Figure 11). Fecundity was reduced for the Rb biotype at 5.5 and 26.4 °C. Overall, the optimum temperature for reproduction was 12°C. Collectively, these responses suggest that 5.5 and 26.4°C are close to the Upper Development (UDT) and Lower Developmental Thresholds (LDT) respectively, where aphid performance is negatively affected, meaning that the temperature range leading to optimum aphid performance is between 12.5-21.4°C. More alates were produced at lower temperatures and the RbKent biotype appeared to produce more alates compared to the WT4850a biotype, but not the WTKent10Pop biotype (data not shown). Photoperiod did not influence development or the r_m value for WT4850a, WTKent10Pop, RbKent, RbUK631 and RbKentPop aphid biotypes (data not shown).

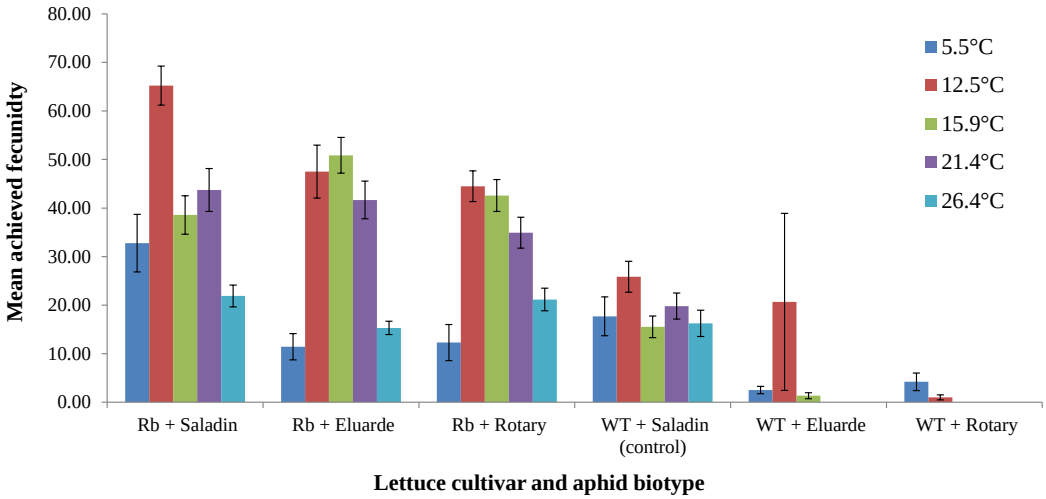


Figure 11. Mean fecundity at five constant temperatures (5.5, 12.5, 15.9, 21.4 and 26.4°C) for each treatment including the SE. Photoperiod (16L:8D).

Estimates of the Lower Developmental Threshold and day-degree requirements suggest that the WT4850a *N. ribisnigri* biotype has a LDT of close to 4.6°C and requires 121.1 day-degrees to reach adulthood (Table 4). These estimates were determined by performing a linear regression between mean rate of development (y dependent variable) and temperature (x independent variable) and determining where the line crosses the x-intercept. The temperatures shown are the mean temperatures in the incubators for each set of tests).

Table 4. Linear regression model, LDT (±SE), upper and lower 95% confidence limits (CL) and day-degree (DD). The temperatures shown are the mean incubator temperatures during each set of experiments. These vary slightly because incubator temperatures fluctuate within a small temperature range.

Treatment	LDT (°C)	Upper CL	Lower CL	Linear regression model	DD
WT + Saladin (12.3, 20.3, 25°C)	4.576 ±0.25	5.071	4.081	Y= -0.03779+0.008257x	121.11
WT + Saladin (12.5-21.4°C)	4.211 ±0.52	5.256	3.166	Y= -0.03111+0.007387x	135.37
Rb + Saladin (12.4-21.4°C)	5.371 ±0.44	6.238	4.505	Y= -0.04489+0.008358x	119.66

Objective 2. Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching

Obtaining and monitoring eggs from the field

Year 2010

When monitoring of the blackcurrant bushes began in November 2010, several aphid eggs had already been deposited in both the large cages and small enclosures. The eggs had been deposited mainly in the angle between a stem and a bud and were initially green in colour, before turning black. The eggs which remained green were unfertilised.

Unfortunately, individuals of a species of aphid other than *N. ribisnigri* were observed depositing eggs on the blackcurrant bushes in one of the large cages. Specimens were collected and sent to Rothamsted Research where they were identified as *H. lactucae.* As a result, the large cages were no longer monitored as it was impossible to distinguish between eggs of *N. ribisnigri* and *H. lactucae* until they hatched in the following spring.

Weekly monitoring of the blackcurrant bushes to record egg hatch began on 6 January 2011, and on 17 February, 20 nymphs were observed on a blackcurrant bush inside Enclosure 2.

Year 2011

In 2011, monitoring for sexual morphs and eggs began earlier, on 5 September 2011. Of the seven enclosures set up, only five had *N. ribisnigri* remaining on the lettuce plants. On 7 October, the first alate *N. ribisnigri* were observed on the blackcurrant bushes in the enclosures and these were assumed to be gynoparae (produce sexual females) as they were not males. However, as there were no oviparae nymphs present, they could have been alate parthenogenetic aphids. An unknown aphid was also present in high numbers on the blackcurrant bushes and on the netting of Enclosures 1 and 2. Specimens of the aphid were collected and sent to Rothamsted Research where they were identified as *Eriosoma ulmi.* Fortunately, this aphid does not lay eggs on *Ribes* species and was migrating back to its winter host (elm) for the winter.

On 14 October, alate *N. ribisnigri* were observed producing nymphs and were therefore assumed to be gynoparae. On 21 October 2011, *N. ribisnigri* males and gynoparae were observed on the blackcurrant bushes. Following this, the first eggs were observed on 28 October, with one egg in Enclosure 4 and two eggs in Enclosure 5, both of which were accompanied by an ovipara (egg laying female). By 11 November, four of the five enclosures contained eggs and many gynoparae and oviparae were depositing nymphs and eggs. On 5 December, approximately 320 eggs were observed on the blackcurrant bushes present in each enclosure (3 bushes per enclosure), with Enclosure 5 containing the most eggs (Figure 12). At this time, large numbers of oviparae were still present on the blackcurrant bushes.

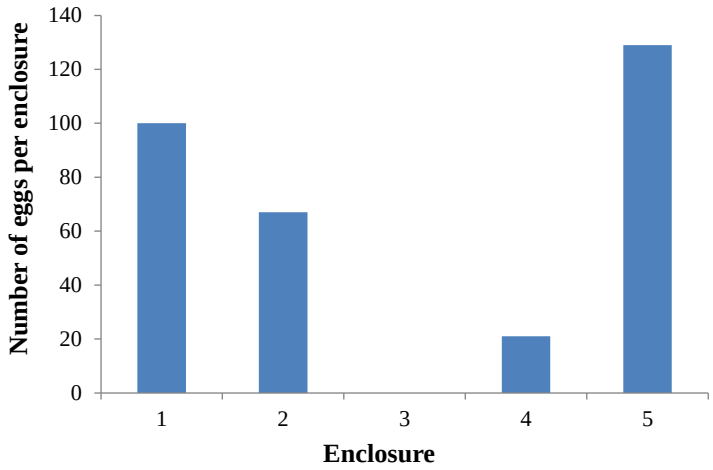


Figure 12. Number of eggs on blackcurrant bushes in each enclosure.

On 6 January 2012, monitoring for egg hatch began. Nymphs were first observed on 24 February and these had hatched since the previous sampling date of 17 February. The buds of the blackcurrant bushes had not started to open by the time the first nymphs hatched. It

was not until 8 March that the buds were opening. At this time, three nymphs were observed in Enclosure 1 and one in Enclosure 5. However, the nymphs were not present on subsequent monitoring dates. No fundatrices (nymphs hatching from eggs) had established on the blackcurrant bushes by 29 March.

Inducing sexual morph production

Following pre-natal and post-natal conditioning under a range of temperatures and photoperiod regimes, adult morphs were recorded as shown in Figure 13.

The only treatment regime which did not lead to production of a sexual morph was 12°C 16L:8D where only apterous and parthenogenetic alate *N. ribisnigri* were observed. Apterous forms were common to all treatments and occurred in the same batches of offspring as gynoparae and males. The various morphs and also eggs are shown in Figure 14.

Males occurred at 15°C 14L:10D, 15°C 13L:11D and 12°C 14L:10D. Parthenogenetic alate *N. ribisnigri* occurred in the same batches as the males.

Gynoparae developed under two rearing regimes. A considerable number of gynoparae were produced at 12°C 13L:11D, while no males were observed. Conditioning at 12°C 14L:10D also resulted in the production of gynoparae but no more than three individuals were observed. Males were also produced. Neither of the regimes at 15°C produced gynoparae, but they did produce males.

As different numbers of aphids were used to produce G_0 offspring for each treatment regime, the differences between each treatment in the numbers produced per batch were not meaningful. Also, the alates which occurred at the rearing regime 15°C 13L:11D were not screened to see if they produced nymphs on lettuce and therefore were not determined as gynoparae or parthenogenetic alates in this experiment.

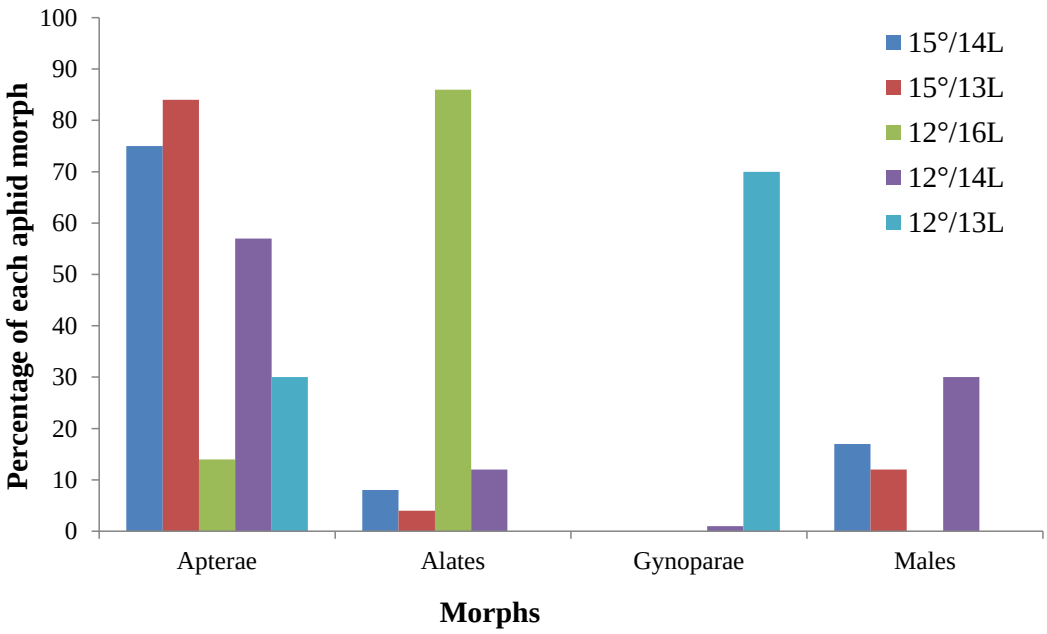


Figure 13. Percentage morphs produced under each rearing regime.

Obtaining eggs in the laboratory

On 25 November 2011 four potted blackcurrant bushes were prepared. On 20 January 2012, when the temperature was reduced to 12 °C, two eggs were observed on the blackcurrant bushes. However, on 2 February 2012, following the temperature decrease, considerably more eggs were observed on three of the four blackcurrant bushes. Thus, whilst a few eggs were produced by Day 56, egg numbers had increased considerably by day 69, once the temperature had been lowered.

As a preliminary experiment suggested that 15°C 13L:11D alone did not result in egg production, while the above second repeat did (although only a low number of eggs were observed), this condition was tested without the temperature change to 12°C. This showed that after 63 days at 15°C 13L:11D no eggs were produced.

On 8 June 2012 a blackcurrant bush was placed at 12°C 13L:11D to see if this regime alone would result in the production of eggs. By 27 July, several eggs had been produced, with more eggs observed on 2 August. Thus eggs were produced between 41-49 days. Unfortunately, it was not possible to determine whether eggs were produced at 12°C14L:10D, as when infested lettuce plants were paired with blackcurrant bushes, an individual aphid, which was not *N. ribisnigri*, was found to be laying eggs. Table 5 summarises the outcomes from this experiment.

a)  b) 

c) 

Figure 14. **a)** Gynoparae, oviparae and males on blackcurrant leaf **b)** sexual morphs and eggs **c)** oviparae and eggs

Table 5. Outcome of the rearing regimes used to screen for the induction of egg production when lettuce plants infested with *N. ribisnigri* were paired with blackcurrant bushes.

Temperature (°C)	Photoperiod	Eggs produced	Days exposed to regime and in some cases when eggs were produced
15	14L:10D	No	63
15 transferred to 12 (on day 66)	13L:11D	No	66 at 15°C
		Yes	31 at 12°C
15 transferred to 12 (on day 56)	13L:11D	Yes	56 at 15°C
		Yes	13 at 12°C
15	13L:11D	No	63
12	14L:10D	Un-determined	
12	13L:11D	Yes	49

Host preference and development of fundatrices

When the fundatrices (Figure 15) were placed onto blackcurrant leaves at 12 and 16°C 16L:8D, the leaves curled quickly and the fundatrices died, meaning that development time to adulthood could not be determined.

On 24 March 2012 the fundatrices placed on lettuce plants developed to adulthood and had produced alate offspring, which were themselves producing nymphs.

On 11 April 2012, the four alates produced by the fundatrices and the eight alate/apterous parthenogenetic *N. ribisnigri* placed on a blackcurrant leaf were dead and had not produced any offspring. However, the alates produced by the fundatrices which were left on the lettuce plant continued to develop and produce offspring.

Diapause termination

Preliminary experiment

To determine when diapause had ended naturally in the field, sampling of the small number of eggs deposited in the field in 2010 was carried out approximately every two weeks which began on 6 January 2011. Table 6 shows the numbers of eggs which had hatched by each monitoring date for eggs which has been sampled over six dates.

On 9 February 2011 the first nymph was observed on a cutting which had been sampled from the field enclosures on 1 February, thus taking approximately eight days to hatch. By the end of the monitoring period, two nymphs out of 13 eggs had hatched on this cutting.

On 21 February 2011, one nymph was observed on one of the cuttings which were sampled on 17 February, taking approximately four days to hatch. Following this, a total of four nymphs hatched on this cutting out of the 14 eggs sampled.

When cuttings were sampled from the field on 25 February 2011, one nymph was already present. Following transfer to 16°C 16L:8D, a further nymph was observed on 1 March, hatching within two days. Two of the four eggs on this cutting hatched.

On the final sampling date in March, three eggs hatched three days after being transferred 16°C 16L:8D. The highest number of eggs which hatched were sampled from the field on 17 February.

Egg mortality was relatively high and appeared to be due to desiccation and fungal infection, particularly when eggs were at the base of the cuttings and close to the sponge. Tinytag® recordings indicated that the mean temperature in the containers held at 16°C 16L:8D was 16.64°C. Recordings of humidity were very variable between 6 January and 1 February 2011 and following replacement of the logger the rest of the experiment was exposed to a relative humidity with a mean value of 73.42%.

Figure 15. Newly-hatched *N. ribisnigri* fundatrix

Table 6. Number of field-produced eggs which hatched following sampling on six occasions. The total number of eggs sampled is also shown.

	Sampling date					
	6/1/11	**20/1/11**	**1/2/11**	**17/2/11**	**25/2/11**	**4/3/11**
Monitoring date						
10/1/11	0					
11/1/11	0					
12/1/11	0					
13/1/11	0					
14/1/11	0					
17/1/11	0					
18/1/11	0					
19/1/11	0					
20/1/11	0					
24/1/11	0	0				
25/1/11	0	0				
26/1/11	0	0				
27/1/11	0	0				
30/1/11	0	0				
1/2/11	0	0				
6/2/11	0	0	0			
7/2/11	0	0	0			
8/2/11	0	0	0			

	Sampling date					
	6/1/11	**20/1/11**	**1/2/11**	**17/2/11**	**25/2/11**	**4/3/11**
9/2/11	0	0	1			
10/2/11	0	0	0			
11/2/11	0	0	0			
14/2/11	0	0	0			
15/2/11	0	0	0			
21/2/11	0	0	1	1		
22/2/11	0	0	0	1		
23/2/11	0	0	0	0		
25/2/11	0	0	0	1		
28/2/11	0	0	0	0	1	
1/3/11	0	0	0	0	1	
2/3/11	0	0	0	1	0	
3/3/11	0	0	0	0	0	
4/3/11	0	0	0	0	0	0
7/3/11	0	0	0	0	0	3
Total hatched	0	0	2	4	2	3
Total eggs sampled	14	10	13	14	4	4

Main experiment

Sampling began on 26 November 2011. Table 7 shows the number of eggs which hatched for eggs sampled on five sampling dates. Eggs were produced under field conditions (Field) or in the laboratory and then transferred to the field (Lab).

On transferring the eggs to 16°C 16L:8D, the first fundatrix hatched by 23 January 2012, having being sampled from eggs produced in the laboratory on 6 January 2012. From this sampling date onwards, at least one fundatrix hatched from each sample of eggs, whether produced in the field or the laboratory. After 13 February, all the laboratory-produced eggs had been used and only field-produced eggs were sampled on 24 February.

Field-produced eggs did not commence hatching until 1 February on blackcurrant plants sampled from the field on 20 January 2012. On later sampling occasions, more field-produced eggs hatched compared with laboratory-produced eggs.

As shown in Table 7, percentage hatch was generally low. In addition, a considerable number of eggs were removed because of fungal infection or desiccation, although losses were reduced following the later sampling dates, when the eggs were not monitored for as long. Because eggs were lost, egg hatch is expressed as a percentage of the number of eggs which appeared 'viable' rather than the total sampled. The greatest proportion of eggs hatched from field-produced samples taken on 24 February, where 45.8% of 'viable' eggs hatched by 27 February.

Table 7. Number of eggs which hatched after being sampled on five occasions. Eggs were field-produced (Field) or produced in the laboratory (Lab). The total number of eggs sampled and the number of eggs removed because of fungus infection or desiccation are also shown, as is the percentage of 'viable' eggs which hatched.

	Sampling date and egg origin								
	06/01/2012		20/01/2012		30/01/2012		13/02/2012		24/02/2012
	Field	Lab	Field	Lab	Field	Lab	Field	Lab	Field
Monitoring date									
13/1/2012	0	0							
16/1/2012	0	0							
20/1/2012	0	0							
23/1/2012	0	1	0	0					
24/1/2012	0	0	0	0					
26/1/2012	0	0	0	2					
30/1/2012	0	0	0	0					
01/2/2012	0	1	1	1	0	0			
03/2/2012	0	0	0	0	1	0			
11/2/2012	0	0	1	0	3	0			
15/2/2012	0	0	0	0	0	1			
17/2/2012	0	0	0	0	1	1	1	0	
20/2/2012	0	0	0	0	0	0	4	1	
23/2/2012	0	0	0	0	0	0	2	0	
27/2/2012	0	0	0	0	0	0	1	0	11

	Sampling date and egg origin								
	06/01/2012		20/01/2012		30/01/2012		13/02/2012		24/02/2012
	Field	Lab	Field	Lab	Field	Lab	Field	Lab	Field
29/2/2012	0	0	0	0	0	0	0	0	0
Total no. hatched	0	2	2	3	5	2	8	1	11
Total no. eggs sampled	27	27	29	33	33	29	28	10	27
Total no. eggs removed	18	21	19	13	6	9	5	2	3
Percentage hatch of 'viable' eggs (%)	0	33	20	20	18.5	10	34.8	12.5	45.8

For field-produced eggs the time required for 50% of the eggs to hatch decreased as the sampling dates became later (Figure 16). For example, only approximately 2 days were required for 50% of the field-produced eggs to hatch when kept at 16°C 16L:8D following sampling on 24 February 2012. While this relationship is less distinct for laboratory-produced eggs due to the lack of data, the trend line is similar to that of the field-produced eggs.

Cumulative hatching curves for both field-produced and lab- produced eggs sampled from the field on different dates and maintained at 16°C 16L:8D are shown in Figure 17. Similarly to Figure 16 it shows that eggs collected from the field at later sampling dates compared to earlier sampling dates, required less time at 16°C 16L:8D before they hatched but also had a higher percentage of eggs hatching sooner. None of the field-produced eggs collected at the earliest sampling date (6 January) hatched when kept for 54 days at 16°C 16L:8D.

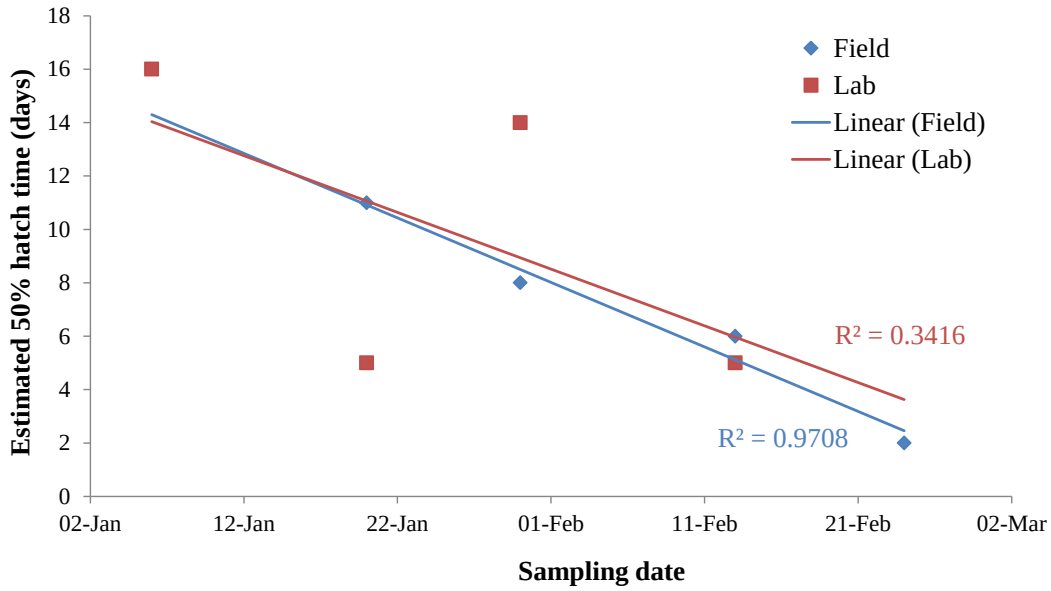


Figure 16. Estimated time (days) until fifty per cent of the field-produced and lab-produced eggs collected from the field at each sampling date hatched, when kept at 16°C 16L:8D.

