FINAL REPORT

To:
Horticultural Development Council
Bradbourne House
Tithe Barn
East Malling
Kent, ME19 6DZ

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

FV 279

Rosemary Collier
WarwickHRI, University of Warwick,
Wellesbourne, Warwick, CV35 9EF

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Commercial - In Confidence

Grower Summary

FV 279

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

Final Report 2007

Project Title	Biology and control of currant-lettuce aphid (*Nasonovia ribisnigri*)
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Signed on behalf of: Warwick HRI

Signature: ……………………………………………………………

Professor Simon Bright
Director and Head of Department

Date: ……………………………..………………

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

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Science section

FV 279:

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

Headline

- Progress has been made in understanding various aspects of the life cycle and survival of the currant-lettuce aphid. This will form the basis of developing potential control measures.
- At present there are no changes to grower practice.

Background and expected deliverables

UK lettuce crops are infested commonly by four species of aphid. Of these, the currant-lettuce aphid, *Nasonovia ribisnigri*, is of greatest economic importance, being difficult to control, particularly on crops that are close to maturity. Imidacloprid seed treatment (Gaucho) is effective against the currant-lettuce aphid, although it may not persist for the full life of the crop. There are sometimes concerns about the crop safety of this treatment. Whilst they may be effective early on, insecticides applied as foliar sprays to hearted crops often have relatively little effect because the aphids are hidden within the foliage.

There is considerable annual (and regional) variation in the abundance of currant-lettuce aphid and resultant crop damage and it is likely that an early warning of the timing of infestation and likely abundance would facilitate a more effective control strategy. Although the nature of the life-cycle of the currant-lettuce aphid is known, almost nothing has been published on the developmental biology of the currant-lettuce aphid and on its relationship with temperature and daylength. This is the type of information that is necessary for a clearer understanding of the life-cycle and to provide the basis for development of a robust forecast and an effective control strategy. The aim of this project is to clarify and ‘quantify’ key aspects of the life cycle of the currant-lettuce aphid in the UK.

Once the currant-lettuce aphid has infested lettuce crops in early summer, aphid numbers increase rapidly for several weeks. In most years, currant-lettuce aphid populations (and populations of other species of aphid) then undergo a mid-season ‘crash’. The reasons for this decline in numbers are not clearly understood. In most years, numbers of the currant-lettuce aphid increase subsequently in late summer and it is generally the dominant pest aphid on lettuce at this time of year. This project will attempt to determine the role of natural enemies and fungal disease in regulating currant-lettuce aphid populations. Whilst we are unlikely to develop a full explanation for the crash in this time-frame, the project should identify areas to focus on in future research.

The project should provide growers with the following:

- Quantification of the life-cycle of the currant-lettuce aphid and, in particular, its overwintering biology.
- A forecast of the timing of key events in the life-cycle/population development of the currant-lettuce aphid.
- Information on the currant-lettuce aphid biology (e.g. the mid-summer crash) that can be used to improve the control strategy for the currant-lettuce aphid.

Summary of the project and main conclusions

The overall aim of this project is to quantify all aspects of the life-cycle of the currant-lettuce aphid and the relationship between the currant-lettuce aphid and its natural enemies on lettuce. The objectives of the project are:

1. To quantify the rate of currant-lettuce aphid reproduction on lettuce at different constant temperatures.
2. To record currant-lettuce aphid and aphid predator numbers and apparent causes of aphid death on lettuce throughout the growing season.
3. To quantify the temperature and / or photoperiod requirements for egg production and egg hatch.
4. To determine whether the currant-lettuce aphid is able to overwinter in its parthenogenetic form on secondary hosts and to identify which secondary host plants might serve as good overwintering sites.

1. To quantify the rate of currant-lettuce aphid reproduction on lettuce at different constant temperatures

This information is necessary for a clearer understanding of the life-cycle and to provide the basis for development of a robust forecast and an effective control strategy. It was obtained through a constant temperature experiment in the laboratory at 16 hours light and 8 hours dark (16L: 8D) in every 24 hours, which is approximately equivalent to the photoperiod in the UK in early to mid-summer. In an experiment to determine the efficacy of reported methods, constant temperature experiments were performed at a single temperature (20°C). A winged female aphid was placed on each of twelve 3-week old lettuce (cv Iceberg) seedlings in pots, allowed to reproduce, and then all but one nymph per plant was removed after 24 hours. Daily observations then ensued and a record was made of mortality, moulting, and the number of new-born aphids produced (new-born aphids were removed from the plants immediately). One of the 12 aphids died before producing any offspring. This aphid died two days after inoculation.