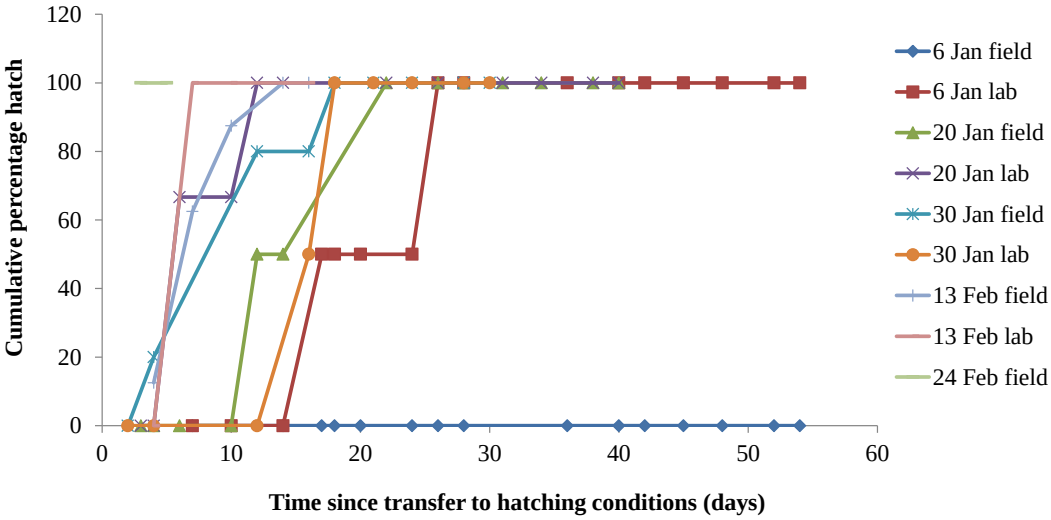


Figure 17. Percentage of fundatrices hatching from field-produced and lab-produced eggs collected from the field at each sampling date and kept at 16°C 16L:8D for up to 54 weeks.

Egg chilling requirements and thermal requirements for hatching

Eggs were chilled at 0°C (actual mean -0.09°C and 89.9% RH) or -5°C (actual mean -4.64°C and 84.9% RH) from 8 June 2012 and then sampled at weekly intervals from 28 June and placed at 16°C 16L:8D (actual mean 16.2°C and 83.8%RH). Few eggs hatched overall. However**,** on 24 August, a single nymph was observed on blackcurrant cuttings sampled from the 0°C D:D regime and placed at 16°C 16L:8D on 2 August (i.e. observed 22 days after being placed at 16°C).

On 2 September, one nymph hatched from eggs kept at 0°C and one nymph from eggs kept at -5°C, all of which were placed at 16°C 16L:8D on 24 August (i.e. nymphs observed 9 days after being placed at 16°C).

Although the point at which diapause terminated cannot be determined from this study, an estimate of the number of day-degrees (D°) required for post-diapause development can still be made using the period of time between the sampling date and date of egg hatch determined in the diapause termination experiment. Day-degrees accumulated between the sampling date and date of egg hatch for field-produced eggs in 2010/2011 and 2011/2012 and also laboratory-produced eggs during 2011/2012 are shown in Figure 18. These were calculated using the Lower Developmental Threshold of 4.6°C determined in this study and minimum and maximum temperatures from the University of Warwick, Wellesbourne meteorological station.

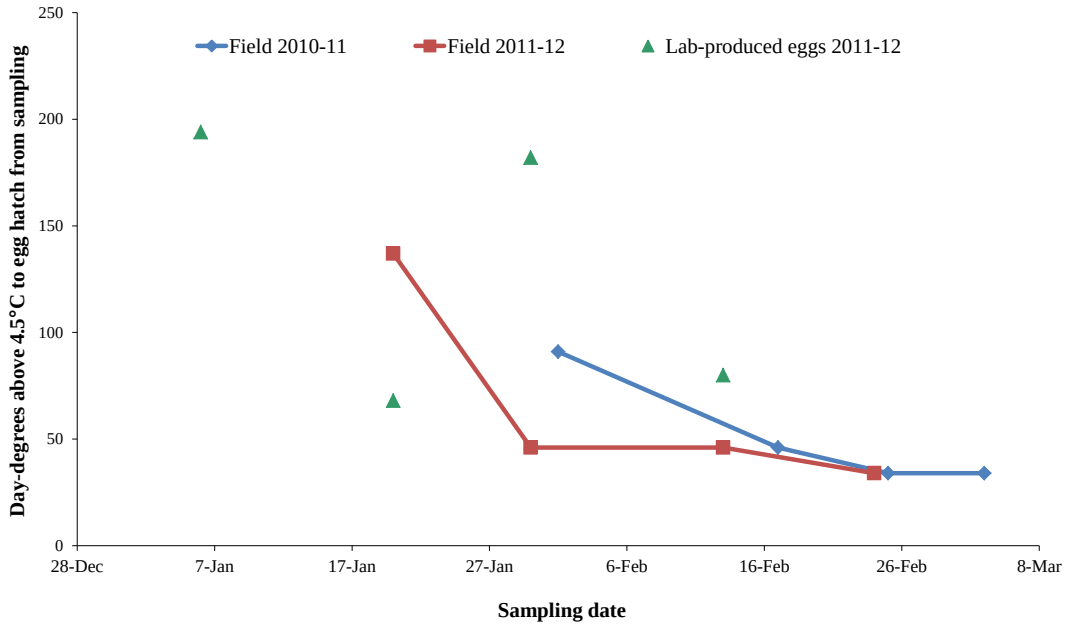


Figure 18. Day-degrees accumulated between the sampling date and date of egg hatch for field-produced eggs in 2010/2011 and 2011/2012 and laboratory-produced eggs during 2011/2012.

Figure 18 suggests that the time required until egg hatch at 16°C 16L:8D became constant at approximately 50D°. Thus it is likely that post-diapause development takes just under 50 D° and that diapause terminates sometime in late January – early February.

Objective 3. Investigate alternative host plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts.

Eight species of plant (mainly wild species) are suitable hosts for both UK WT and Rb *N. ribisnigri* (e.g. Figure 19). This includes *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis*. The performance of WT and Rb *N. ribisnigri* biotypes was similar regardless of the host plant.

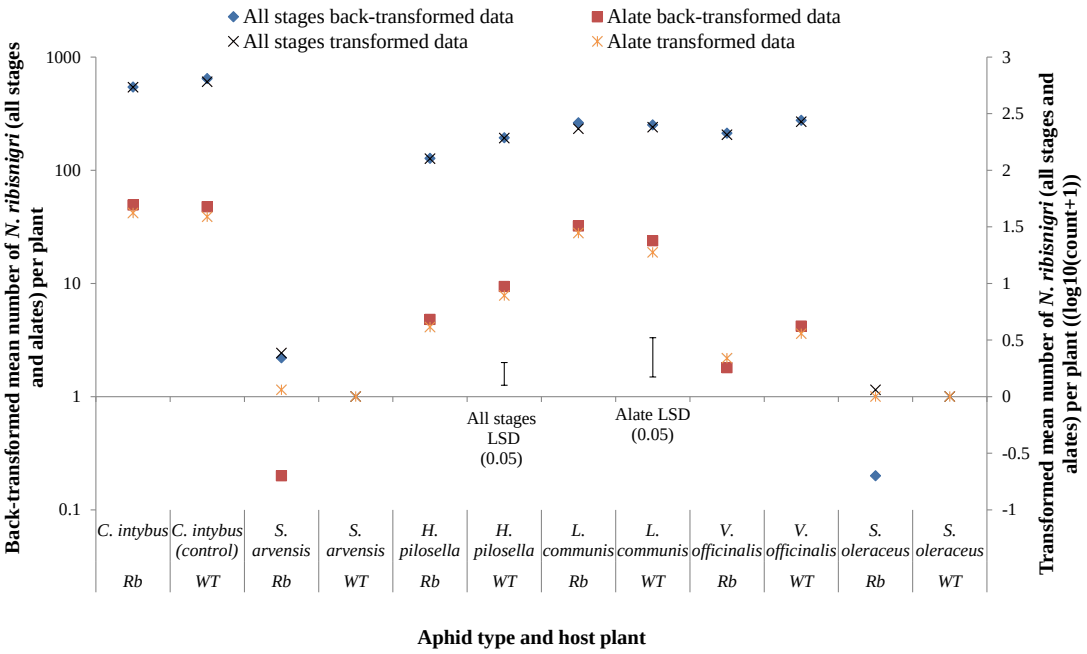


Figure 19. Transformed and back-transformed mean numbers of alate and all stages of WT and Rb *N. ribisnigri* present after three weeks in Batch one.

Figure 20 shows the back-transformed mean number of *N. ribisnigri* per plant present on each sampling date for the host plants sampled from the sheltered and unsheltered sites during the winter of 2011-12. WT *N. ribisnigri* survived throughout the winter and were still present in March, with unsheltered *V. arvensis* and sheltered *C. intybus* supporting a mean of 1 and 3.5 aphids per plant respectively on the last sampling date. While these means were small, this study confirms the ability of *N. ribisnigri* to overwinter successfully.

The mean numbers of *N. ribisnigri* per plant recorded throughout the winter varied depending on sampling date. When comparing Figure 20 to the temperature recordings illustrated in Figure 21, peak numbers of aphids coincided with peaks in temperature, particularly for *N. ribisnigri* on unsheltered *V. arvensis*. For example, a period of warmer weather occurred from 18 December 2011 until 8 January 2012 and this coincided with an

increase in numbers of *N. ribisnigri* on the host-plants. These numbers remained high on some weeds until a period of colder weather occurred at the beginning of February, resulting in a sharp decline in aphid numbers. They then began to increase again but numbers fluctuated with variations in temperature.

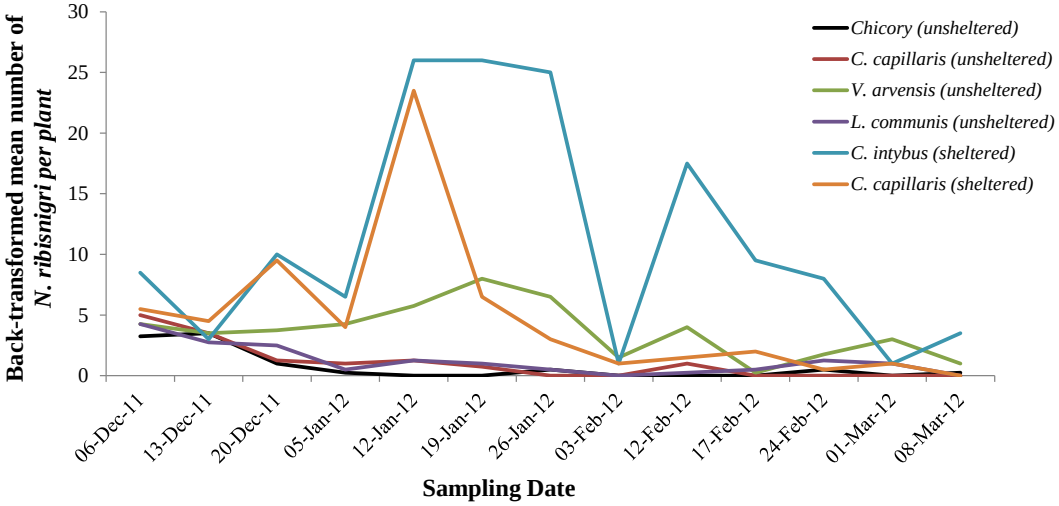


Figure 20. Back-transformed mean number of WT *N. ribisnigri* for unsheltered *C. intybus, C. capillaris, V. arvensis* and, *L. communis* and sheltered *C. intybus* and *C. capillaris.*

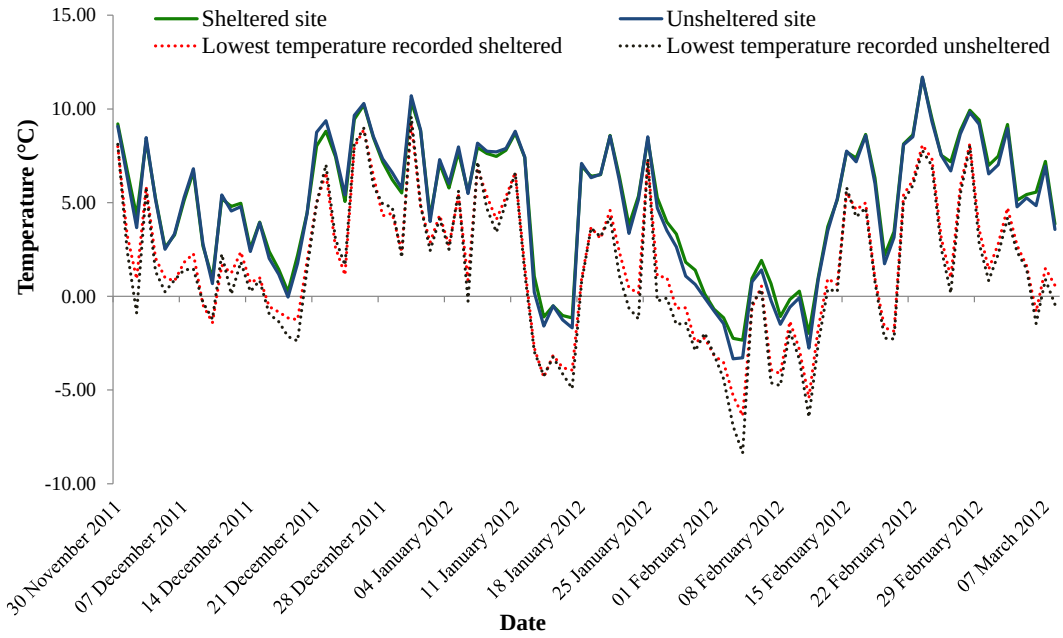


Figure 21. Average daily temperatures and lowest recorded daily temperatures (°C) from 30 November 2011 to 7 March 2012 at both sheltered and unsheltered sites.

Objective 4. Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi.

6.1 Field trial 2010

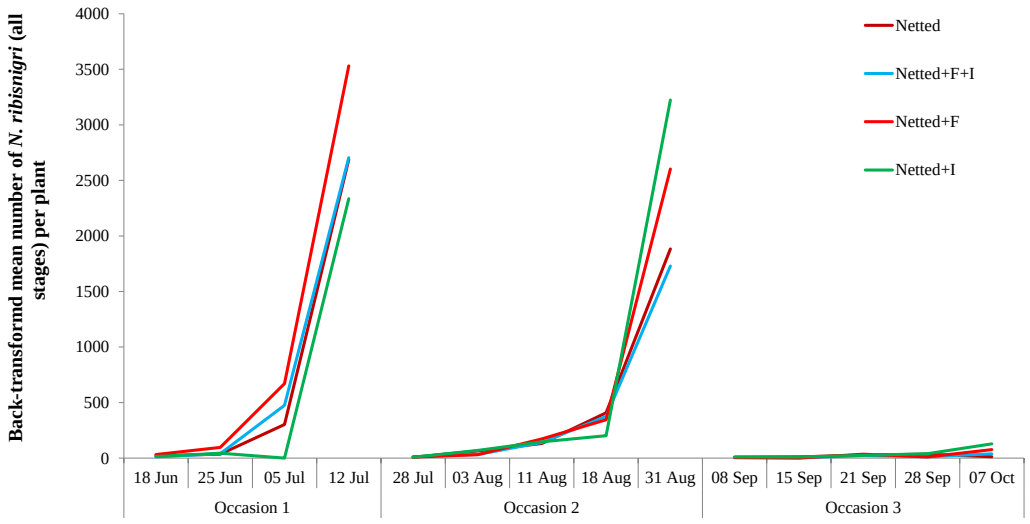
Aphids

Figures 22a and b show the back-transformed mean number of *N. ribisnigri* (all stages) recorded per plant for netted treatments and open plots at each sampling date over the three field trial occasions. Netted treatments had significantly higher numbers of *N. ribisnigri* compared to open and control treatments, particularly for Occasions 1 and 2. During Occasion 1 and 2, aphid numbers per plant on the netted treatments were initially low following inoculation and then continued to increase rapidly up to the last sampling date for that occasion. Aphid numbers were higher on the first occasion than the second. During Occasion 3, numbers of *N. ribisnigri* remained low. During sampling a high number of alates *N. ribisnigri* were observed on the underside of the nets.

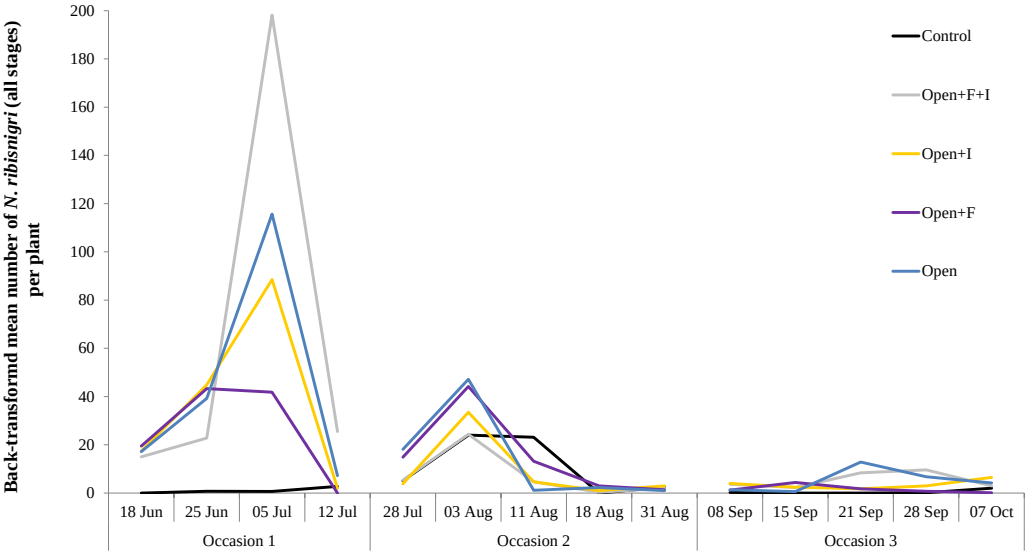
When considering the open plots, aphid numbers during Occasion 1 increased from 18 June until 5 July (Figure 22b). However, by 12 July a dramatic decrease in aphid numbers had occurred. Numbers decreased in the Open+F+I treatment from 198.17 aphids per plant to 25.50 per plant, on the Open+I treatment from 88.50 per plant to 2 per plant, on the Open+F treatment from 41.83 per plant to 0 per plant and on the open control plot (non-inoculated) from 115.67 to 7.25 per plant. Unfortunately, data for a fifth sampling week on Occasion 1 plots were not collected as severe rainfall made the plants too wet to sample. Therefore, it is not known whether this decline continued. This population crash was not observed on the netted plots.

A similar, but smaller, decrease was also observed on Occasion 2 where an increase in numbers of *N. ribisnigri* was observed on 28 July and 3 August for the open and control plots, which was then followed by a decline on 11 August, after which aphid numbers remained low for the remainder of the sampling occasions. Aphid numbers on plants in the netted plots peaked later than those in open treatments in Occasions 1 and 2. Aphid numbers also peaked on Occasion 3 in the netted plots, but no clear peak was identified for the open plots.

The back-transformed mean numbers of *N. ribisnigri* on the control plants throughout Occasion 1, 2 and 3 that had not been infested artificially generally remained low, peaking at only 24 aphids per plant on 3 August (Figure 22b).



a)



b)

Figure 22a-b. Back-transformed mean number of *N. ribisnigri* (all stages) per plant recorded on **a)** netted treatments and **b)** open plots, at each sampling date during three field trials (Occasions 1-3).

The numbers of alate *N. ribisnigri* recorded on each of the netted plots (Figure 23), show a similar pattern when compared with the mean number of non-alate *N. ribisnigri* (includes apterae and nymphs) in Figure 24. Both alate and non-alate *N. ribisnigri* displayed population peaks on 12 July and 31 August for Occasions 1 and 2, but the numbers of alates per plant were much lower than for the numbers of non-alate stages per plant. Whether a further increase in numbers of alate and non-alate *N. ribisnigri* would have been observed for netted treatments following 12 July is again unknown as a fifth sampling date was not

possible. Those treatments with the highest number of non-alate aphids per plant did not always result in the highest number of alates per plant.

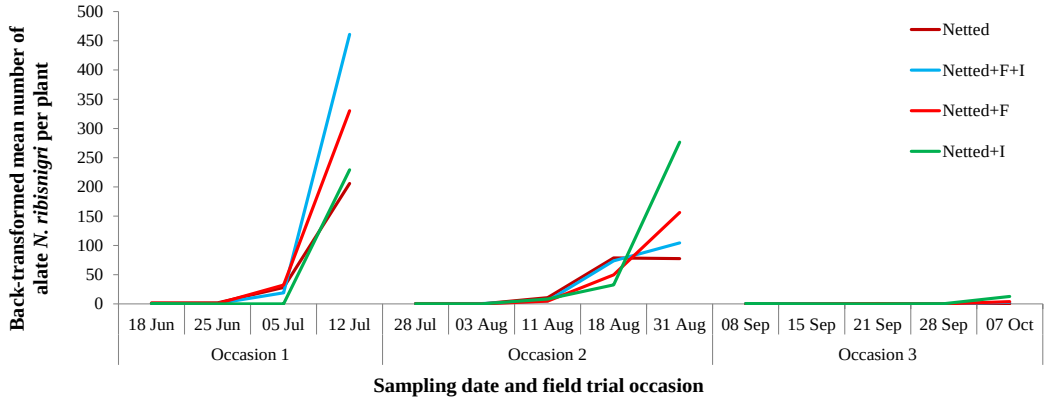


Figure 23. Back-transformed mean number of alate *N. ribisnigri* per plant for the four netted plots at each sampling date during three field trials.

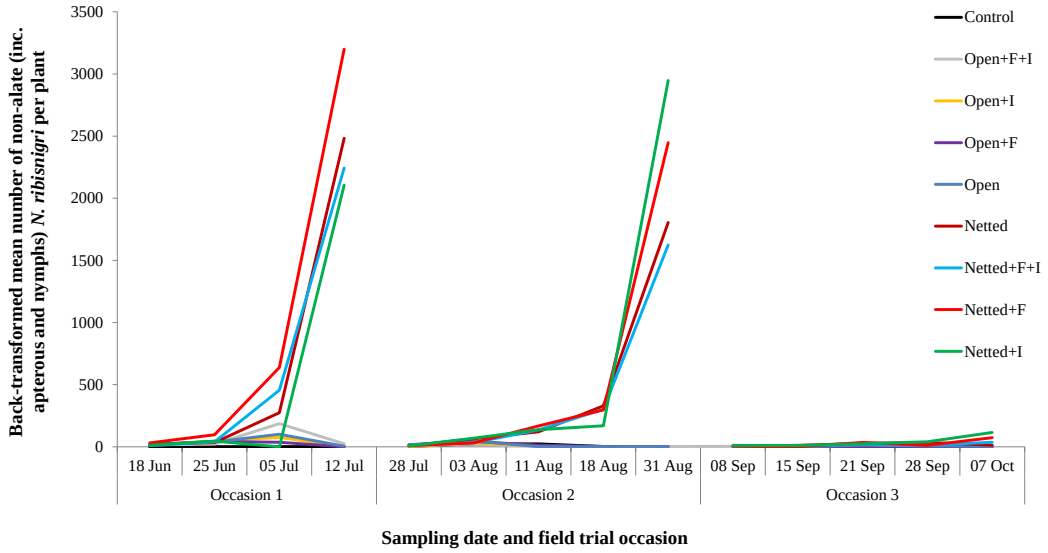


Figure 24. Back-transformed mean number of non-alate *N. ribisnigri* (including apterae and nymphs) per plant for the nine treatments at each sampling date during three field trials.

Figure 25a shows the relationship between the numbers of alate and non-alate *N. ribisnigri* for the netted plots for each of the field trial occasions. In the case of Occasion 1 and 2, as the numbers of non-alate forms increased, the numbers of alates also increased. The data for Occasion 3 do not show such a strong relationship as numbers of non-alate and alate aphids remained low.

Performing Pearson R correlations between the numbers of non-alate and alate aphids for each occasion demonstrated a strong and positive correlation coefficient (r) for Occasions 1 (r= 0.83, d.f. 30, p<0.001) and 2 (r= 0.93, d.f. 40, p=0.000) with highly significant probabilities

of there being a relationship between them. The coefficient for Occasion 3 was lower, as expected, (r= 0.76, d.f. 40, p<0.001) but still demonstrated a relationship. Figure 25b shows the data and fitted lines for Occasions 1 and 2 with both X and Y variables transformed using LOG (data+1).

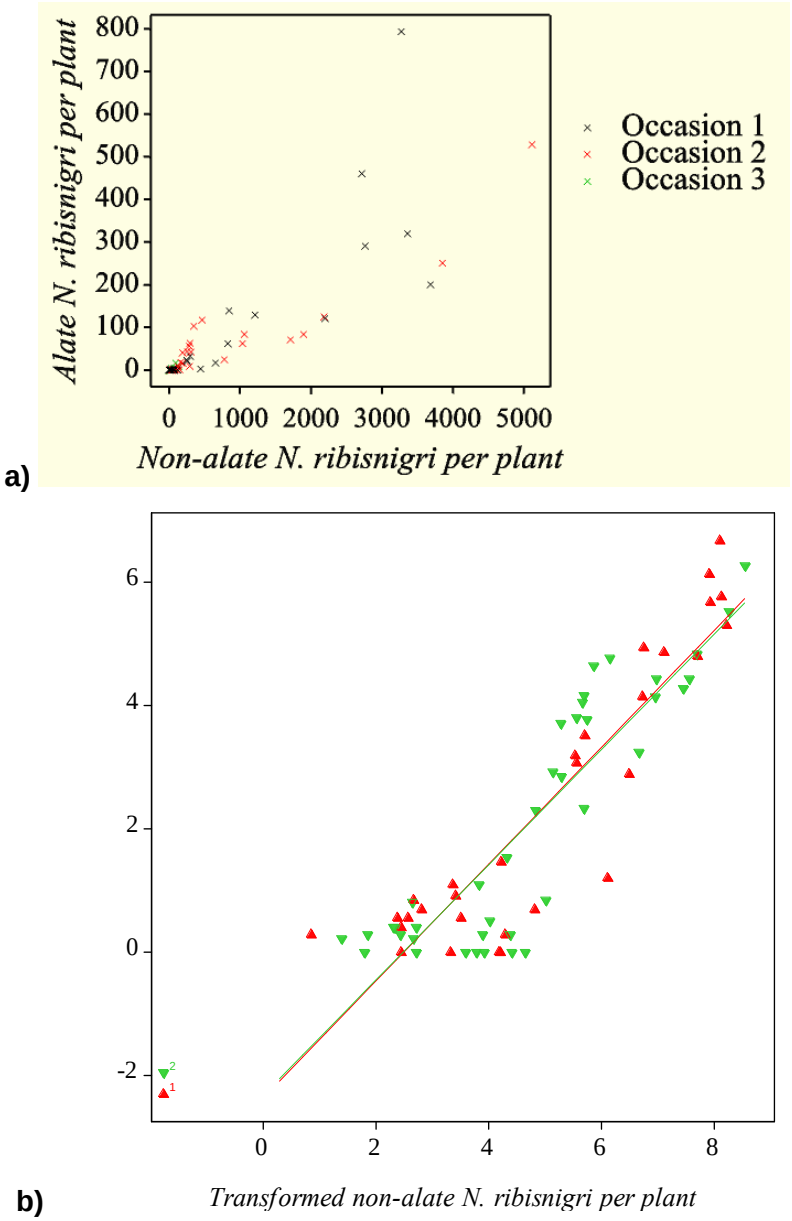


Figure 25 a-b. **a)** Scatter graph of untransformed counts of alate and non-alate *N. ribisnigri* for each of the three field trial occasions for the netted plots **b)** Linear regression of the number of alates and non-alate *N. ribisnigri* (LOG(data+1) for Occasions 1 and 2 for the netted plots.

Figure 26 shows the mean percentage of the total population per plant which were alates at each sampling date during the three field trial occasions in the netted treatments. Generally, during Occasion 1 and 2 the percentage of alates increased at each sampling date, except on 31 August 2010 (Occasion 2) where the percentage of alates decreased by 16%. During

Occasion 3 no clear trend was observed and the percentage of alates fluctuated. The percentage of alates in the total population never exceeded 20%.

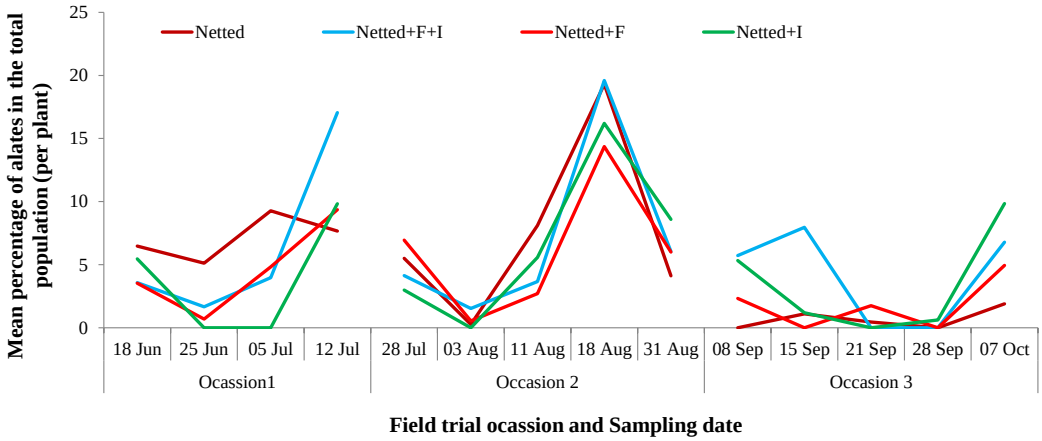


Figure 26. Mean percentage of the total population per plant which were alates at each sampling date during three field trials for the netted treatments.

With the open plots, similar patterns were observed when comparing non-alate and alate *N. ribisnigri* (Figure 24 and 27). Unlike the netted plots, peak numbers occurred on 5 July and a decline was observed by 12 July.

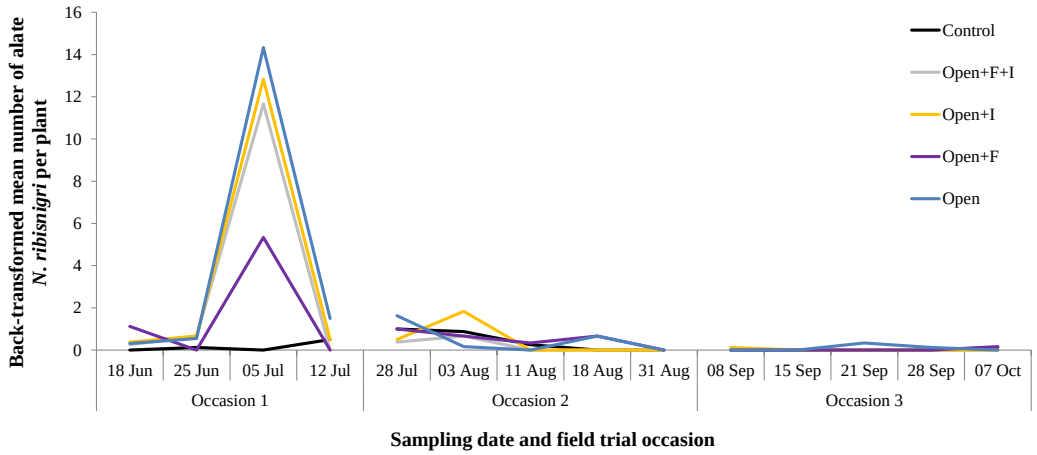
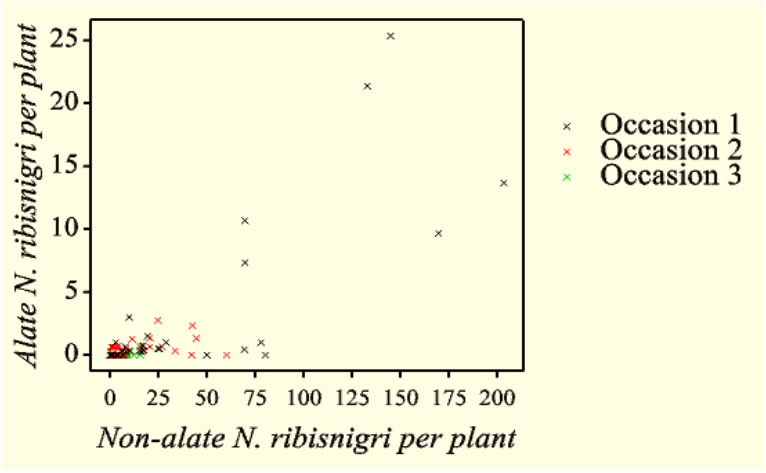


Figure 27. Back-transformed mean number of alate *N. ribisnigri* per plant for the four open plots at each sampling date during the three field trials.

Figure 28a shows the relationship between alate and non-alate *N. ribisnigri* for the open plots for each of the field trials. This is similar to the netted plots, but not as strong. When Pearson R correlations were performed, a high positive correlation was observed between the number of alate and non-alate *N. ribisnigri* for Occasion 1 (r= 0.79, d.f. 32, p<0.001) but the relationship was weaker for Occasions 2 (r= 0.44, d.f. 40, p=0.004) and 3 (r= 0.40, d.f. 40, p=0.01). Figure 28b shows the fitted lines for Occasions 1 and 2 with both X and Y variables transformed using (LOG(data+1)).

a)



b)

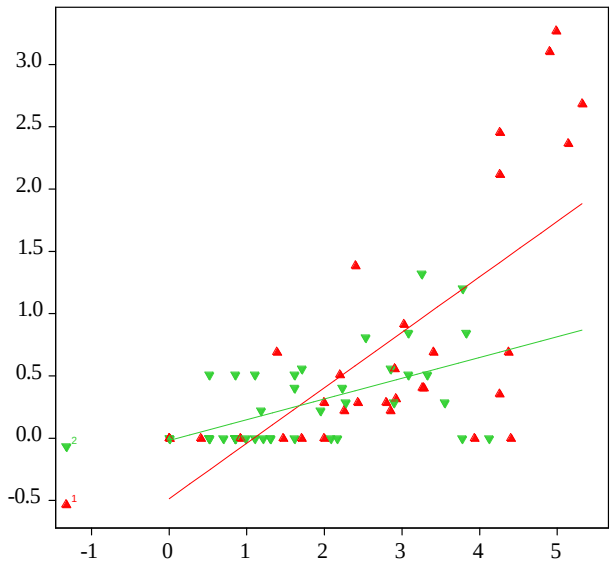


Figure 28 a-b. a) Scatter graph of untransformed counts of alate and non-alate *N. ribisnigri* for each of the three field trial occasions for the open plots **b)** Linear regression of the number of alates versus non-alate *N. ribisnigri* for Occasions 1 and 2 for the open plots.

Figure 29 shows the mean percentage of the total population per plant which were alates at each sampling date during the three field trial occasions in the open plots. During Occasion 1 the percentage of alates in all treatments increased until 5 July 2010. Following this the percentage alates in two treatments (Open+I and Open) continued to increase while the other treatments decreased (Open+F+I and Open+F). During Occasion 2, two treatments (Open and Open+F) increased to a peak on 18 August 2010, while few alates were observed in the other treatments. During Occasion 3, the percentage of alates only exceeded 3% on 7 October 2010 when 100% alates were observed. This was because only one aphid (an alate) was found.

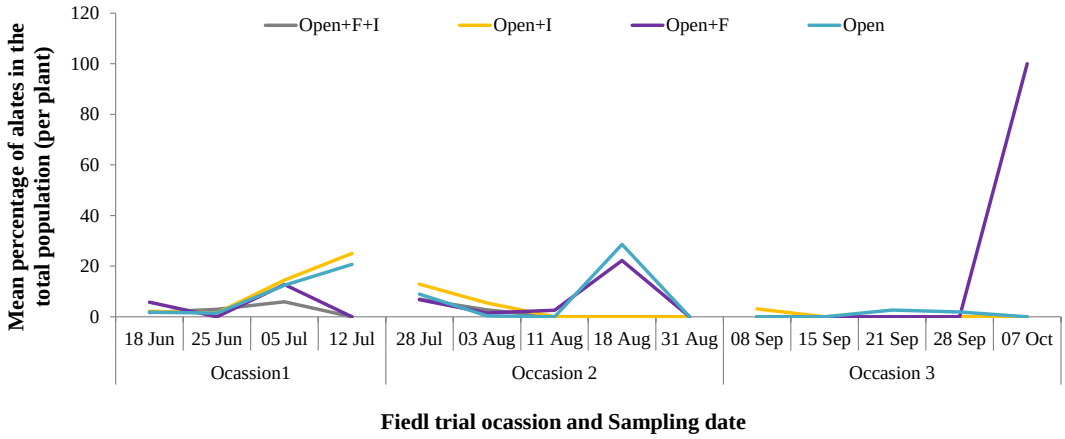
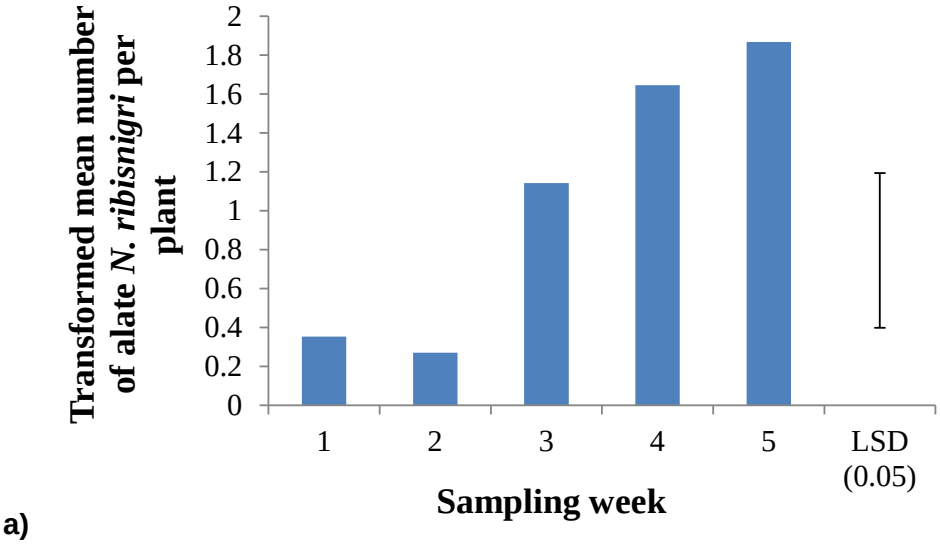
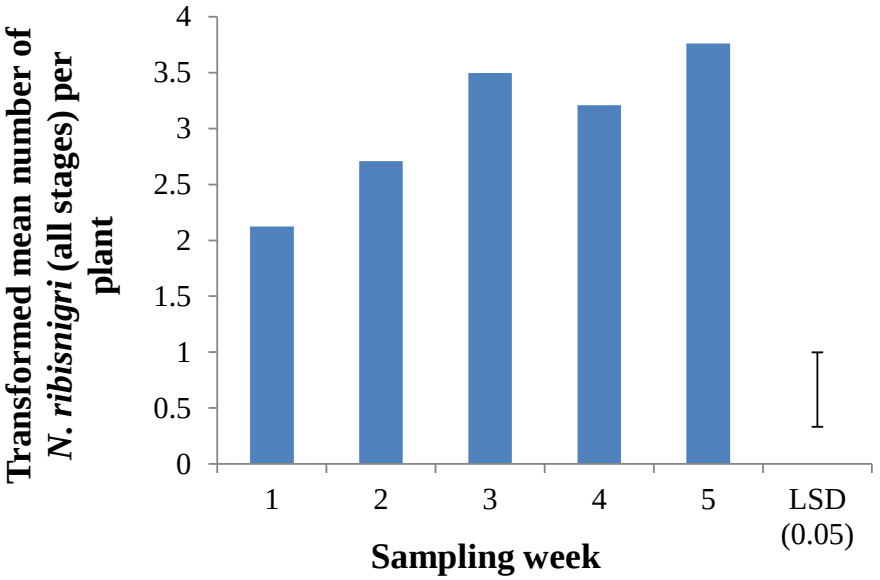


Figure 29 Mean percentage of the total population per plant which were alates at each sampling date during three field trials in the open plots.

An ANOVA was performed on the numbers of alate and all stages of *N. ribisnigri* recorded. A significant effect of sampling week was observed for both alate ($F(4,18)= 7.40$, $p=0.001$) and all stages of *N. ribisnigri* per plant ($F(4,18)= 8.77$, $p<0.001$). Figures 30a and b show the effect of sampling week on the numbers of alates and all stages of *N. ribisnigri,* respectively. Later sampling weeks had significantly more aphids than Sampling Week 1.



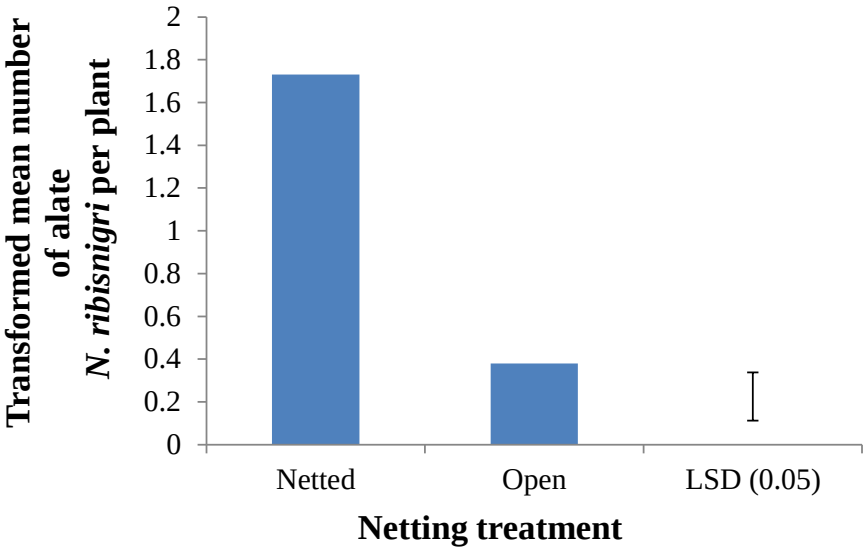
a)



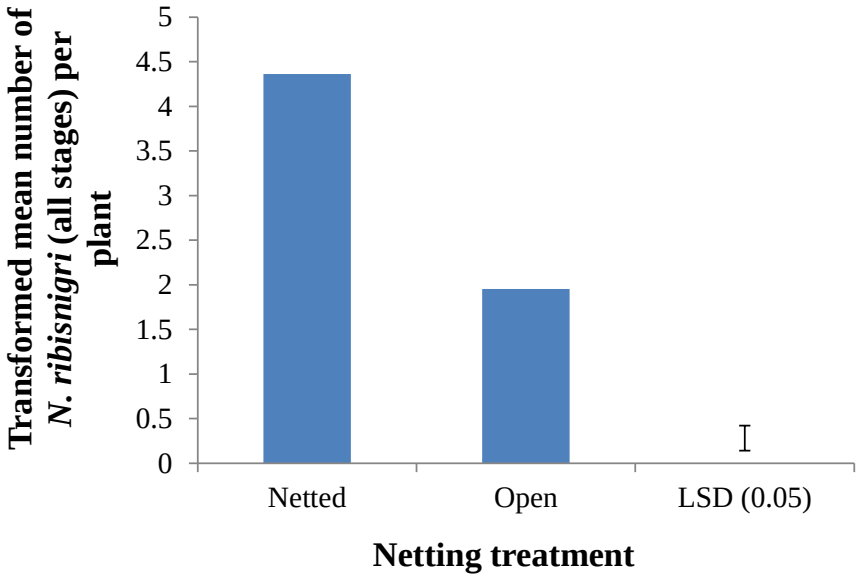
b)

Figure 30 a-b. Transformed mean number of **a)** alates **b)** all stages of *N. ribisnigri* per plant from the ANOVA for the effect of sampling week.

A significant effect of netting treatment was observed for both alate ($F(1,159)= 140.98$, $p<0.001$) and all stages of *N. ribisnigri* ($F(1,159)= 286.77$, $p<0.001$). Figures 31a and b show the effect of netting treatment on the numbers of alate and all stages of *N. ribisnigri,* respectively, where netted plots always had higher numbers of aphids than open plots.



a)



b)

Figure 31 a-. Transformed mean number of **a)** alate **b)** all stages of *N. ribisnigri* per plant from the ANOVA for the effect of netting treatment.

An interaction was observed between netting treatment and sampling week for both alate ($F(4,159)= 34.90$, $p<0.001$) and all stages of *N. ribisnigri* ($F(4,159)= 43.02$, $p<0.001$). When considering the interactions between sampling week and netting treatment, significantly more alate (Figure 32) and all stages of *N. ribisnigri* (Figure 33) were recorded per plant on netted plots than on open plots, except for Sampling Weeks 1 and 2. From Sampling Week 3 onwards, the difference between netted and open plots increased each week, with the numbers of aphids increasing on plants in netted plots but not in open plots. No effects of fungicide or insecticide application were observed.

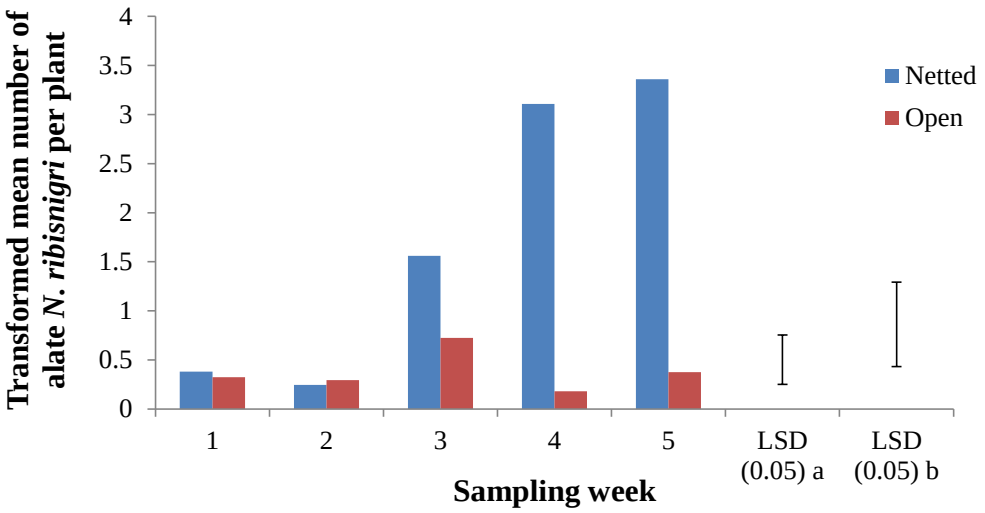


Figure 32. Transformed mean number of alate *N. ribisnigri* per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

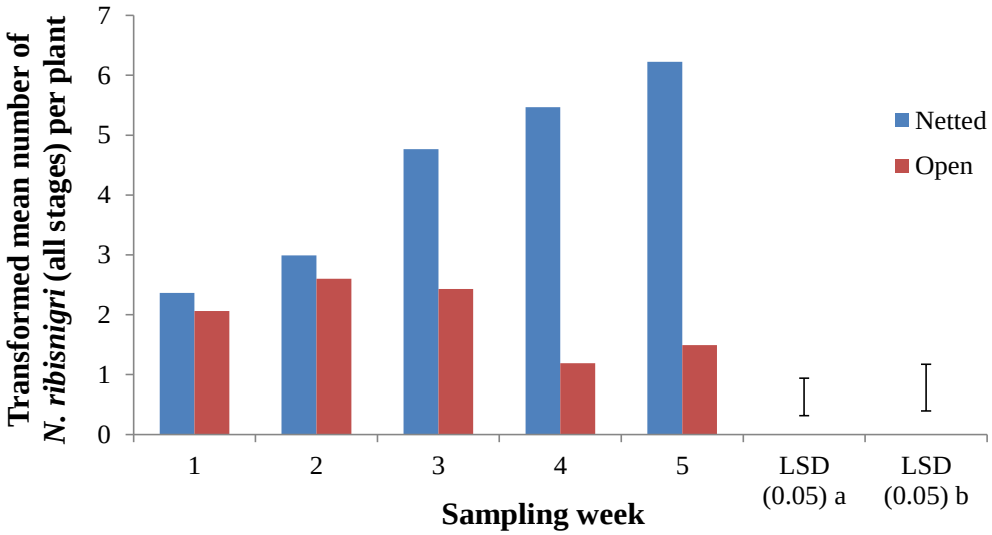


Figure 33. Transformed mean number of all stages of *N. ribisnigri* per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

An ANOVA was also performed on the number of natural enemies recorded to determine whether there was an effect of netting or fungicide or insecticide treatment.