Finding - Developmental time (time from birth of nymphs to adult) was 8 days at 20°C, and total offspring per aphid was 35.

2. To record currant-lettuce aphid and aphid predator numbers and apparent causes of aphid death on lettuce throughout the growing season.

The aim of this objective was to record aphid numbers (including cadavers and apparent cause of death) and aphid natural enemy numbers on lettuce throughout the growing season, and to subsequently analyze the data for correlation and possible causation regarding changes in aphid numbers. In summer 2006, lettuce was planted out into plots in the field and was either (1) inoculated with currant-lettuce aphids and covered with insect-proof netting, or (2) inoculated with currant-lettuce aphids and left uncovered, or (3) not inoculated and not covered.

The first planting in 2006 took place in the week beginning Monday 19 June. However, inoculation with wingless aphids did not lead to their establishment on the lettuce plants. By the time of the second planting, in the week beginning Monday 24 July, it was suspected that the seed used in plantings 1 and 2 may have been treated with insecticide. Some plants from the second planting were therefore inoculated with aphids in the laboratory and colonization was compared with that on other lettuce seedlings. Once again, aphid populations did not establish on these plants, neither in the field nor in the laboratory. They did establish on the other lettuce plants, leading to the conclusion that the seed used for field trials on 19 June and 24 July was insecticide-treated.

The third planting (using different seed) took place in the week beginning 21 August 2006. Aphids did establish, but this planting was too late in the year to provide any usable information regarding the aphid crash, which typically occurs in late July or early August.

Finding – What it did show was that aphid numbers rose strongly in the covered environment between mid-September and mid-October, and that this rise was not matched in the lettuce plots that were left uncovered. The presumption in the design of this experiment is that by covering the lettuce, aphid natural enemies are excluded, and hence if higher numbers of aphids are seen on covered lettuce, this is due to lack of predation or parasitism. There are other effects of covering a crop with netting that also need consideration, however. Perhaps the most important is that winged aphids are forced to remain in the plot, probably adding significantly to the numbers compared with an open plot. Also, the micro-climate within a covered plot may at times be more favourable to the aphids. The impact of heavy rain, for example, is dissipated at the surface of the net and hence aphids are not knocked off the plants as they might otherwise be. In a similar fashion, strong winds have less of an impact in a covered plot.

There was a decline in the numbers of aphids in all three plots at the end of the study period, from mid- to late October. The autumn 'peak' in aphid numbers occurred on 10 October in the covered plot and on 24 October in the open plots.

3. <u>To quantify the temperature and / or photoperiod requirements for egg production and egg hatch</u>

In late autumn, shorter days and lower temperatures lead to the production of winged 'migrants' and both male and female aphids are produced. These migrants leave the lettuce and settle on currant (*Ribes*) species such as blackcurrant, redcurrant and gooseberry. When they have settled on the currant plants, the females produce wingless females, which mate with the males and lay eggs. The eggs are deposited most frequently in the angle between the stem and a bud. The overwintering egg stage is an adaptation that allows the currant-lettuce aphid to withstand the cold, wet weather of winter. The eggs spend the winter in diapause (a form of hibernation), during which they will not hatch, even if exposed to relatively high temperatures. Once diapause ends, spring temperatures determine when the eggs will hatch, which is usually in March or April. The newly-hatched aphids complete one or two generations on the currant plant before winged forms migrate to lettuce in May or June.

One of the first aims of this project was to develop a technique for obtaining overwintering eggs so that their development could be studied in more detail. In August 2006, lettuce plants infested with currant-lettuce aphid were enclosed in an aphid-proof cage with *Ribes* bushes, most of which were blackcurrant, but also some gooseberry. The currant bushes were monitored weekly for aphids.

Finding - Currant-lettuce aphids were first seen on the bushes on 17 October, when both winged and wingless forms were observed. Eggs were first observed approximately 4 weeks later, on 15 November. By this time, although not counted and recorded, the number of wingless aphids per currant bush was as high as 50, although numbers per plant were very variable, with a small number of plants having < 5 aphids per plant. The variation in the number of eggs per plant was more extreme, with > 100 eggs on some plants and none on others.

The migration of the currant-lettuce aphid to its winter host observed in this experiment occurred in October / November and this coincides exactly with the literature on this subject. Although aphids were not counted on the currant after 27 October, they were still monitored, as already reported. It was considered that a good indicator of migration to the currant was the number of winged aphids attached to the roof of the cage on a still, sunny day: By mid-November this rough guide to migration was showing that the migration 'peak' had passed. By 7 December no winged aphids had been seen attached to the roof of the cage for one week. By this time also, 33 of the 55 currant bushes had no viable foliage remaining.