Parasitized aphids

There was a significant effect of netting ($F(1,159)= 8.50$, $p=0.004$) on the mean number of parasitized aphids per plant. As shown in Table 8, significantly more parasitized aphids per plant were found in open plots.

Table 8. Transformed mean number of parasitized aphids per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.247
Open	0.432
LSD	0.1259
d.f.	159

There was an interaction between netting treatment and sampling week on the number of parasitized aphids per plant ($F(4,159)= 9.67$, $p<0.001$). Figure 34 shows the interaction, where significantly more parasitized aphids were found on open plots compared with netted plots in Sampling Weeks 1-4. In Sampling Weeks 4 and 5, the number of parasitized aphids in netted treatments increased, possibly indicating that they were present in treatments where they were supposed to be excluded. In Sampling Week 5 more parasitized aphids per plant were observed in netted plots.

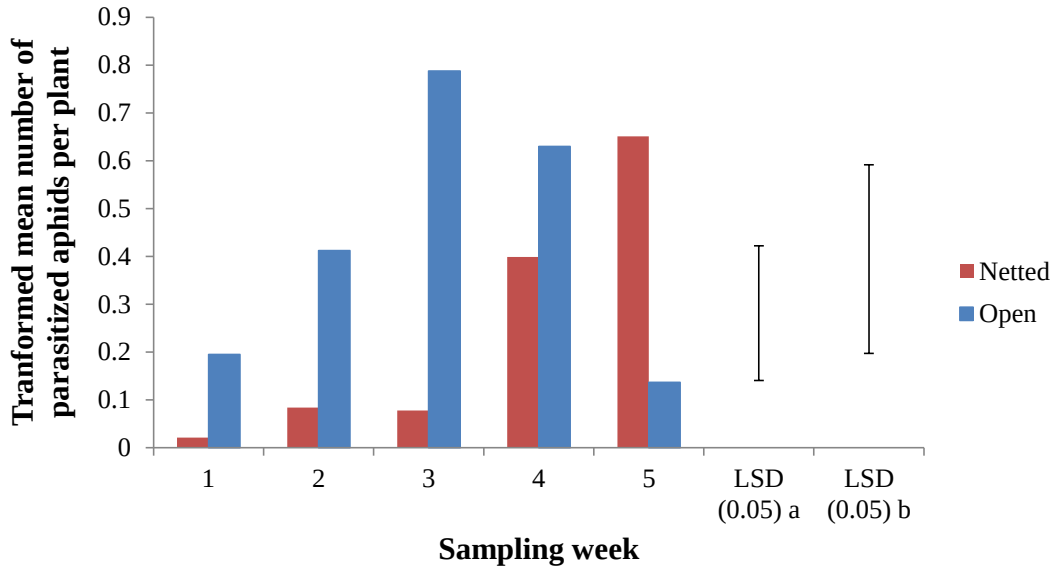


Figure 34. Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

The ANOVA indicated that there was a significant interaction between fungicide and insecticide on the number of parasitized aphids per plant ($F(1,159)= 6.09$, $p=0.015$). However, Figure 35 illustrates the means and LSD of this output which does not support this significant effect. This anomaly can be described as a cross-over interaction where there were no individual effects of insecticide and fungicide but a major interaction between the two treatments. This suggest that not applying both insecticide and fungicide, or applying both, reduced the number of parasitized aphids present, while applying them individually was beneficial and increased the number of parasitized aphids.

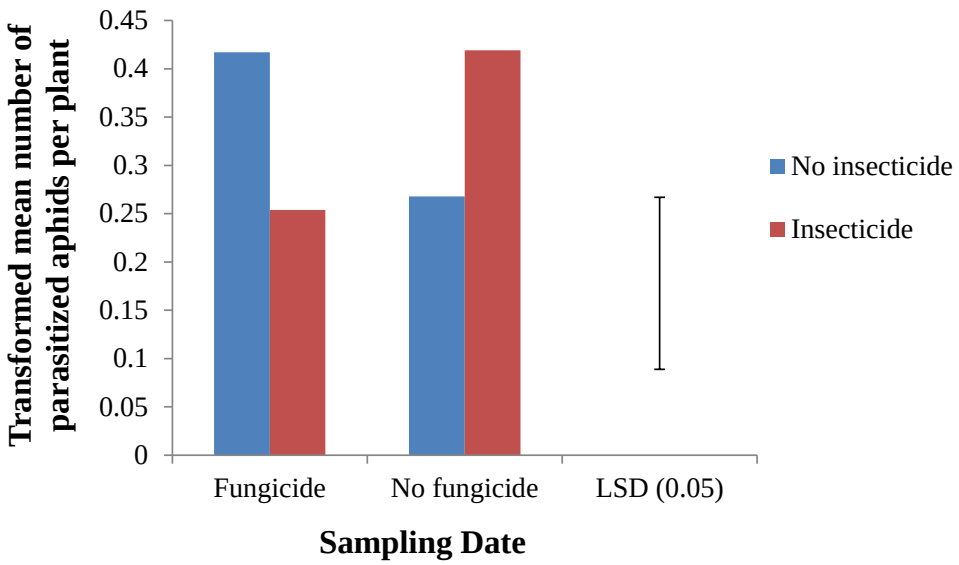


Figure 35. Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between fungicide and insecticide.

An interaction between netting, fungicide and insecticide treatments on the number of parasitized aphids per plant was observed ($F(1,159)= 9.45$, $p=0.002$). As shown in Figure 36, netted plots with neither or both fungicide and insecticide applications had significantly less parasitized aphids than those treated with either fungicide or insecticide. The numbers of parasitized aphids in open plots were similar regardless of the insecticide or fungicide treatment.

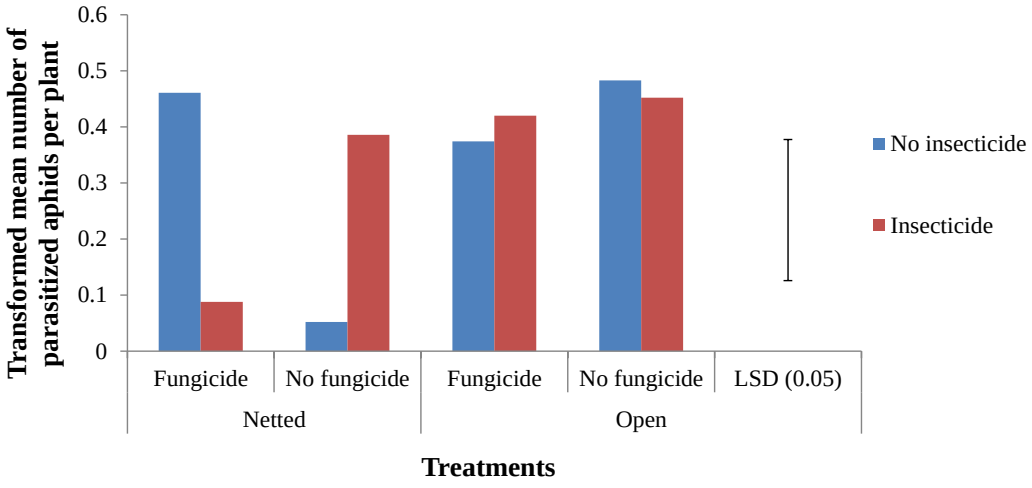


Figure 36. Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between netting, fungicide and insecticide treatments.

Syrphid larvae

A significant effect of netting treatment on the number of syrphid larvae per plant ($F(1,159)= 56.88$, $p<0.001$) was observed. As shown in Table 9, significantly more syrphid larvae per plant were observed in open plots.

Table 9. Transformed mean number of syrphid larvae per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.027
Open	0.315
LSD	0.0755
d.f.	159

An interaction between netting treatment and sampling week was also observed ($F(4,159)= 7.34$, $p=0.001$). As shown in Figure 37, significantly more syrphid larvae were observed per plant in open plots in Sampling Weeks 3-5, while significantly fewer syrphid larvae were present in Sampling Weeks 1 and 2 than in Sampling Weeks 3-5.

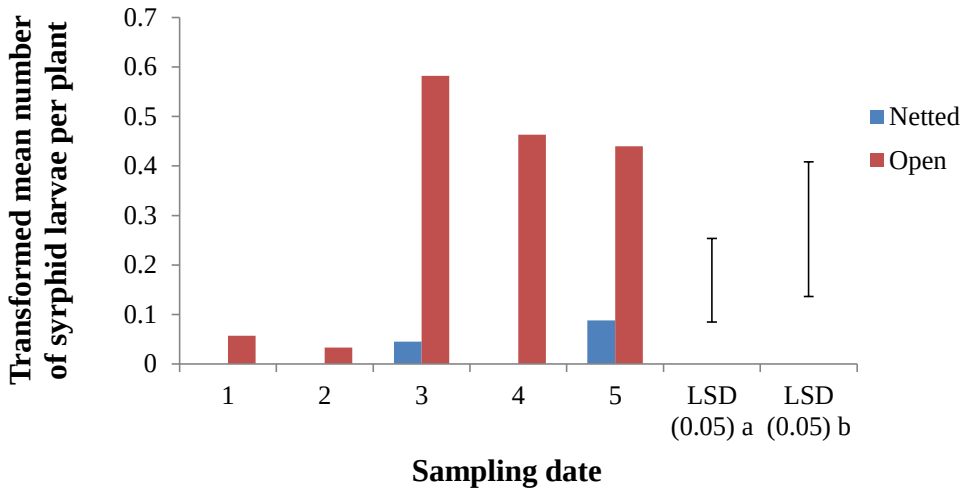


Figure 37. Transformed mean number of syrphid larvae per plant from the ANOVA for the interaction between sampling week and netting treatment. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Anthocoridae

A significant effect of netting (Table 10) was observed, with more Anthocoridae found per plant in open plots ($F(1,159)= 35.31$, $p<0.001$).

Table 10. Transformed mean number of Anthocoridae per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.002
Open	0.157
LSD	0.0515
d.f.	159

An interaction was observed between netting treatment and sampling week ($F(4,159)= 4.52$, $p=0.002$). As shown in Figure 38, more Anthocoridae were observed on open plots, particularly during Sampling Weeks 3-5. For open plots, significantly less Anthocoridae were present in Sampling Weeks 1 and 2 compared with Sampling Weeks 3-5. However, there were too many zero values in the data set for the ANOVA analysis to provide good estimates.

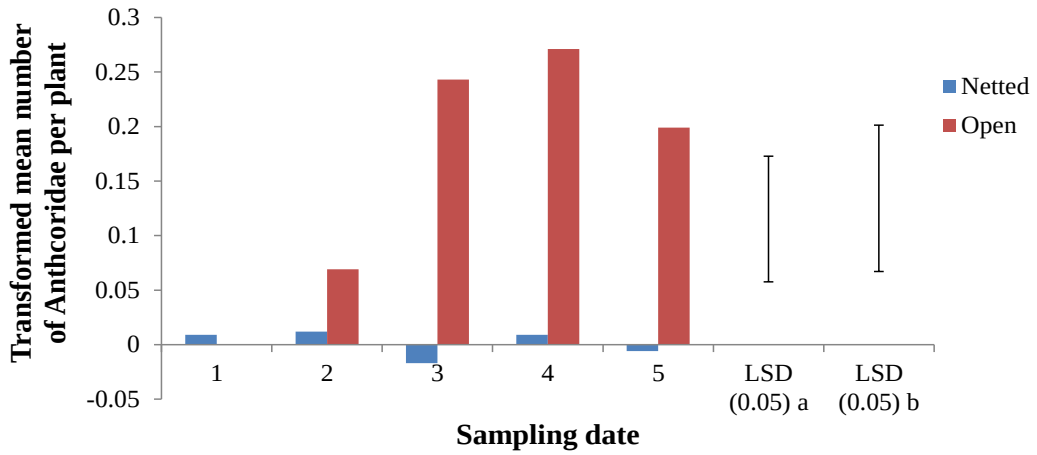


Figure 38. Transformed mean number of Anthocoridae per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

A significant effect of fungicide on the numbers of Anthocoridae was observed ($F(1,159)= 4.96$, $p=0.027$); more were present on plants from fungicide treated plots (Table 11).

Table 11. Transformed mean number of Anthocoridae per plant from the ANOVA for the effect of fungicide.

Treatment	Mean
Fungicide	0.108
No fungicide	0.050
LSD	0.0515
d.f.	159

As shown in Figure 39, there was an interaction between fungicide and insecticide ($F(1,159)= 4.36$, $p=0.038$). There were less Anthocoridae per plant from plots which were treated with insecticide only, or from untreated plots, than from plots treated with both insecticide and fungicide treatments.

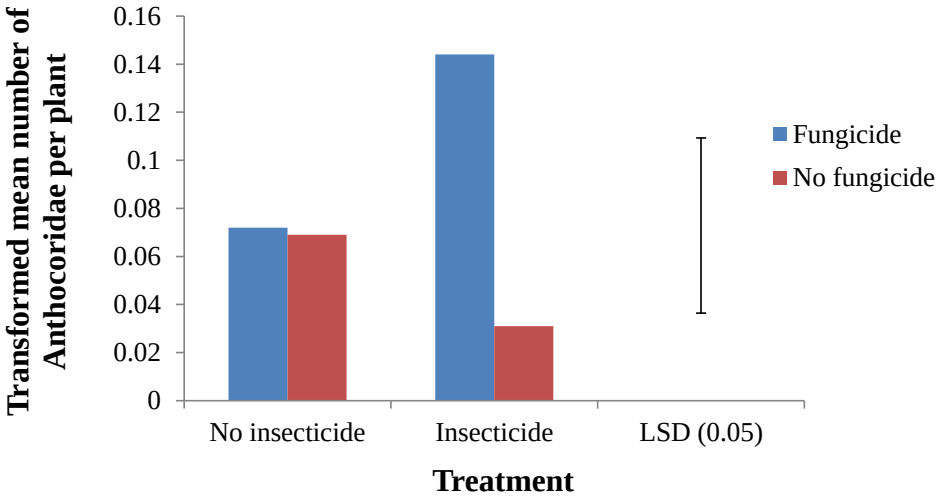


Figure 39. Transformed mean number of Anthocoridae per plant from the ANOVA for the interaction of insecticide and fungicide.

Neuroptera

The numbers of Neuroptera were very low during the trial, resulting in negative values for some of the means, indicating a lack of data.

Araneae

There was a significant effect of sampling week ($F(4,18)= 10.42$, $p<0.001$) on the numbers of spiders. Significantly more spiders were found in Sampling Weeks 3-5 (Table 12).

Table 12. Transformed mean number of Araneae per plant from the ANOVA for the effect of sampling week.

Sampling Week	Mean
1	0.060
2	0.089
3	0.347
4	0.425
5	0.473
LSD	0.1770
d.f.	18

There was a significant effect of netting treatment ($F(1,159)= 41.68$, $p<0.001$) and more spiders were found in plants from open plots (Table 13).

Table 13. Transformed mean number of Araneae per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.159
Open	0.398
LSD	0.0730
d.f.	159

There was an interaction between sampling week and netting treatment ($F(4,159)= 4.42$, $p=0.002$). With the exception of Sampling Week 1, significantly more spiders were found on plants from open plots (Figure 40), and the difference increased from Sampling Week 2-4. There were more spiders on plants from open plots in Sampling Weeks 3, 4 and 5 compared with Sampling Week 1. For plants from netted plots, there were more spiders in Sampling Week 5 than in Sampling Week 1.

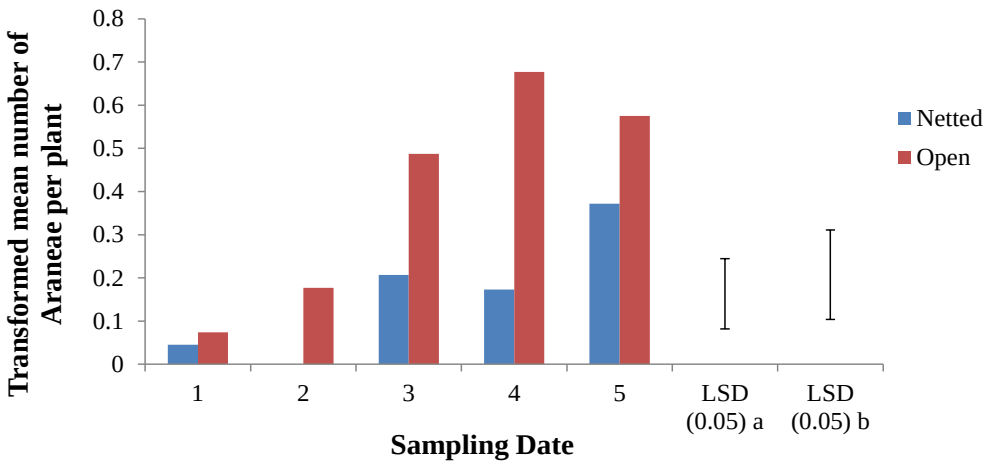


Figure 40. Transformed mean number of Araneae per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Total number of natural enemies

When the numbers of Anthocoridae, Coccinellidae, Neuroptera, parasitized aphids and syrphid larvae were summed, there was an overall effect of sampling date ($F(4,18)= 4.94$, $p=0.007$). Table 14 shows that there were significantly more natural enemies in Sampling Weeks 3-5 compared with Sampling Week 1.

Table 14. Transformed mean number of total natural enemies per plant from the ANOVA for the effect of sampling week.

Sampling Week	Mean
1	0.130
2	0.280
3	0.649
4	0.779
5	0.701
LSD	0.3814
d.f.	18

When considering the effect of netting treatment, significantly more natural enemies were observed per plant from open plots ($F(1,159)= 49.86$, $p<0.001$) (Table 15).

Table 15. Transformed mean total number of natural enemies per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Netted	0.268
Open	0.748
LSD	0.1342
d.f.	159

There was an interaction between sampling week and netting treatment ($F(4,159)= 8.31$, $p<0.001$). There were significantly more natural enemies in Sampling Weeks 2, 3 and 4 in plants from open plots (Figure 41). Between sampling weeks, plants from open plots contained significantly more natural enemies in Sampling Weeks 3, 4 and 5 than in Sampling Week 1. Significantly more natural enemies were found in plants from netted plots in Sampling Week 5 than in Sampling Week 1.

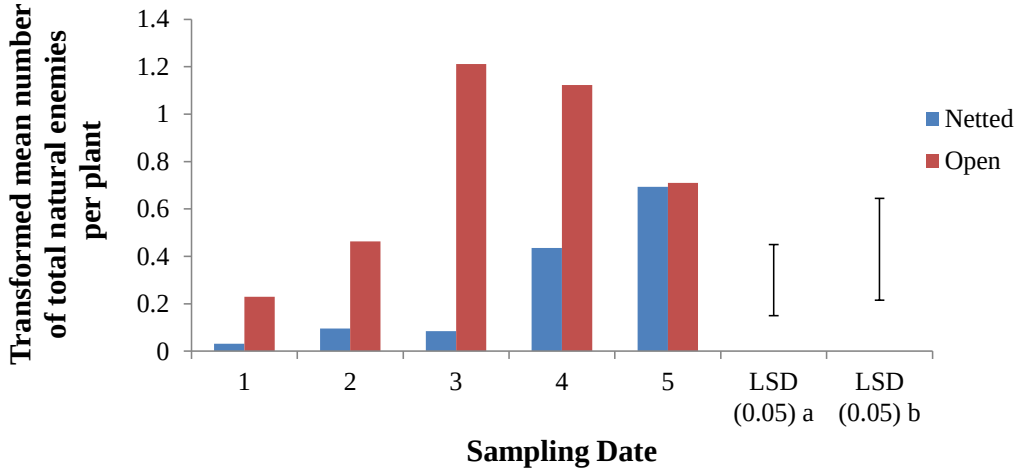


Figure 41. Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between sampling week and netting. LSD (a) used for comparison between treatments in the same sampling week and LSD (b) used for comparison between means in different sampling weeks.

Figure 42 shows the slight interaction between insecticide and fungicide treatments ($F(1,159)= 4.01$, $p=0.047$) where the number of natural enemies found in plants taken from plots with no application of fungicide and insecticide were significantly different to those taken from plots where only fungicide was applied.

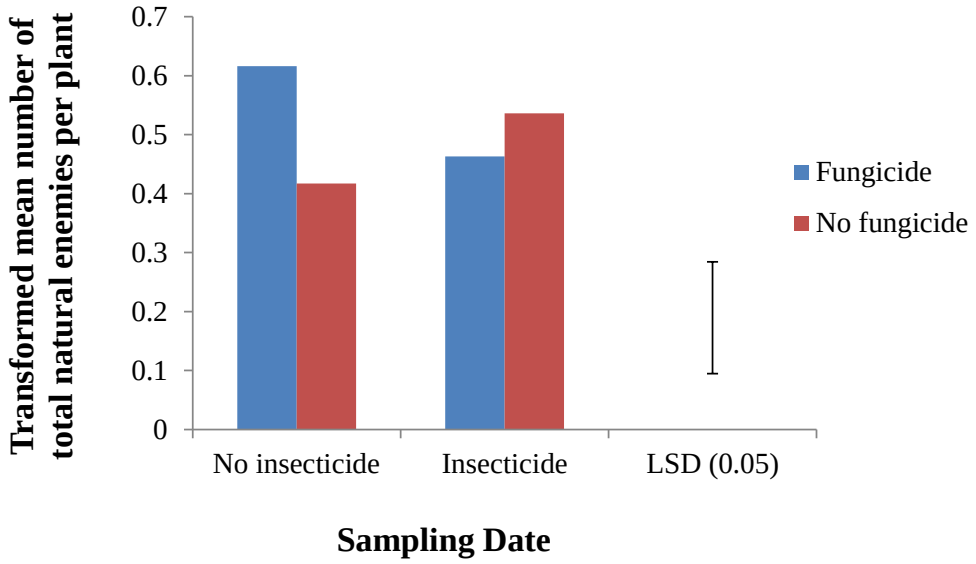


Figure 42. Transformed mean number of natural enemies per plant from the ANOVA analysis for the interaction of insecticide and fungicide.