Although the winter migration event was complete before December, wingless aphids still populated the lettuce plants in the cage at this time. The population of currant-lettuce aphid remained in the deteriorating lettuce, which was left in the ground for the purpose of monitoring after the currant plants and the cage were removed in early December. New host plants were planted or placed around the original lettuce through December (lettuce) and January (lettuce and potential weed hosts), to provide the aphids with alternative hosts. Predictably, the lettuce plants did not prosper; they either died completely or remained alive with a tiny ‘stub’ of leaves. Although the weed hosts remained alive, none provided the enclosed head typical of lettuce and hence left any colonizing aphids open to the elements. In spite of this, aphids were still present, albeit at very low numbers, until 27 February, after which none were seen. It is also noteworthy that the aphids appeared to be reproducing right up to their demise in February.

The eggs overwintering on currant bushes at Wellesbourne were subjected to different regimes of daylength and temperature to discover the conditions likely to induce diapause completion and egg-hatch in the spring.

Finding - In a preliminary trial, a regime of 16L: 8D and constant 16°C induced egg hatch in early February 2007 in the currant-lettuce aphid eggs laid in October and November 2006.

4. To determine whether the currant-lettuce aphid is able to overwinter in its parthenogenetic form on secondary hosts and to identify which secondary host plants might serve as good overwintering sites

In warmer climes (e.g. Australia), it appears that female currant-lettuce aphids continue to produce nymphs throughout the year, rather than producing sexual forms in autumn that lay eggs. It is thought that the same thing happens in southern Britain during mild winters when, in the absence of lettuce crops, the aphid reportedly makes use of weed species such as chicory, hawkweed, and speedwell. Potential alternative hosts are being assessed for their suitability as hosts for currant-lettuce aphid at a range of summer and winter temperatures. Preliminary tests in which weeds and lettuce were made available to the aphid in an enclosed container showed that after 4 weeks at 20°C the currant-lettuce aphid numbers on speedwell (190 per plant) were equivalent to those on lettuce (192 per plant) but that numbers on chicory were lower (123 per plant).

Finding -The most important conclusion to draw from the above is that both weed species (speedwell and chicory) are potential hosts and their suitability for overwintering necessitates field trials at appropriate temperatures.

Financial benefits

- There are no direct financial benefits from this project at this stage.

- Lettuce crops occupy over 5,000 ha of land each year and are worth currently about £70M per annum (Defra Basic Horticultural Statistics 2005). Without adequate insecticidal control, a conservative estimate is that about 10% of crops would be rendered unmarketable by aphids.

- The cost-benefit relationship could be in the order of £21K/£7M, which equates to about 1:350, or 0.3% of the estimated benefits in a single year.

Action points for growers

- At present there are no changes to grower practice.

Science Section

Introduction

This work was undertaken during the first year of a PhD studentship and the following introduction is a summary of the Literature Review done towards the beginning of the PhD project.

Aphids are major pests of outdoor lettuce crops, and in the UK, the currant-lettuce aphid, *Nasonovia ribisnigri*, is one of three important species that colonize the foliage of lettuce (Collier *et al.,* 1999; Collier & Harrington, 2001), the others being the potato aphid, *Macrosiphum euphorbiae*, and the peach-potato aphid, *Myzus persicae*. Unlike these other pests of crop plants, *N. ribisnigri* is specific to lettuce. It prefers the foliage of the lettuce heart and because of this it is often unaffected by treatment with insecticide sprays, which do not penetrate to the centre of the lettuce head. Additionally, some clones of *N. ribisnigri* are resistant to insecticides (Denholm et al., 2004). The problem most commonly reported by growers is infestations of tens to low hundreds of aphids in healthy-looking mature lettuce heads. This is a problem for the grower because lettuce heads are rejected by supermarket buyers if more than a handful of aphids are present per plant.

Aphids either complete a 'whole' life-cycle that includes sexual reproduction leading to overwintering as an egg or, if conditions are favourable for continued feeding, an 'incomplete' life-cycle that involves reproduction without sex. These life-cycles are termed *holocyclic* and *anholocyclic*, respectively. According to Blackman & Eastop (2000), *N. ribisnigri* follows an holocyclic life-cycle.

In holocyclic life-cycles, aphids either remain on the same plant or group of plants for the entirety of their life-cycle (i.e. they are monoecious), or the sexual forms of the aphid reproduce on a different plant / group of plants to the non-sexual forms (heteroecious).This attribute is set by aphid physiology and hence is not influenced by external factors such as temperature. *Nasonovia ribisnigri* is heteroecious: sexual reproduction takes place on currant (*Ribes*) species and non-sexual reproduction on lettuce (and related plants).