Finally, an interaction between netting treatment, insecticide and fungicide ($F(1,159)= 9.42$, $p=0.003$) was observed which again confirms that numbers of natural enemies were generally higher in open plots (Figure 43). When compared with one another, plants from open treatments had similar numbers of predators regardless of whether fungicide and/or insecticide had been applied. However, netted plots had a lower number of natural enemies when fungicide and insecticide were combined or when they were both absent.

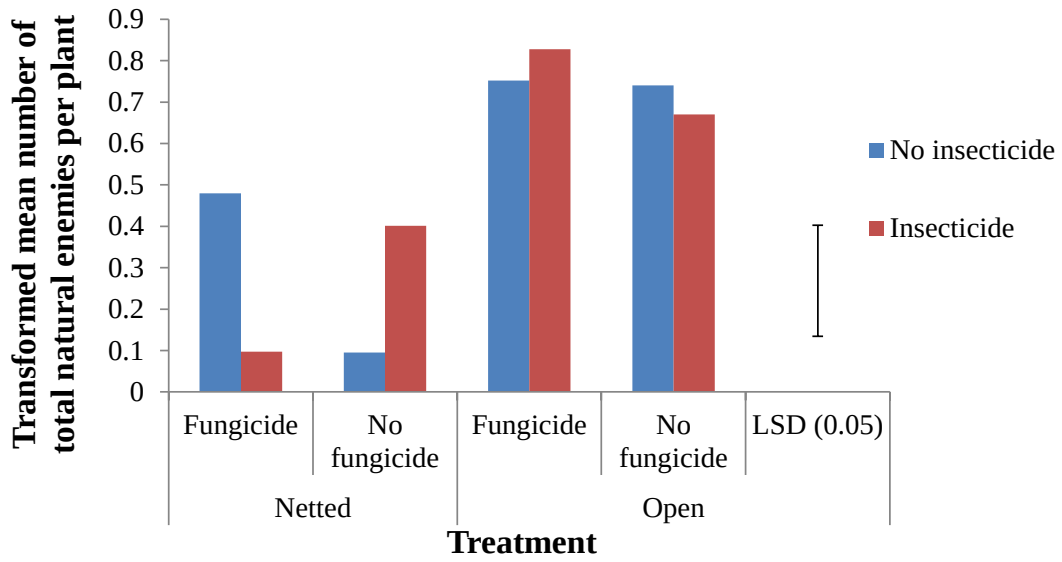


Figure 43.Transformed mean number of natural enemies from the ANOVA for the interaction of netting, insecticide and fungicide.

When analysing the data on the presence of natural enemies, the main peak was around 5 and 12 July, where a decline in aphid numbers had been identified in open plots. When plotting the back-transformed numbers of *N. ribisnigri* (all stages) per plant and the numbers of Anthocoridae, Neuroptera, parasitized aphids, Araneae and syrphid larvae per plant, only a relationship between parasitized aphids and *N. ribisnigri* (all stages) per plant was observed on 5 July (Figure 44). The relationship shows that the number of parasitized aphids per plant increased with the number of *N. ribisnigri* (all stages) per plant. However, two extreme responses can be observed in this relationship. One occurs in one of the replicate plots for treatment Open+I, where extremely high numbers of parasitized aphids compared to other treatments occurred, when *N. ribisnigri* numbers were also high. The other anomaly occurs in one of the replicate plots for treatment Open+F+I where no parasitized aphids were observed when *N. ribisnigri* numbers were high.

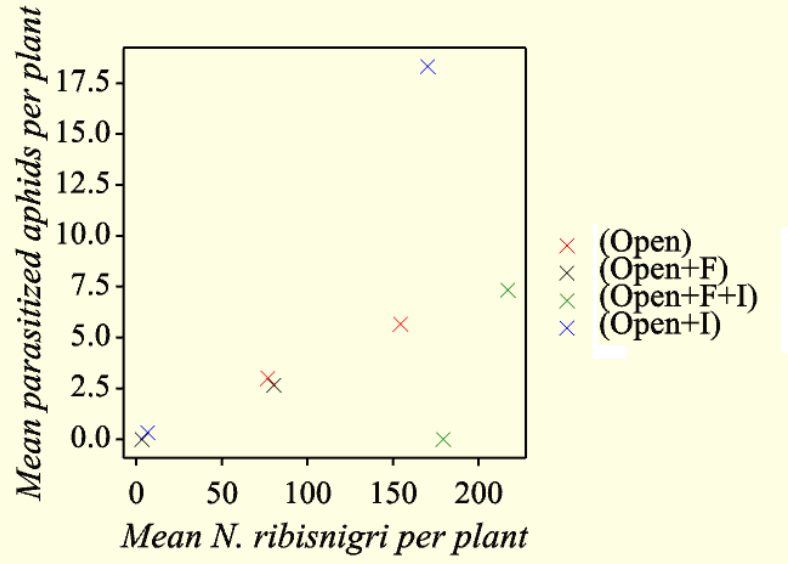


Figure 44. Back-transformed mean number of *N. ribisnigri* (all stages) per plant and mean number of parasitized aphids per plant on 5 July 2010 sampling date.

As each of these observations is based on the destructive sampling of approximately four plants, these anomalies could be explained by parasitoids parasitizing or not parasitizing aphids more on these lettuce plants by chance, compared to other lettuce plants in other treatment plots. When these anomalies were removed, a linear regression indicated a relationship of 1 parasitized aphid per 30 *N. ribisnigri* (Figure 45). A strong and positive correlation coefficient was calculated (r=0.10, observations 6, p<0.001).

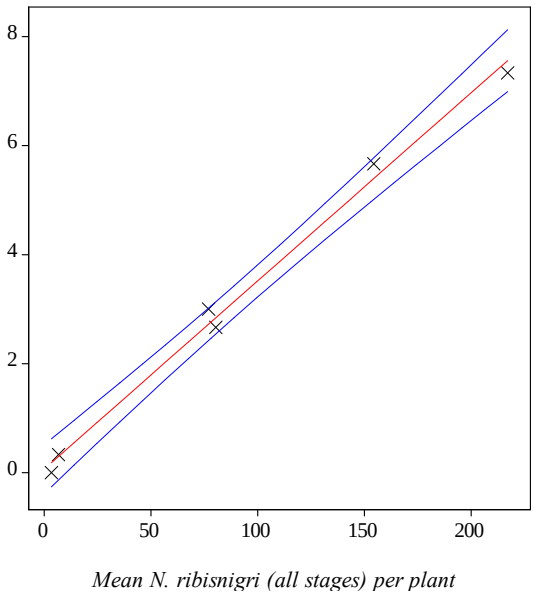
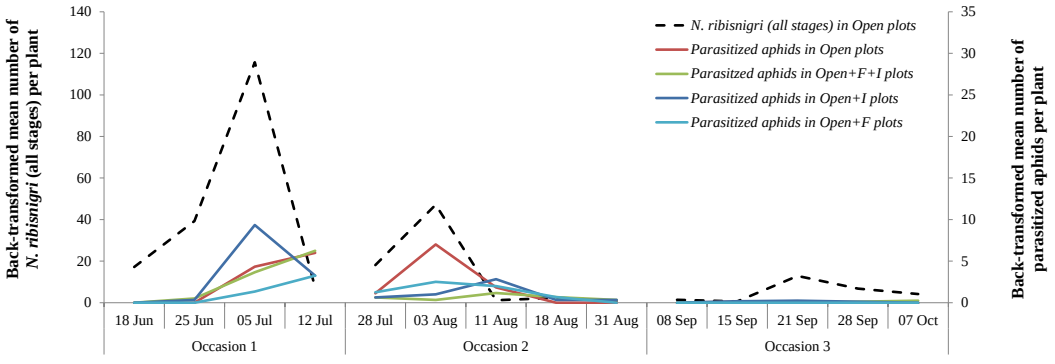


Figure 45. Linear regression of the back-transformed mean number of *N. ribisnigri* and parasitized aphids per plant on 5 July 2010 sampling date.

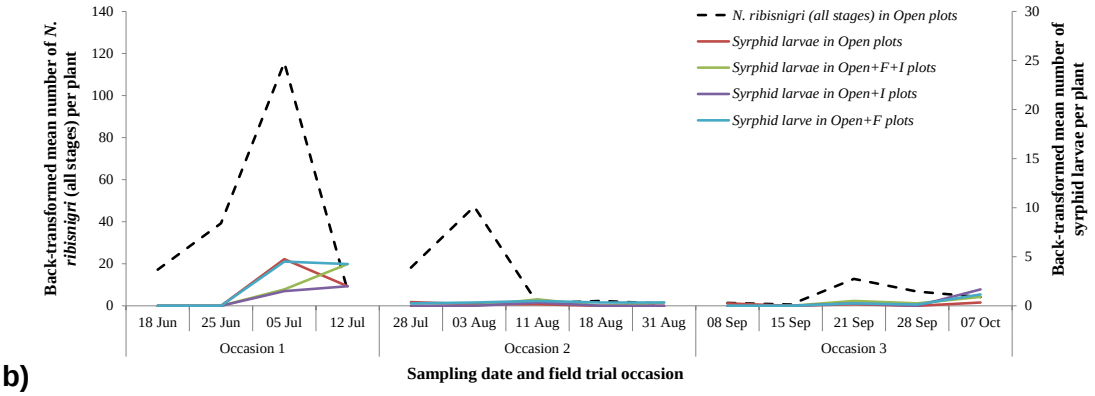
When the number of natural enemies recorded at each sampling date was investigated, parasitic wasps (evaluated through the number of parasitized lettuce aphids) and syrphid larvae were the most prevalent natural enemies in the open plots. The numbers of parasitized aphids were higher than the numbers of syrphid larvae, but both peaked on 5 July when the numbers of *N. ribisnigri* also peaked (Figure 46a and b). However, on 12 July, aphid numbers had declined considerably whilst the parasitized aphids and syrphid larvae were still present but in lower numbers than those seen on 5 July. Whether these parasitized aphids were *N. ribisnigri* is unknown.

Other predators associated with aphids, such as the Anthocoridae, were recorded in lower numbers, as illustrated in Figure 46c, where *Anthocoris nemorum* was the most prevalent species. The Hemerobiidae and Chrysopidae (Neuroptera) were also present in low numbers as shown in Figure 46d; the majority of these were identified as *Micromus variegatus* (Chrysopidae).

When considering the activity of generalist predators such as the Araneae, they were present throughout the three field trials, but displayed one of their two population peaks on 12 July, as the number of all stages of *N. ribisnigri* decreased (Figure 46e). Throughout the trial, some natural enemies were rarely observed, particularly mobile natural enemies such as the Coccinellidae, Hemerobiidae and Chrysopidae.



a)



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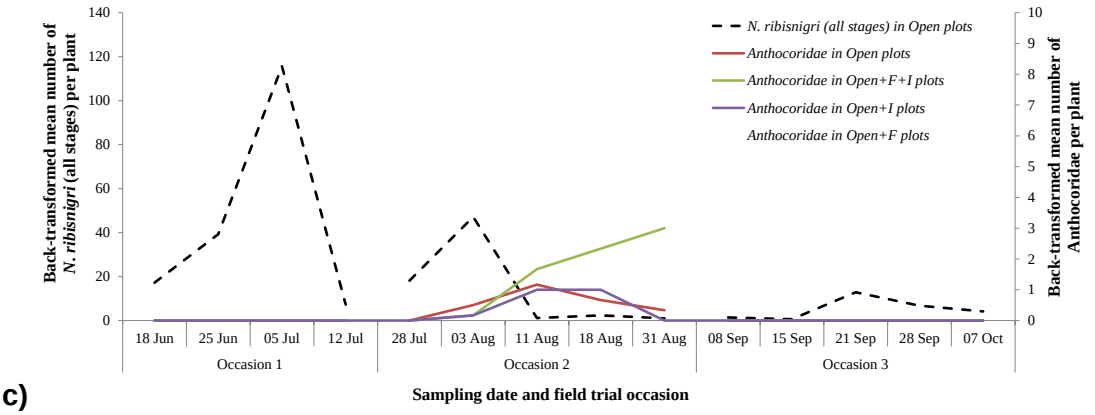

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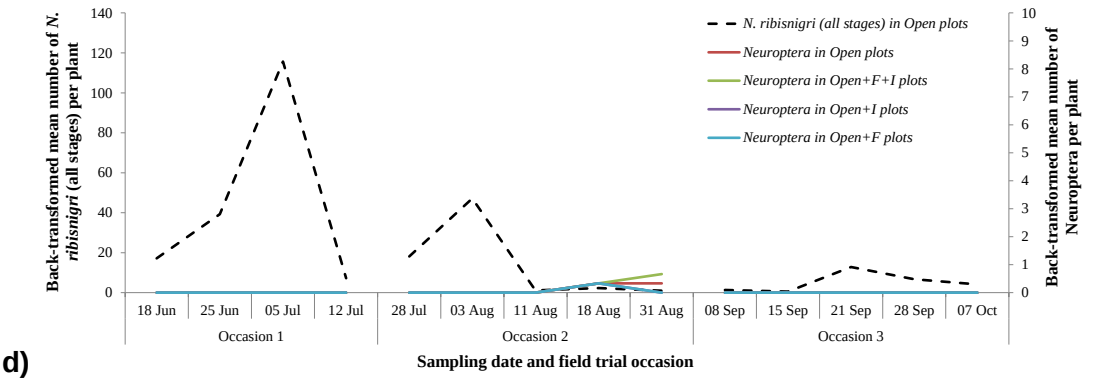

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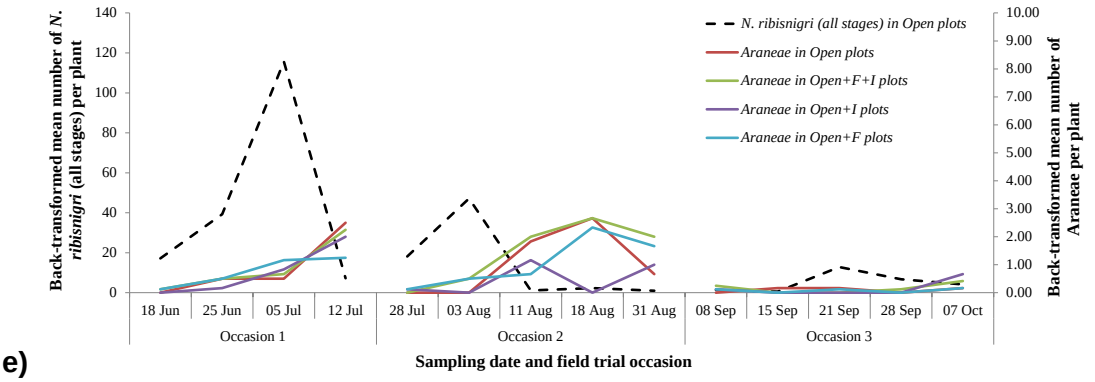


e)

Figure 46a-e. Back-transformed mean number per plant of *N. ribisnigri* and **a)** parasitized aphids **b)** syrphid larvae **c)** Anthocoridae **d)** Neuroptera and **e)** Araneae per plant on each of the open treatments.

Weather

Temperature, humidity and rainfall records were obtained from the University of Warwick, Wellesbourne meteorological station. Figure 47 shows the mean maximum and minimum temperatures and mean relative humidity recorded during each month. The maximum mean temperature was 23.2ºC (for July) and the minimum mean temperature was 9.8ºC (for June). Mean relative humidity increased gradually through the summer, from 65.86 to 78.70 %. The highest mean monthly rainfall occurred in August (4.14mm) and the least in July (0.66mm) (Figure 48).

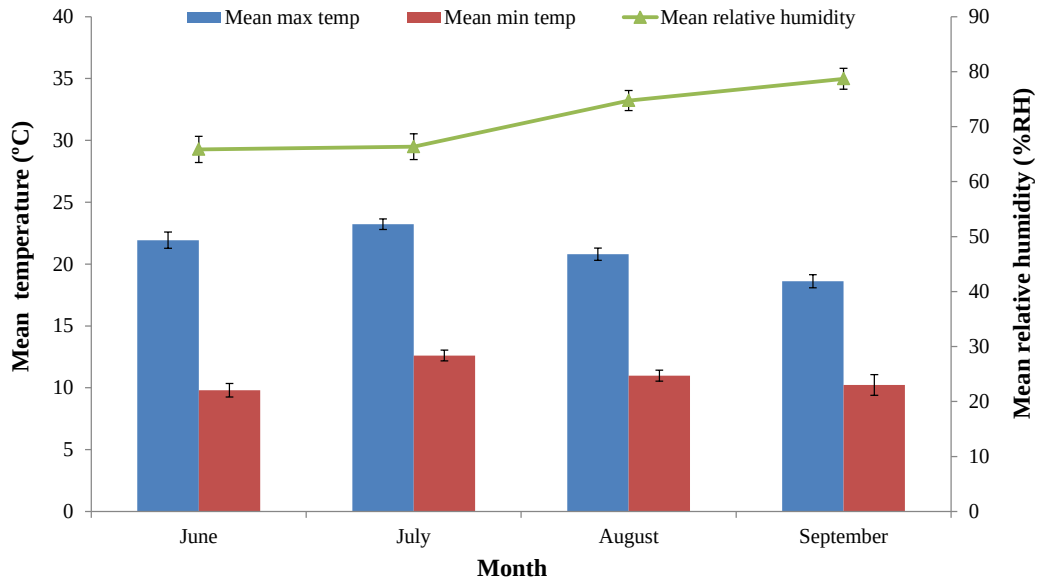


Figure 47. Mean (±SE) maximum and minimum temperatures (ºC) and mean (±SE) relative humidity (%RH) recorded during each month.

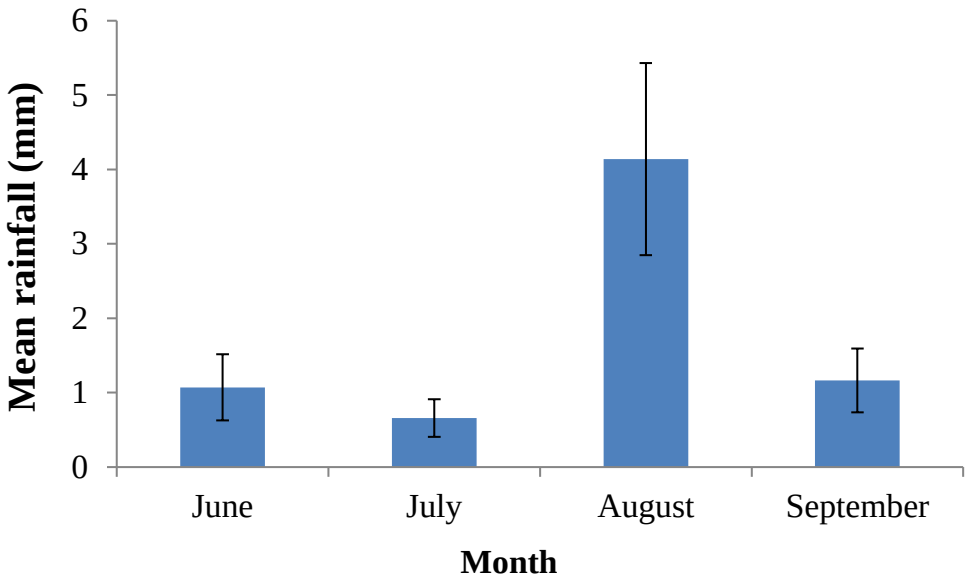


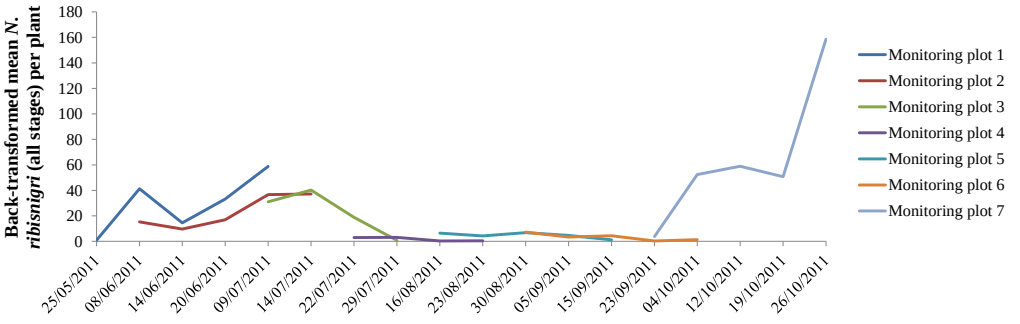
Figure 48. Mean (±SE) rainfall (mm) recorded during each month.

Field trial 2011

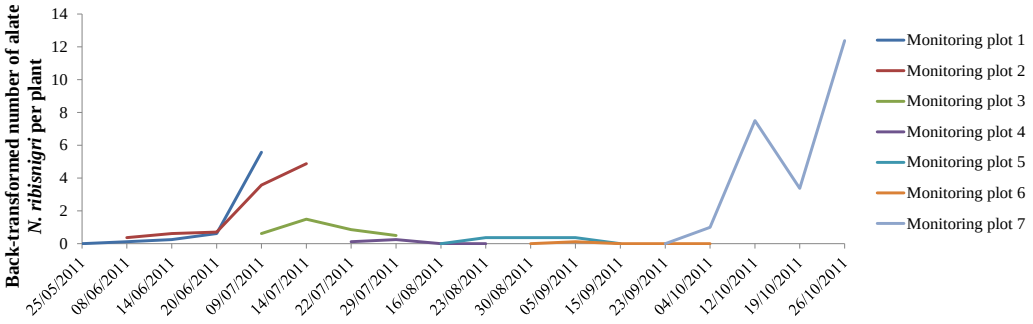
Monitoring plots

Aphids

Figure 49a shows the mean number of *N. ribisnigri* (all stages) per plant on each sampling date for the seven monitoring plots. The largest numbers of *N. ribisnigri* occurred late in the season on 26 October 2011 with a second, smaller, peak on 9 July 2011. Very low numbers of aphids were found between these peaks, following a decline starting on 14 July 2011. Numbers began to increase again from 23 September 2011. This suggests that the mid-summer crash occurred between 14-29 July 2011. The numbers of alates per plant followed a similar pattern (Figure 49b). Figure 6.2.7c shows the mean percentage of the total population per plant which were alates at each sampling date. This shows that the percentage of alates in the population increased to up to 13% by 14 July 2011 and then up to 60% by 29 July 2011. Following this the percentage of alates decreased to 0% and continued to fluctuate below 13%.



a)



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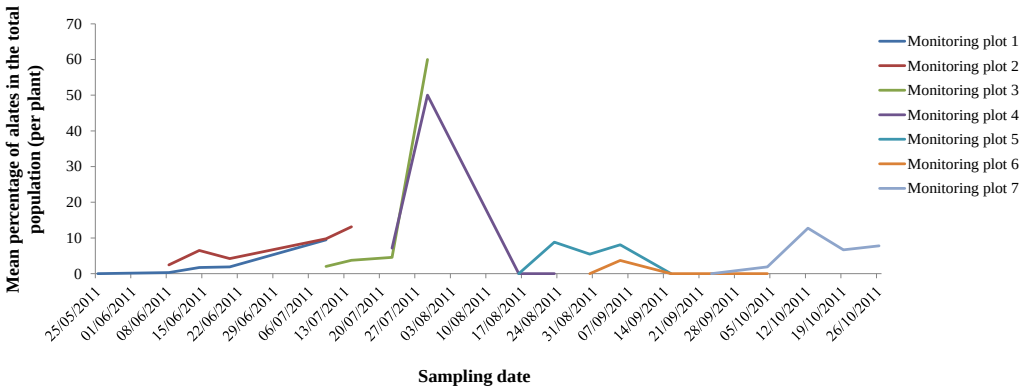


c)

Figure 49 a-c. Back-transformed mean **a)** number of *N. ribisnigri* (all stages) **b)** alate *N. ribisnigri* **c)** percentage of alates in the total population, recorded per plant on each sampling date from May to October 2011 in the seven monitoring plots.

Macrosiphum euphorbiae was present in much higher numbers than *N. ribisnigri*, but it also displayed a mid-summer crash following a peak in numbers on 14 July (Figure 50).

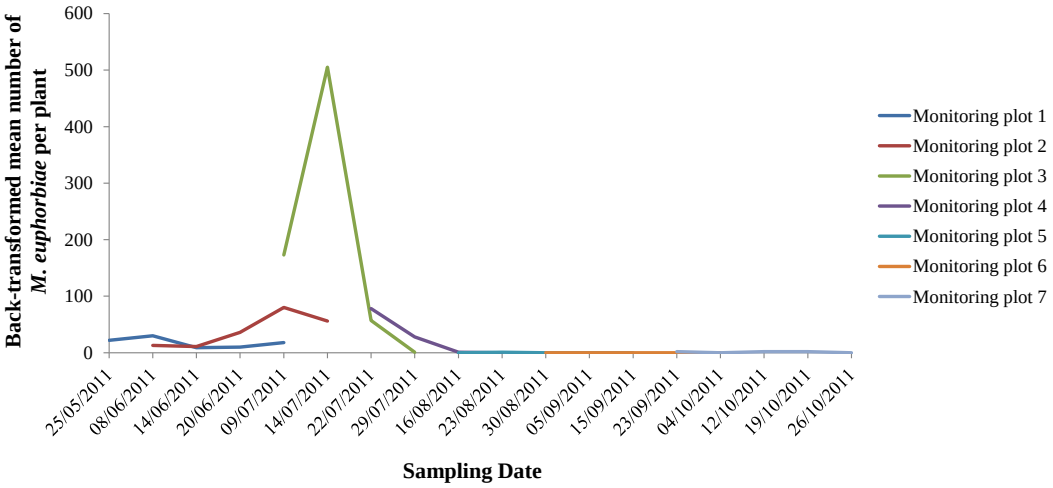


Figure 50 Back-transformed mean numbers of *M. eurphorbiae* recorded on each sampling date from May to October 2011 in the seven monitoring plots.

Myzus persicae was present in similar numbers to *N. ribisnigri* but displayed erratic changes in abundance, with peaks on 20 June, 14 and 29 July in Plots 2, 3 and 4 respectively (Figure 51). Like *N. ribisnigri,* numbers also increased from 23 September.

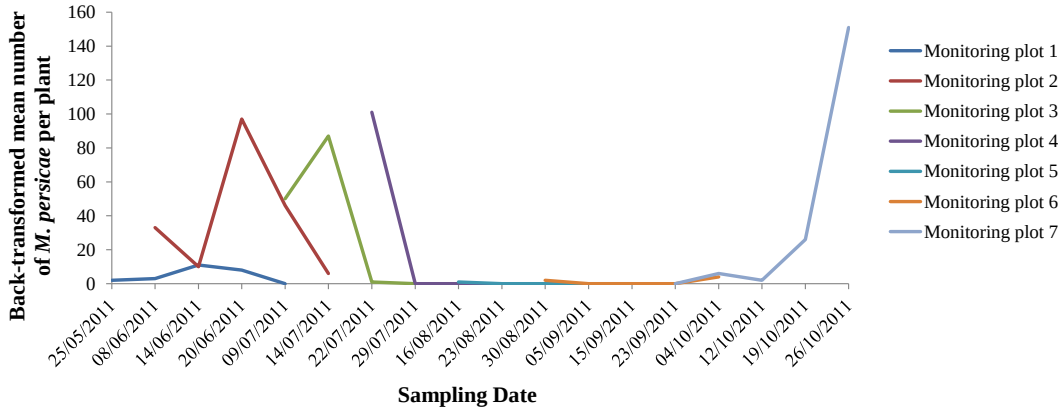


Figure 51 Back-transformed mean numbers of *M. persicae* recorded on each sampling date from May to October 2011 in the seven monitoring plots.

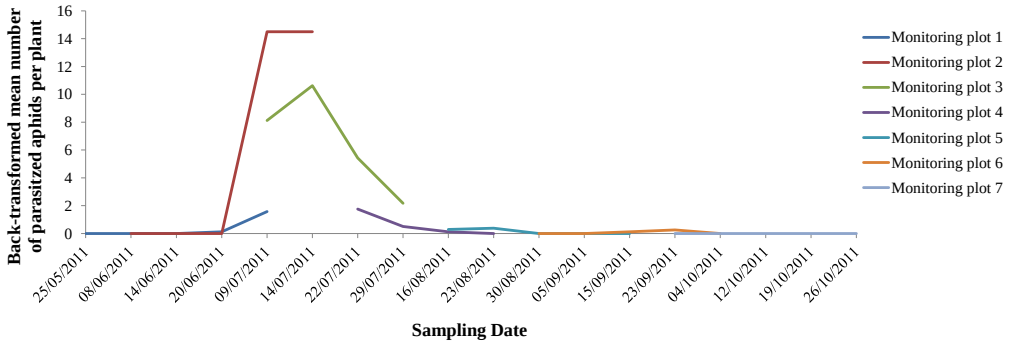
Natural enemies

Parasitized aphids were the most prevalent natural enemies with a mean of 14.5 parasitized aphids per plant on 9 and 14 July (Figure 52a). Following this peak in numbers, the mean

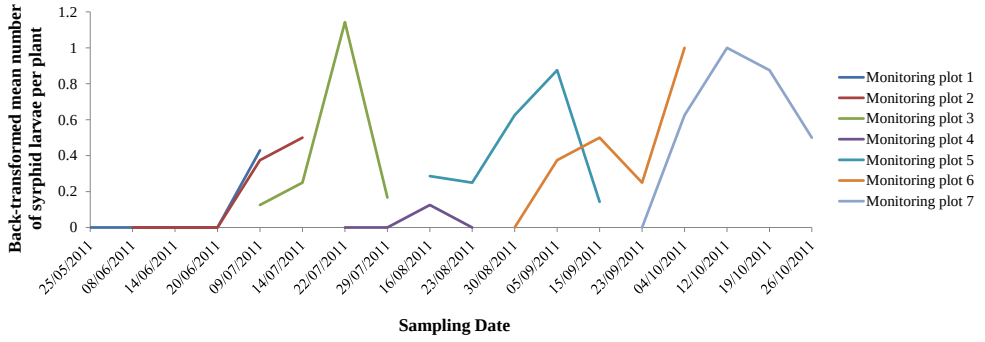
number of parasitized aphids per plant decreased and remained low for the remaining sampling dates.

Syrphid larvae were observed from 20 June onwards, peaking on 22 July with a mean of 1.14 syrphid larvae per plant (Figure 52b). The numbers of syrphid larvae were very low and there were no clear fluctuations in numbers.

Aphids infected with entomopathogenic fungi were also counted and the mean number of infections peaked on 14 July, with five infected aphids per plant (Figure 52c). Infection by entomopathogenic fungi was observed only between 20 June and 29 July. Numbers of Coccinellidae, Anthocoridae and Neuroptera were very low so data is not presented.



a)



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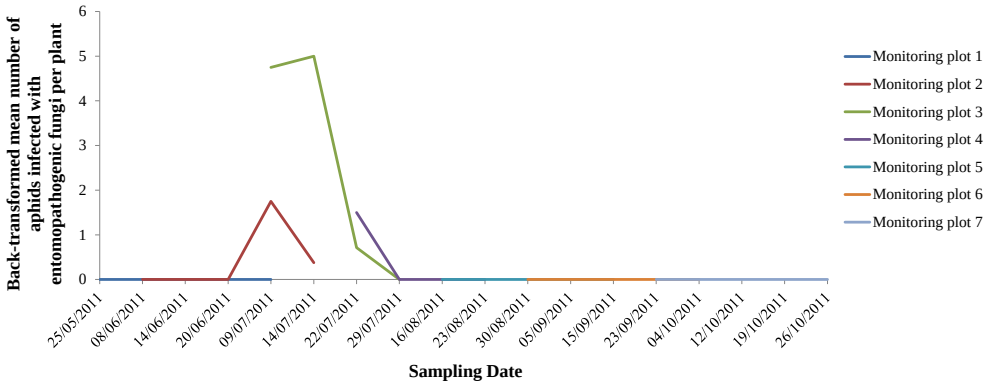


c)

Figure 52 a-c Back-transformed mean number of **a)** parasitized aphids per plant **b)** syrphid larvae per plant and **c)** aphids infected with entomopathogenic fungi per plant on each sampling date from May to October in the seven monitoring plots.

Water traps

Figure 53 shows the total numbers of natural enemies found in the water traps near the monitoring plot. Syrphid adults were the most numerous, with a maximum of 80 recorded on 4 August. Maximum counts of Coccinellidae and Anthocoridae were much lower, with 11 Coccinellidae on 29 July and six Anthocoridae on 16 August. Counts of Neuroptera never exceeded one on any sampling date and have not been shown.

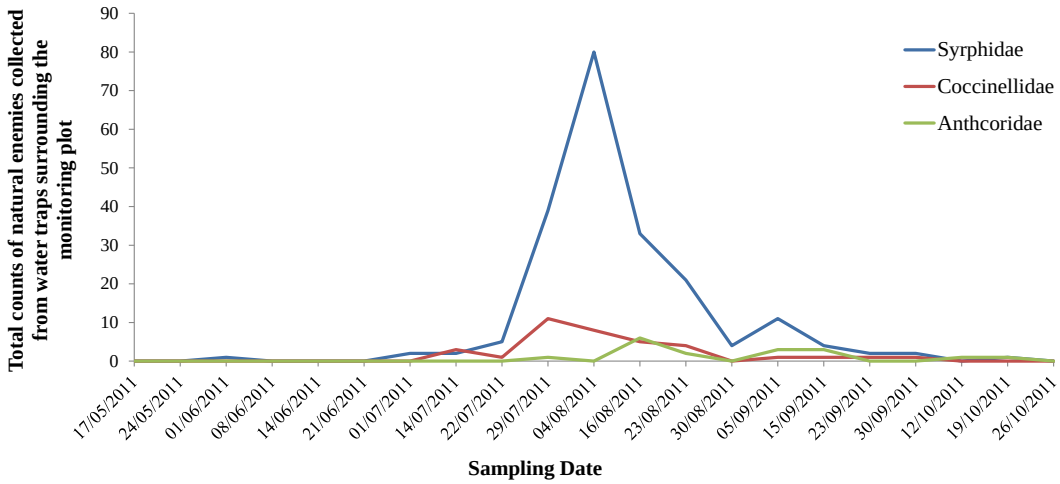


Figure 53 Counts of Syrphid adults, Coccinellidae and Anthocoridae recorded in monitoring plot water traps.

Field trial 2011- Trial plots

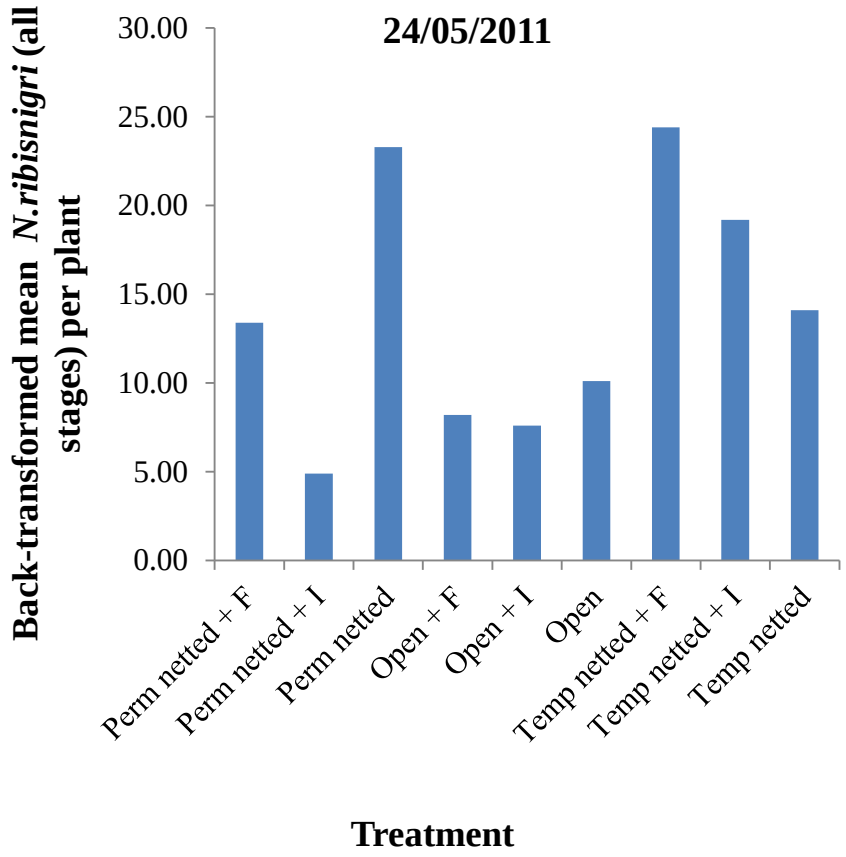
Aphids

Figure 54 a-e shows the back transformed mean number of *N. ribisnigri* (all stages) per plant on the nine treatments at the end of each field trial carried out in May, June, July, August and September. Only data for lettuce plants inoculated with *N. ribisnigri* were included, as aphid numbers were significantly lower on plants which had not been inoculated.

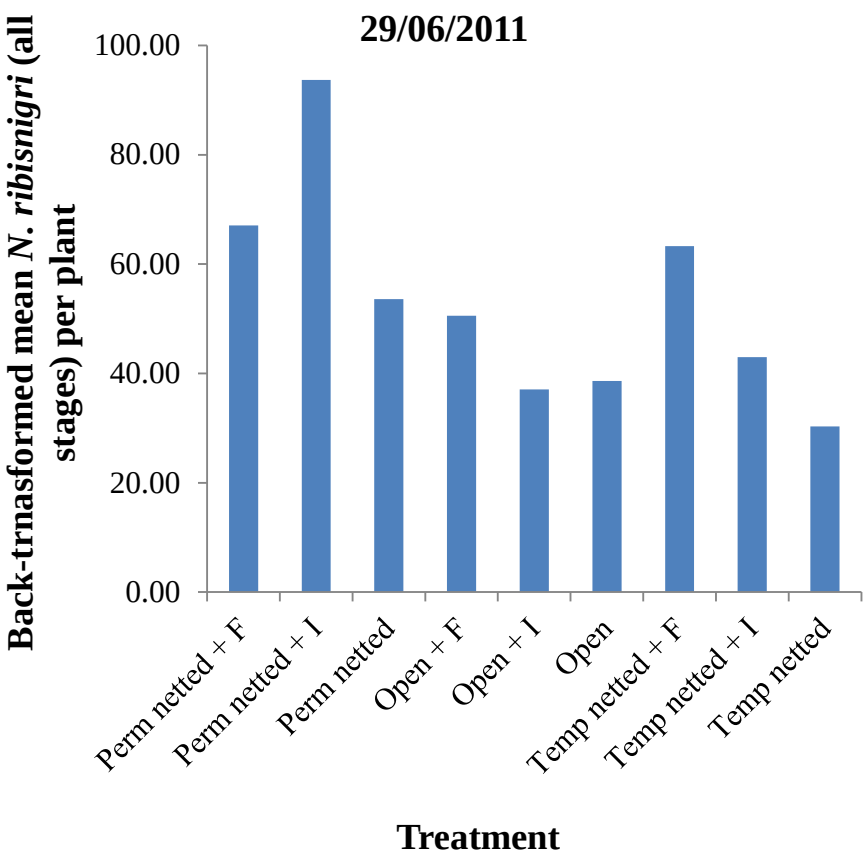
Each trial provides an indication of the impact on *N. ribisnigri* from the introduction of natural enemies and entomopathogenic fungi by comparing the numbers of aphids at the end of the trial in temporarily netted plots, with those in permanently netted and open plots.

As shown in Figure 54 b and e, the highest numbers of aphids were found in the June and September trials, reaching 93.7 and 113.30 aphids per plant respectively in netted plots. Unfortunately, the trial during May was severely damaged by hares. The lowest number of aphids observed occurred during the trials in July and August which were infested with means of 3.3 and 1 *N. ribisnigri* per plant respectively, in the open treatments.

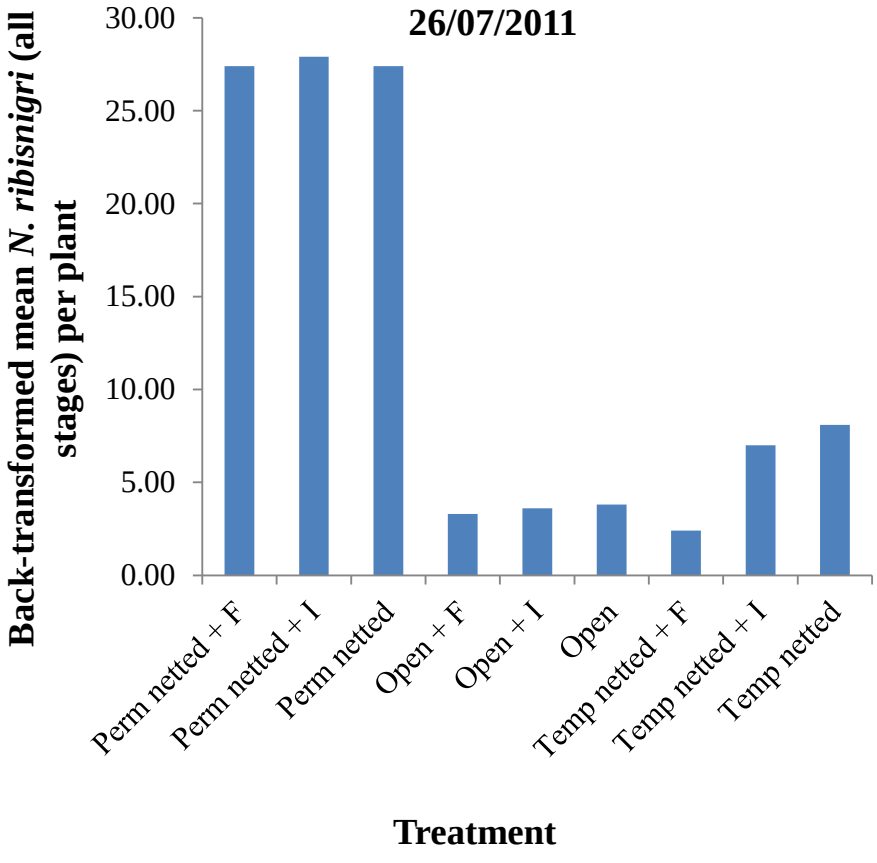
The trial in July had the lowest mean numbers of *N. ribisnigri* per plant in temporarily netted plots. The largest difference between permanently netted and temporarily netted treatments was a decrease of 25 aphids per plant between the Temp Netted+F and Perm Netted+F treatments. The monitoring plots indicated that the mid-summer crash occurred around 14 July and this period was covered by the trial in July.



a)



b)



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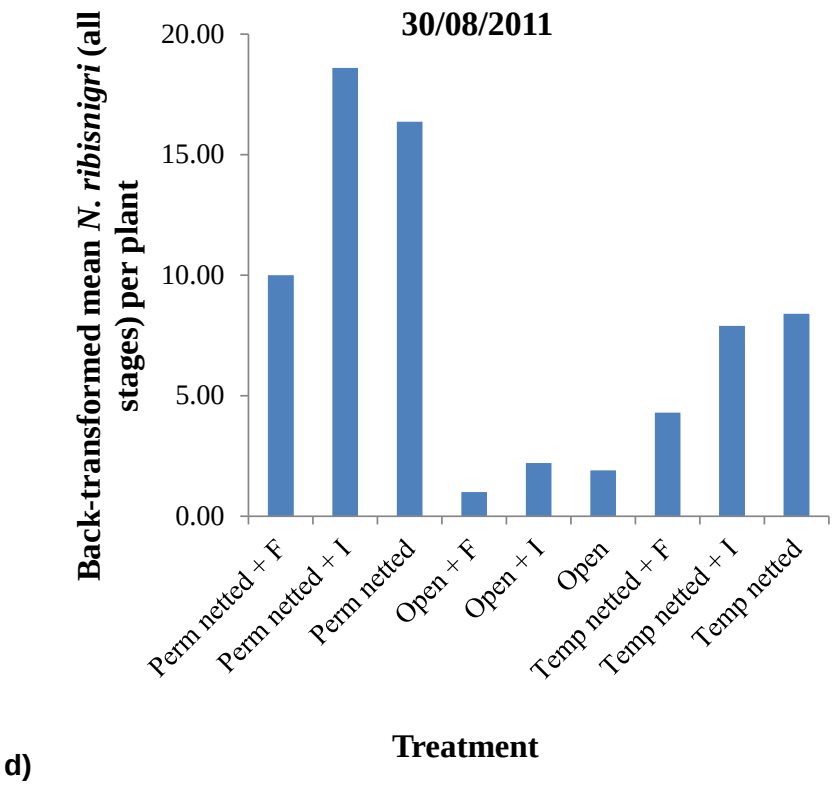
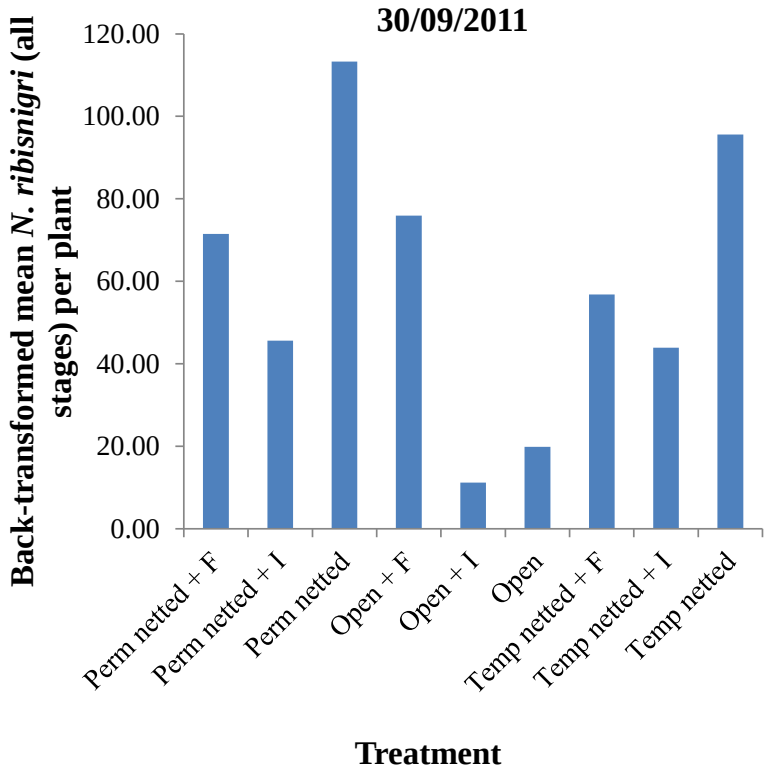


d)



e)

Figure 54 a-e. Back-transformed mean number of *N. ribisnigri* (all stages) sampled per plant at the end of each field trial on **a)** 24 May **b)** 29 June **c)** 26 July **d)** 30 August and **e)** 30 September 2011.

An ANOVA was performed on all stages of *N. ribisnigri* per plant in each field trial. As shown in Table 16 there was an overall effect of field trial month, with June and September having significantly more aphids per plant ($F(4,5)= 111.59$, $p<0.001$). The netting treatment had an effect on the number of *N. ribisnigri* with open plots having significantly less aphids per plant than both temporarily netted and permanently netted plots (Table 17) ($F(2,40)= 31.30$, $p<0.001$). There were also significantly less aphids in the temporarily netted plots compared to the permanently netted plots.

Table 16. Transformed mean of *N. ribisnigri* (all stages) per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	2.573
June	3.867
July	2.208
August	1.863
September	3.772
LSD	0.2890
d.f.	40

Table 17. Transformed mean total number of *N. ribisnigri* (all stages) per plant from the ANOVA for the effect of netting treatment.

Treatment	Mean
Permanently Netted	3.383
Temporarily Netted	2.927
Open	2.259
LSD	0.2890
d.f.	40

As shown in Figure 55, there was an interaction between field trial month and netting on the number of *N. ribisnigri* (all stages) per plant ($F(8,40)= 4.22$, $p<0.001$). Only in July and August was there a significant difference between permanently netted plots and both temporarily netted and open plots. Aphid numbers per plant from permanently netted plots were significantly different to those from open plots in July, August and September. No significant effects of fungicide or insecticide application were observed.

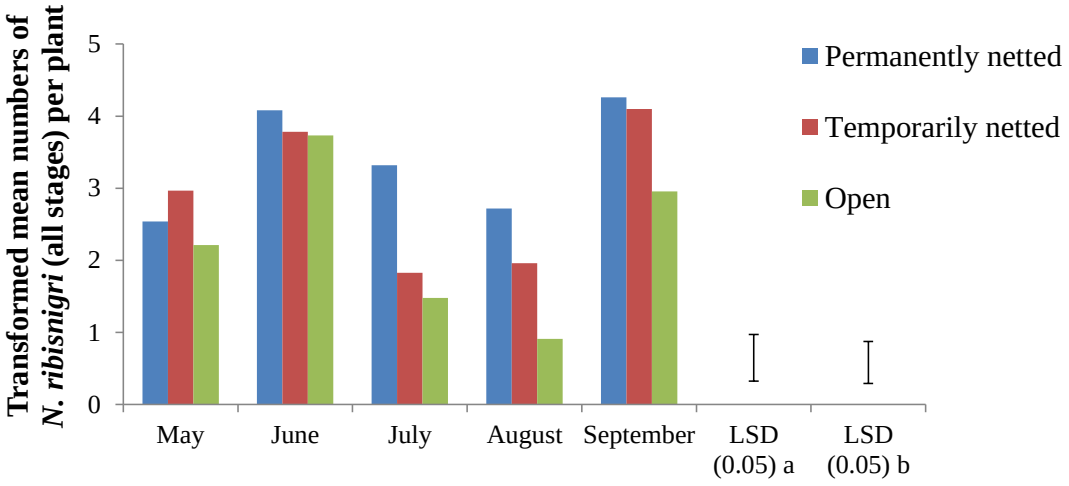


Figure 55. Transformed mean number of *N. ribisnigri* (all stages) per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

When analysing the mean number of alate *N. ribisnigri* per plant, only an effect of field trial month was observed ($F(4,5)= 30.11$, $p=0.001$) (Table 18). Alate numbers in field trial months May, July and August were significantly lower than those in June and September

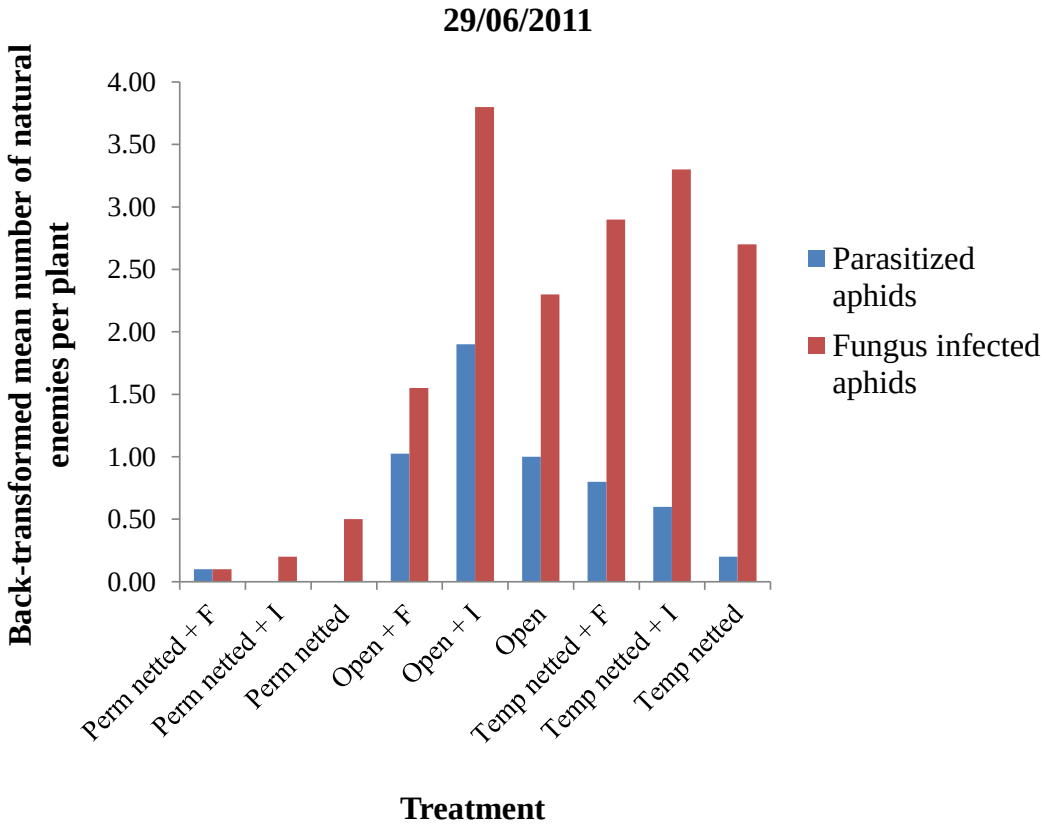
Table 18. Transformed mean number of alate *N. ribisnigri* per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0.170
June	0.501
July	0.125
August	0.193
September	0.496
LSD	0.1228
d.f.	5

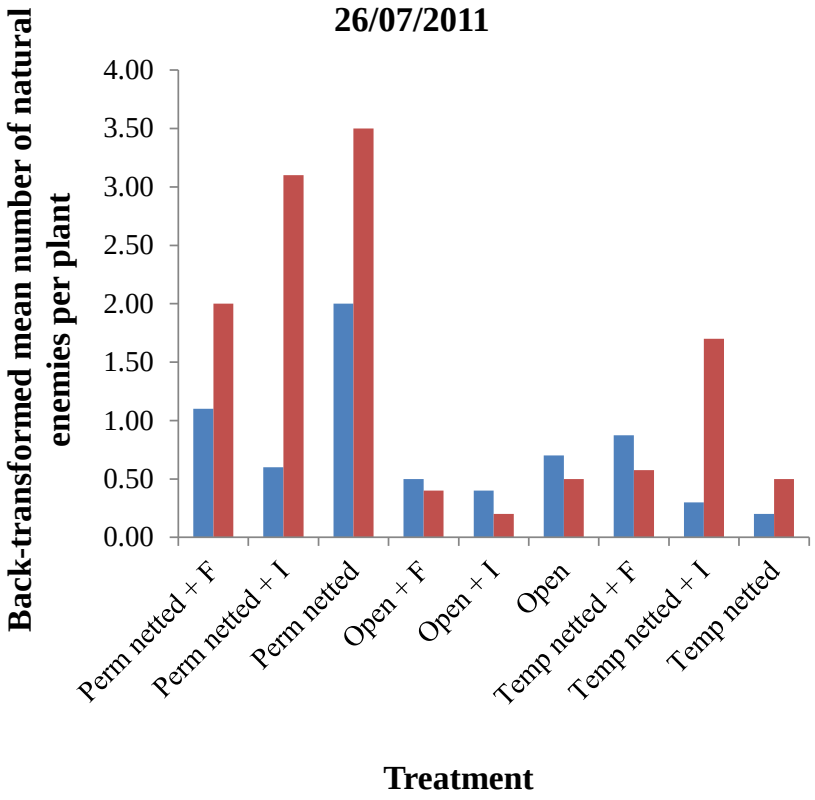
Natural enemies

When considering the numbers of natural enemies found on plants from each of the nine treatments in each field trial month (data on inoculated lettuce plants only) most 'types' of natural enemy were present in very low numbers for all the treatments (mean less than one).

Figure 56 shows the mean number of parasitized aphids per plant and aphids infected with entomopathogenic fungi per plant which were present in the June-September field trials. No natural enemies or aphids infected with entomopathogenic fungi were recorded during the May field trial. In the June trial (Figure 56a), more infected and parasitized aphids were observed in the temporarily netted and open plots compared to any other field trial month, whilst in the July trial there were more infected and parasitized aphids in the permanently netted plots. In August and September there were fewer infected and parasitized aphids and they were not present in all of the treatments.



a)



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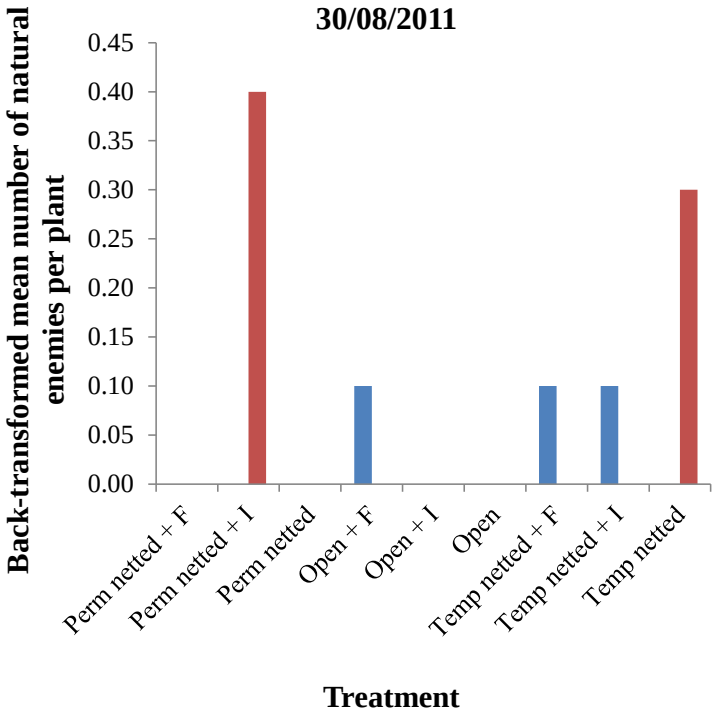

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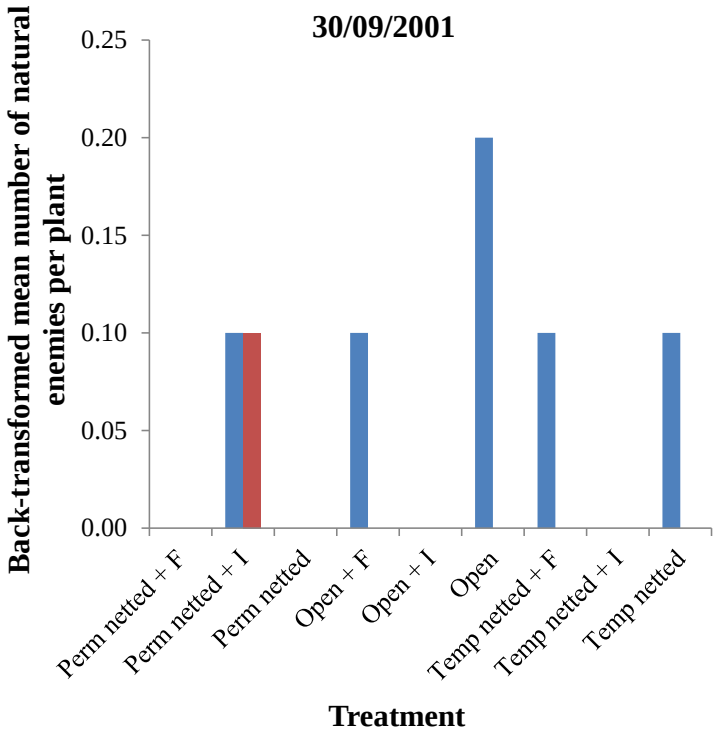

d)

Figure 56 a-d. Transformed mean number of parasitized aphids and aphids infected with entomopathogenic fungi per plant sampled from inoculated lettuce plants on **a)** 29 June **b)** 26 July **c)** 30 August and **d)** 30 September 2011.

While the numbers of natural enemies were generally low, an ANOVA was performed on the numbers of parasitized aphids, infected aphids and total natural enemies recorded in each field trial month. Other families of natural enemies recorded, including Anthocoridae, Coccinellidae, syrphid larvae and Araneae, were not analysed individually as the means were less than one due to numerous zero values. No clear relationships were observed between any of the natural enemies and numbers of *N. ribisnigri.*

Parasitized aphids

There was a significant effect of field trial month ($F(4,5)= 20.41$, $p=0.003$) on the number of parasitized aphids per plant (Table 19). Significantly more parasitized aphids were observed in June and July.

Table 19. Transformed mean number of parasitized aphids per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0
June	0.405
July	0.500
August	0.030
September	0.059
LSD	0.1890
d.f.	5

There was an interaction between field trial month and netting treatment ($F(8,40)= 9.19$, $p<0.00$)) (Figure 57). The June trial saw significantly more parasitized aphids on temporarily netted and open plots compared with the permanently netted plots, while in July there were significantly more parasitized aphids in permanently netted plots. The May, August and September trials produced fewer parasitized aphids from all netting treatments.

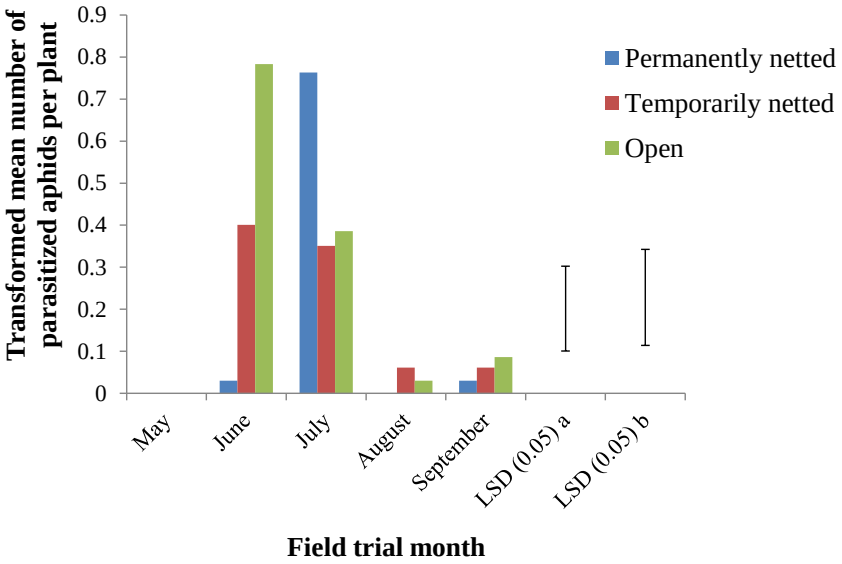

Figure 57. Transformed mean number of parasitized aphids per plant from the ANOVA for the interaction between field trial month and netting. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Entomopathogenic fungi

There was a significant effect of field trial month ($F(4,5)= 121.27$, $p<0.001$) on the number of infected aphids per plant (Table 20).

Table 20. Transformed mean number of aphids infected with entomopathogenic fungi per plant from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0
June	0.999
July	0.691
August	0.059
September	0.010
LSD	0.1531
d.f.	5

There was an interaction between field trial month and netting treatment on the number of infected aphids per plant ($F(8,40)= 13.86$, $p<0.001$) (Figure 58). The June trial produced significantly more infected aphids per plant on temporarily netted and open plots compared with the permanently netted plots, while in July there were significantly more in permanently netted plots. The May, August and September trials produced fewer infected aphids from all netting treatments. No effect of insecticide or fungicide was observed.

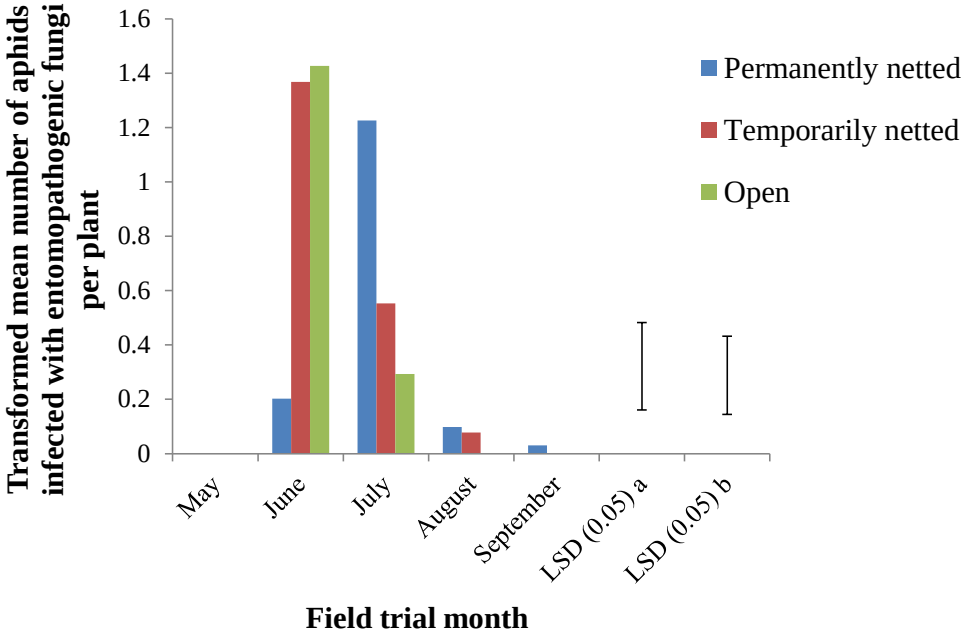

Figure 58. Transformed mean number of aphids infected with entomopathogenic fungi per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Total number of natural enemies

When the numbers of Anthocoridae, Coccinellidae, parasitized aphids and syrphid larvae were summed there was an overall individual effect of field trial month ($F(4,5)= 23.70$, $p=0.002$), netting ($F(2,40)= 8.39$, $p<0.001$) and treatment ($F(2,40)=3.66$, $p=0.035$)). Significantly more natural enemies per plant were observed in July (Table 21). In addition, significantly more natural enemies were observed in open plots compared with permanently netted and temporarily netted plots as (Table 22) and there were significantly less natural enemies in plants from insecticide-treated plots than from the control or from fungicide-treated plots (Table 23).

Table 21. Transformed mean number of natural enemies from the ANOVA for the effect of field trial month.

Field trial month	Mean
May	0.000
June	0.413
July	0.604
August	0.082
September	0.226
LSD	0.1839
d.f.	5

Table 22. Transformed mean number of natural enemies from the ANOVA for the effect of netting treatment.

Netting treatment	Mean
Permanently Netted	0.181
Temporarily netted	0.258
Open	0.357
LSD	0.0869
d.f.	40

Table 23. Transformed mean number of natural enemies from the ANOVA for the effect of treatment.

Spray treatment	Mean
Control	0.303
Fungicide	0.294
Insecticide	0.198
LSD	0.0869
d.f.	40

Interactions were observed between netting treatment and field trial month ($F(8,40)= 8.53$, $p<0.001$) and treatment and field trial month ($F(8,40)=2.87$, $p=0.013$). Figure 59 shows the interaction between field trial month and netting treatment. In all months except July the highest numbers of natural enemies per plant were found in the open plots. During July there were significantly more natural enemies in permanently netted plots. In June, there was a significant difference in the numbers of natural enemies per plant between all the netting treatments in that month.

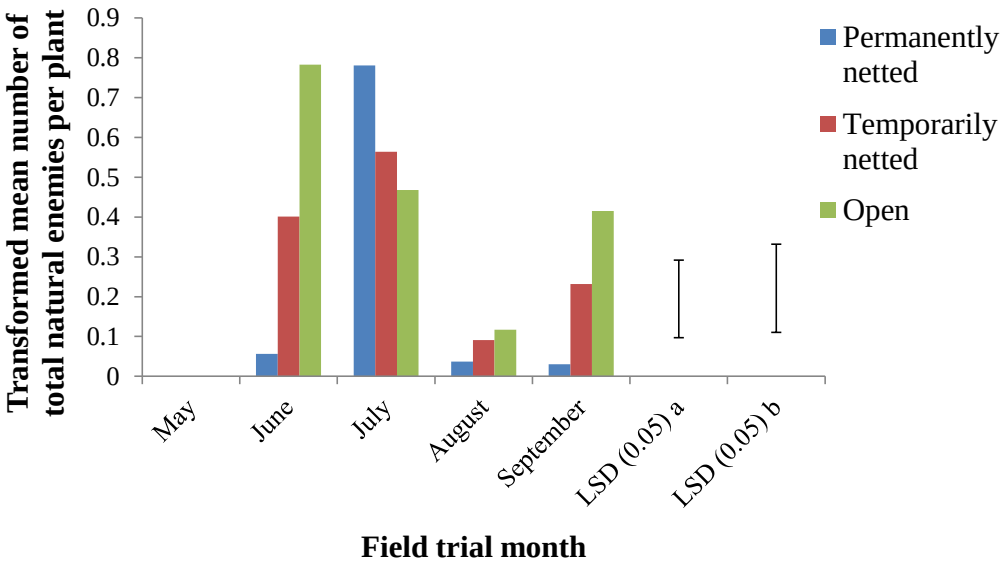

Figure 59. Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between field trial month and netting treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Figure 60 shows the interaction between field trial month and spray treatment. During July and September, the control plots contained significantly more natural enemies than those sprayed with insecticide. In June the opposite effect was observed, with the control having significantly less natural enemies than the plots treated with insecticide. Numbers of natural enemies in the control plots never differed from the plots sprayed with fungicide. Only during July was there a significant difference between fungicide and insecticide treatments.

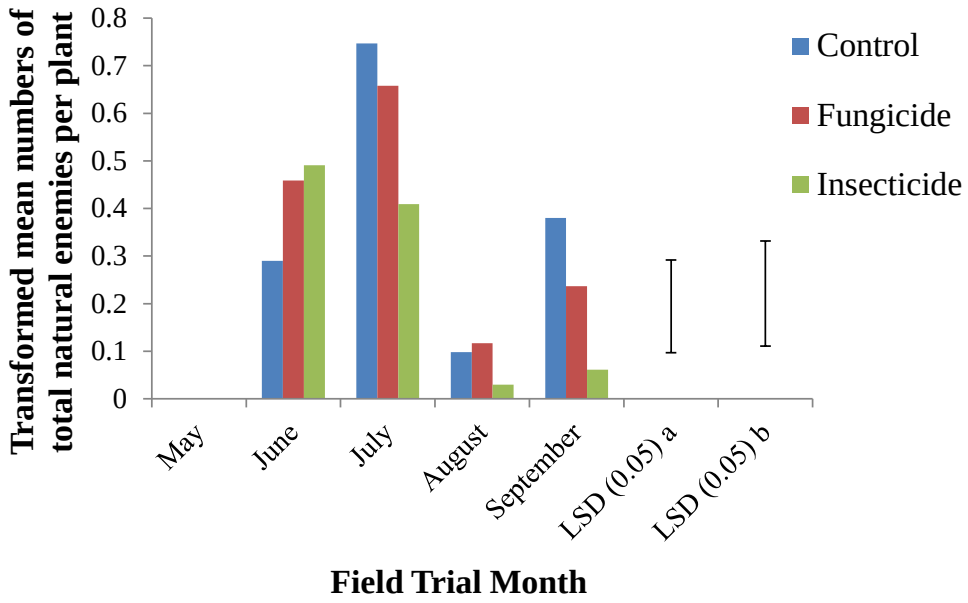

Figure 60. Transformed mean total number of natural enemies per plant from the ANOVA for the interaction between field trial month and spray treatment. LSD (a) used for comparison between treatments in the same field trial month and LSD (b) used for comparison between means in different field trial months.

Water traps

Figure 61 shows the total numbers of natural enemies in water traps located near to the field trial. Peak numbers of syrphid larvae occurred around the 29 July (21). Coccinellidae and Anthcoridae were the only other natural enemies captured, but they never exceeded more than 1 or 2 at each sampling date.

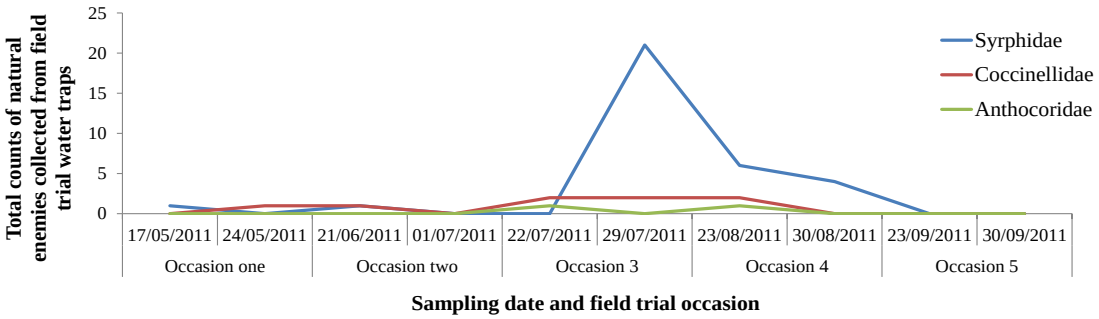

Figure 61 Total numbers of syrphid larvae, Coccinellidae and Anthocoridae captured in water traps.

When combining data from water traps close to both monitoring and field trial plots, *Syrphus ribesii* was the most common syrphid species. *Sphaerophoria scripta* and *Episyrphus balteatus* were also common. Only two coccinellid species were recorded, which were *Coccinella 7-punctata* and *Propylea 14-punctata.*

Temperature

Figure 62 shows the mean temperature recorded from the field trials in June, July, August and September for netted and open plots. The data for the May field trial month has not been included as the Tinytags© malfunctioned.

Higher temperatures were consistently recorded in netted plots compared to open plots with the largest observed difference of 1.18°C in July. The highest mean temperature of 17.27°C was recorded in netted plots during the July trial. The lowest mean temperature (15.49°C) was recorded during the September trial.

Humidity increased gradually each month in both the netted and open plots, with September having the highest humidity. Except for the June trial, humidity was higher in netted plots compared to open plots, with the largest observed difference occurring during August (3.77%).

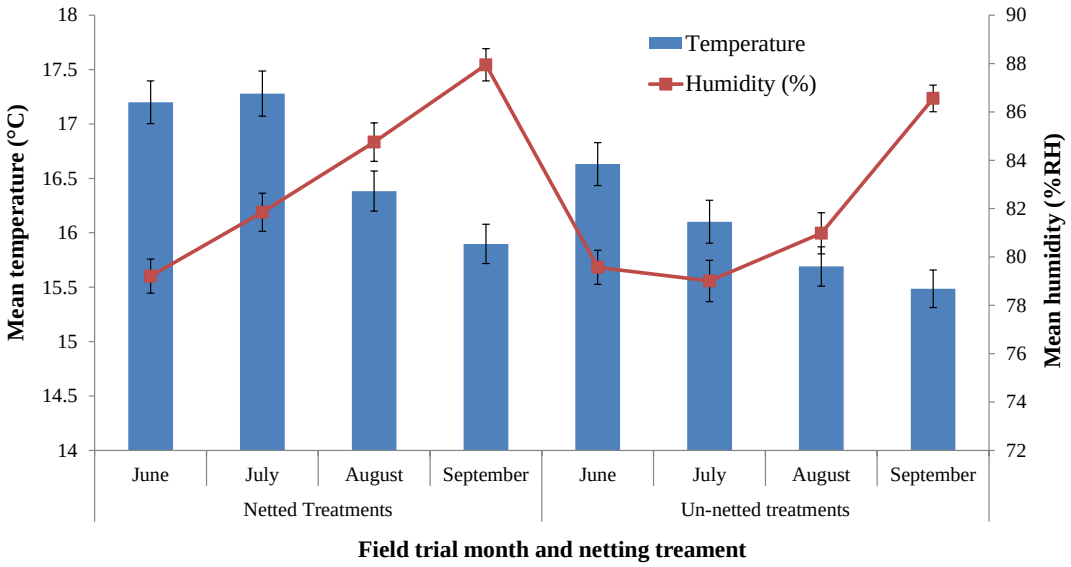

Figure 62. Mean (±SE) temperature (°C) and humidity (%RH) recorded during each field trial for open and netted plots.

Rainfall

Rainfall records were collected from the University of Warwick, Wellesbourne meteorological station. Figure 63 shows the mean rainfall and the maximum daily rainfall during each field trial. The highest mean monthly rainfall occurred in August (2.91mm). The maximum rainfall for one day during each field trial month was 10.5, 6.3, 16.7 and 7.3mm on 24 June, 16 July, 24 August and 16 September 2011 respectively.

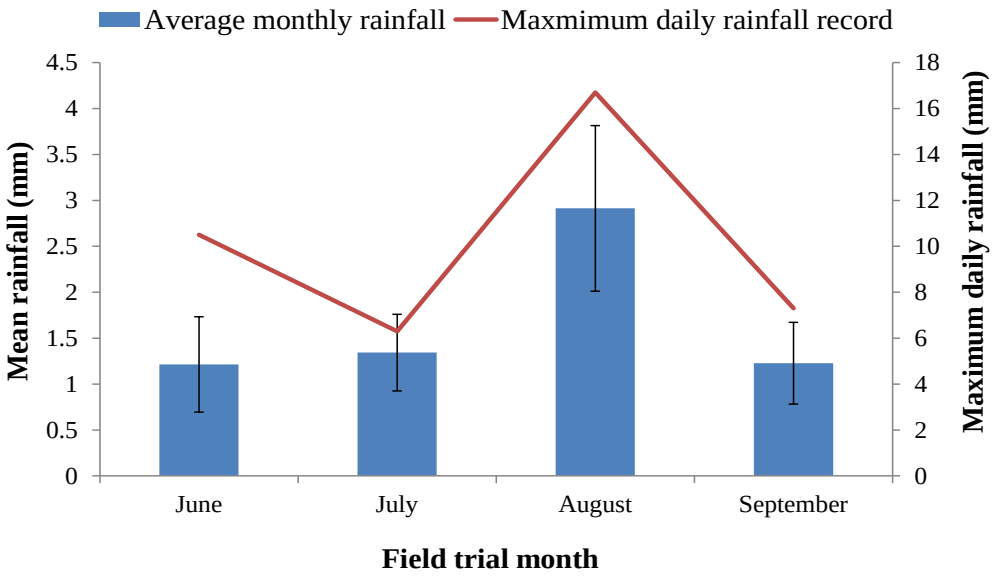

Figure 63. Mean (±SE) rainfall (mm) and the maximum daily rainfall (mm) recorded during each field trial.

Discussion

Nasonovia ribisnigri is a serious pest of lettuce, where its presence can lead to unmarketable produce and financial losses for growers. Its significance as a pest is exacerbated by its preference to feed in the centre of lettuce heads, where it is protected from the effects of certain insecticides and natural enemies. Furthermore, the effectiveness of current control measures is threatened by the continuous reduction in the number of active ingredients available for insect control, and the development of insecticide resistant and host-plant resistance-breaking biotypes. Given these pressures, the need for new methods of control has never been more important.

Prior to this study, little information was available on the biology and behaviour of *N. ribisnigri*, which is essential for the development of control measures and the effective timing of their application. Therefore, the specific objectives of this study set out to provide some of this essential knowledge to aid the development of an integrated pest management strategy and to refine some of the components within it. The objectives set out were as follows:

1) Investigate the effects of photoperiod and temperature on the development of parthenogenetic aphids.

2) Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching.

3) Investigate alternative host-plants (to lettuce) and confirm whether *N. ribisnigri* can use them as overwintering hosts.

4) Investigate the population dynamics of *N. ribisnigri* in response to natural enemies and entomopathogenic fungi.

The outcomes of this study and achievement of these objectives will now be addressed in turn:

1. Investigate the effects of photoperiod and temperature on the development of parthenogenetic summer aphids.

The effects of temperature and photoperiod on the developmental parameters of WT *N. ribisnigri* were described. This included optimum temperatures and lower and upper developmental thresholds (agreeing with those determined in a similar study by Diaz and Fereres (2005)). These were determined using the data collected in this study, which described a linear relationship between development rate and temperature, allowing for the estimation of the day-degree requirements for development from nymph to the final adult moult (which was again similar to those determined by Diaz and Fereres (2007)). Prior to this study, the method used for predicting the population development of *N. ribisnigri* in the UK was based on a day-degree model, using the Lower Developmental Threshold for *P. bursarius* (Collier, *et al.,* 1994). This forecast can now be refined, using the values determined specifically for *N. ribisnigri*, to provide a more accurate forecast of its activity.

Future work could continue to improve the accuracy of the forecast by increasing the data set used to determine the linear relationship. Investigations could also be made into the use of non-linear models which may describe the relationship better and provide a more accurate forecast. In addition, this study showed that photoperiod did not influence development, and estimates were similar between Rb and WT *N. ribisnigri,* meaning that these factors do not need to be considered in the development of the forecast.

This study raised questions about the effectiveness of aphid-resistant cultivars at lower temperatures, where the control provided by the Nr-gene appeared to fail. However, as the ambient temperature fluctuates in the field, and is likely to be above 15ºC for at least some of the period during which lettuce crops are grown, resistance will still be provided against WT *N. ribisnigri*. As a breakdown in resistance was not observed in the field prior to the 'arrival' of the new resistance-breaking biotype, it seems likely that the temperature sensitivity of the Nr-gene is unlikely to threaten the control of WT *N. ribisnigri*. Despite this,

the effects of temperature, particularly fluctuating temperatures, on the performance of new resistant cultivars should be analysed to clarify this.

2. *Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching*

A phenological forecast of egg development and hatching in the spring would provide important information on the activity of *N. ribisnigri* and indicate when it is likely to migrate to lettuce crops. This information would support growers in deciding when to apply control measures including, possibly, the use of seed treatments or resistant cultivars, depending on how far in advance *N. ribisnigri* activity can be predicted. The forecast would also be useful if effective control of *N. ribisnigri* could be achieved by controlling *N. ribisnigri* fundatrices and offspring on the winter host; though whether this would be worthwhile requires further research.

Unfortunately, the data collected during this study were insufficient to support the development of a forecast for egg hatch. This study has however provided methods to initiate sexual morph production and produce eggs in the laboratory, which were unavailable prior to this study. These methods can now be used in the future to determine the relationship between egg development and temperature, which can be used in turn to estimate the LDT and develop a forecast. Monitoring of the winter lifecycle in this study also provided basic information on the overwintering biology of *N. ribisnigri*, confirming the timings of key events such as migration, egg laying and egg hatch.

3. *Investigate alternative host-plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts.*

Various weeds can provide alternative sources of refuge for pests. The data collected in this study confirmed that there are several alternative hosts that WT and Rb *N. ribisnigri* can utilise in the summer. A selection of these host-plants also supported overwintering parthenogenetic *N. ribisnigri* between November and March.

This study confirmed that *N. ribisnigri* may overwinter as nymphs/adults, an attribute which could have implications for the timing of their spring migration, as aphids overwintering in the active stages continue development as soon as temperatures exceed the Lower Developmental Threshold. It is likely that aphids would migrate to lettuce crops ‘sooner’ and develop larger summer populations than those overwintering as eggs. Removal of potential winter host-plants would remove possible refuges for *N. ribisnigri* but consideration must be

given to their ‘other’ roles, for example, as a nectar source for natural enemies during the summer.

Finally, this study confirmed that temperature and host-plant location were the key factors determining aphid survival during the winter, with a combination of sheltered plants and mild winters resulting in enhanced survival and potentially larger spring populations.

4. *Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi*

The monitoring of *N. ribisnigri* populations during 2010 and 2011 recorded the occurrence of the mid-summer crash, which has been described for various aphid species. In this study, in both years, high natural enemy numbers were observed prior to the decline, suggesting that this was one of the most important regulating factors for *N. ribisnigri* populations. While entomopathogenic fungi, syrphid larvae and parasitoids were present in the highest numbers during these trials, future work should focus on determining the effects of individual predator species.

Emigration was also determined to be an important factor regulating aphid populations as the percentage of alate aphids was observed to increase prior to the mid-summer crash in both field trial years. As this study only analysed the potential for emigration to occur, future work should implement methods to monitor ‘real time’ emigration to confirm its role in the mid-summer crash.

Unfortunately, given the resource constraints of the project, the species of parasitoids and fungus specifically affecting *N. ribisnigri* were not recorded. Future work should aim to identify these natural enemies, as effective biological control agents might be recognized, which could be introduced or enhanced as part of an IPM strategy.

Like various other studies, this study has failed to identify a single factor which resulted in the mid-summer crash, but it has identified significant factors involved. Due to its complex nature it is uncertain whether the mid-summer crash will ever be understood fully, but achieving this would allow researchers to predict when aphids will decline naturally, therefore avoiding unnecessary insecticide applications. Idealistically, identifying the factors responsible could facilitate the re-creation of these conditions in the field to induce an aphid decline when required.

Life cycle

Until now, the lifecycle of *N. ribisnigri* has always been described generally, with details of its primary and secondary host-plants and the timing of its migration between them. However, using the information collected in this study a more detailed life-cycle can be provided:

Nasonovia ribisnigri reproduces asexually throughout the summer months on *Lactuca* spp. and other broad leave weeds including *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis.* Development occurs at temperatures above the estimated Lower Developmental Threshold of 4.6°C, where development from nymph to the final adult moult takes approximately 121 day-degrees with temperatures exceeding 26°C becoming deleterious to development.

As temperature and day length decrease in autumn, alate males are produced initially, followed by alate gynoparae (observed to be produced together at 12°C 13L:11D), which migrate around mid-October to the winter host (*Ribes* species). The gynoparae then produce female oviparae which lay eggs after mating with males found on the winter host.
Once the eggs have been deposited, usually in the angle between a stem and a bud, they enter a state of diapause, which terminates naturally in the field between late-January and early-February. However, the preponderance of temperatures below the Lower Developmental Threshold for egg development delays hatching until late February.

Once the eggs have hatched, the fundatrices develop and begin reproduction, feeding from the nutrient rich buds of the primary host-plant. Once the offspring develop into alate adults, migration to the secondary host occurs and the primary host-plant is no longer accepted as a suitable host for colonisation (no nymphs are produced).

It has also been confirmed that *N. ribisnigri* can overwinter as active aphids (adults and nymphs) in the Midlands, on 'alternative' host-plants to lettuce, particularly *V. arvensis*.

Conclusions

1. ***Investigate the effects of photoperiod and temperature on the development of parthenogenetic aphids.***

- This study confirms that temperature is a significant factor affecting the developmental time, developmental rate, intrinsic rate of increase (r_m), fecundity and the propensity to become alate of both WT and Rb *N. ribisnigri*.

- Between 5.5 and 26.4°C, development time decreased with increasing temperature and only at 5.5°C did variation in development times occur with the control (WT4850a on cv.Saladin) being significantly different to Rb biotypes on cv. Saladin and Rb cv. Rotary.
- At lower temperatures, some aphids from the WT4850a biotype survived on Nr-gene cultivars, although their longevity, fecundity and development time were compromised.
- The r_m increased with increasing temperature up to 26.4°C, where the r_m decreased or did not increase further.
- The WT4850a and RbKent biotypes had similar development times and r_m values.
- The control treatment exhibited poor survival when compared to the Rb biotypes, demonstrating poor pre-reproductive survival and continued poor survival during the reproductive phase, particularly at 5.5 and 26.4°C. The Rb biotypes exhibited comparable poor longevity only at 5.5°C. The optimum temperature for longevity was 15°C.
- The control treatment exhibited consistently poor achieved fecundity. However, RbKent biotypes had significantly higher fecundity than the control between 12.5 and 21.4°C. Fecundity was reduced for the Rb biotypes at 5.5 and 26.4 °C. Overall, the optimum temperature for reproduction was 12°C.
- Collectively, these responses suggest that 5.5 and 26.4°C are close to the UDT and Lower Developmental Threshold, where aphid performance is negatively affected, meaning that the temperature range leading to optimum aphid performance is between 12.5-21.4°C.
- More alates were produced at lower temperatures and the RbKent biotype appeared to produce more alates compared to the WT45850a biotype, but not the WTKent10Pop biotype.
- Photoperiod did not influence development time or the r_m value. However, inter-clonal variation was observed, particularly between the control treatment and RbUK631 and RbKent09Pop biotypes. This occurred at each rearing regime for development times, but only at 20°C 14L:10D and 20°C 16L:8D for the r_m value.
- Estimates of the LDT and DD suggest that the WT4850a *N. ribisnigri* biotype has a Lower Developmental Threshold of 4.6°C and requires 121.1 day-degrees to reach adulthood.

2. ***Investigate the conditions required to stimulate development of sexual morphs, egg production, termination of egg diapause and egg hatching.***

- Diapause terminates in the field during mid-late January but temperatures below the Lower Developmental Threshold prolonged post-diapause development and hatching until early February.
- 12°C 13L:11D can induce males and gynoparae of *N. ribisnigri* which produce eggs after approximately 49 days.
- It was estimated that post-diapause development takes just under 50DD using a Lower Developmental Threshold of 4.6°C at 16°C.
- *Nasonovia ribisnigri* fundatrices can survive and reproduce on lettuce but their offspring can no longer colonise *R. nigrum.* Parthenogenetic summer alates and apterous *N. ribisnigri* cannot colonise blackcurrant.

3. ***Investigate alternative host-plants (to lettuce) and confirm whether N. ribisnigri can use them as overwintering hosts.***

- This study confirmed that eight species of plant (mainly wild species) are suitable hosts for both UK WT and Rb *N. ribisnigri*. This includes *C. intybus, C. capillaris, L. communis, H. aurantiacum, H. pilosella, V. arvensis, V. spicata* and *V. officinalis*.
- The performance of WT and Rb *N. ribisnigri* biotypes was similar regardless of the host-plant.
- *Nasonovia ribisnigri* (WT4850a) survived and reproduced during a winter in central England. The results indicate that abundance will be determined by the suitability of the host-plant and the severity of the winter.

4. ***Investigate the population dynamics of N. ribisnigri in response to natural enemies and entomopathogenic fungi.***

- The mid-summer crash was observed during both trial years occurring between the 5-12 July 2010 and 14-29 July 2011.
- Netting treatments reduced the number of natural enemies in plots but did not completely exclude them. Netting treatments also influenced the microclimate and could have resulted in more aphids infected with entomopathogenic fungi during 2011.

- Fungicide and/or insecticide treatments did not reduce the numbers of natural enemies or aphids infected with entomopathogenic fungi during the 2010 trial. However, during 2011 the insecticide treatment was observed to reduce natural enemies in each trial month except June.
- Data on temperature and rainfall could not explain the decline in aphid numbers in 2010 or 2011. However, the intensity and duration of the wind and rain could not be excluded as a possible contributing factor.
- While alate numbers were low prior to the mid-summer crash, their increased percentage as part of the total population suggested emigration could be a significant contributing factor to the mid-summer crash, but further work is required to determine to what extent. During 2011, emigration as a result of poor plant quality was considered unlikely as the aphid decline still occurred in monitoring plots which contained lettuce plants of various ages.
- In both years natural enemies increased prior to the mid-summer crash indicating they play a role in suppressing aphid populations. Further work is required to determine the level of suppression natural enemies can achieve.
- Due to the variation in the species of natural enemies present each year, this study suggests that it is a community of natural enemies which contribute to the mid-summer crash, rather than the activity of one or two key natural enemies.

Knowledge and Technology Transfer

British Leafy Salads Association Conference- 14th November 2012, Kingsgate Conference Centre in Peterborough. Oral presentation.

HDC 3rd Studentship Conference- 4-5th July 2012. Norton Park Hotel, Winchester - Oral presentation

Aphid Special Interest Group- Royal Entomological Society- 18th April 2012, The James Hutton Institute, Dundee. Oral presentation.

School of Life Sciences Student Symposium- 26-28th March 2012. Oral presentation.

IOBC Working Group: Integrated Protection of Field Vegetables conference- 25-28 September 2011. Southern Sweden - Oral presentation

HDC 2nd Studentship Conference- 5-6th July 2011. East Malling Research, Kent - Oral presentation

HDC Open Day - Protecting your Field Veg Crop, 30 June 2011. STC, Yorkshire - Oral presentation

School of Life Sciences Student Symposium- 26-27th May 2010. Presented poster

Royal Entomological Society, Postgraduate Forum Meeting, 2nd-3rd February. The Royal Hotel, Hull- Poster presentation

British Leafy Salads Association Meeting- 5th October. Farmers Club, Whitehall – Oral presentation

1st Annual HDC Studentship Conference - 23-34th February 2010, Lincolnshire. Poster presentation

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Glossary

Table A1 Abbreviations used in this study and their meaning.

Abbreviation	Meaning
CE	Controlled Environment
CL	Confidence Limit
Cv. or Cvs.	Cultivar or Cultivars
DD	Day-degree
Exp.	Experiment
IPM	Integrated Pest Management
IRU	Insect Rearing Unit
LDT	Lower Developmental Threshold
LOG	Logarithm
LSD	Least Significant difference
n	number
Rb	Resistance-breaking
RH	Relative Humidity
r_m	Intrinsic rate of increase
SE	Standard Error
Sp. or Sp.	Species
T	Total
Tn	Possible observations
Treat.	Treatment
UDT	Upper Developmental Threshold
WT	Wild-type

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