In heteroecious aphids like *N. ribisnigri*, the spring migration from the sexual (primary) host to the secondary host, although influenced by crowding, host quality and temperature, is predominantly triggered by the increase in day length (Dixon, 1998). *Nasonovia ribisnigri* in the UK typically migrates to lettuce and other Asteraceae in May or June. Once settled on the secondary host, the winged colonizers produce wingless morphs and these morphs continue viviparous parthenogenesis, producing either wingless or winged morphs themselves. At the end of the summer these same morphs begin to produce sexual morphs that will return to the primary host, and this switch is triggered by decreasing daylength. High temperatures can prevent this switch, however. The *N. ribisnigri* sexual morphs (winged males and winged females (gynoparae)) fly to *Ribes* species, whereupon the gynoparae produce mating, egg-laying females (oviparae) that are invariably wingless. These oviparae mate with the males and the oviparae lay overwintering eggs. The following spring, a highly fecund, wingless female (a fundatrix) emerges from each egg and parthenogenetically reproduces on the *Ribes*.

In addition to temperature and photoperiod effects on the timing of the production of sexual forms, each aphid species has a limited range of temperature over which it can reproduce (Dixon, 1998). Diaz and Fereres (2005) performed laboratory experiments at constant temperatures with parthenogenetic *N. ribisnigri* to establish life table statistics and found that most optima were achieved at 20°C or 24°C. Development time was shortest at 24°C (6.5 and 7.3 days for apterae (wingless adult aphids) and alatae (winged adult aphids), respectively, compared with 8.0 and 9.2 days at 20°C) and hence the potential for population increase was highest at this temperature. However, fecundity was highest at 20°C (29.5 and 23.6 nymphs per female for apterae and alatae, respectively, compared with 22.1 and 19.7 at 24°C). Survivorship was also marginally higher at 20°C (60 of 60 nymphs survived to adult compared with 59 of 60 at 24°C), and deteriorated significantly at temperatures above 24°C. They also found that less alatae were produced at temperatures of 16°C and below (< 7% alatae compared with > 50% at 20°C and 24°C).

Aphid populations often decline precipitously or ‘crash’ in mid to late summer before recovering slightly in the autumn (Hulle et al., 1999). Data from lettuce crops indicate that “within a locality, populations of all aphid species crash at the same time” irrespective of the physiological age of the host plant (Collier & Harrington, 2001), which appears to suggest a causal factor external to both the plants and the aphids. *Nasonovia ribisnigri* seems better able to recover from the ‘crash’ than other species: of three species infesting lettuce it dominated the autumn peak (Collier and Harrington, 2001). Elevated mortality caused by natural enemies is thought to at least contribute to, and perhaps cause, the aphid crash (Karley et al., 2004).

Nasonovia ribisnigri, like other aphids, is prey to a number of specialist and generalist predators, including coccinellid adults, coccinellid larvae, chrysopid larvae, Heteroptera and aphidophagous spiders. It is only recently that parasitoids that specifically attack *N. ribisnigri* have been identified. *Praon volucre* is one (Anon, 2005), and *Aphidius hieraciorum* is another. *Aphidius hieraciorum* was studied in greater detail and it was found that it prefers to oviposit in the first and second aphid instars. Parasitized aphids can be recognized as swollen brown mummies that stick to the leaf.

The most common causative fungi in natural epizootics of Aphididae are two Zygomycetes in the order Entomophthorales; *Erynia neoaphidis* (also known as *Pandora neoaphidis*), and *Neozygites fresenii* (Pell et al., 2001). Infections can be identified by testing for the fungus or by the visible changes in the aphid, eventually producing 'cadavers' or 'mummies' that cling to the leaf surface via mycelial threads. Fungal growth within the aphid mummy causes the mummy to burst open, whereupon resting spores explode onto the leaf and are picked up by other aphids on the leaf. Some researchers hypothesize that this is the cause of the aphid 'crash' as infection and a subsequent decline in aphid numbers has been shown to be potentially very rapid: Steinkraus et al. (1999) found that an infection of *N. fresenii* caused aphid numbers to decline by 80% in 4 days. While most researchers consider entomopathogenic fungi as primary pathogens, there is some debate as to whether the fungal infection is predicated on, and therefore secondary to, a primary viral pathogen. Whatever the case, a fungal epizootic event is a credible cause for the summer aphid crash, as infection can spread quickly and aphid numbers can therefore fall rapidly and precipitously.

There is, however, no consensus on the cause of the summer aphid crash. A UK study of *Macrosiphum euphorbiae* and *Myzus persicae* on potato (ANON, 2002) determined that aphid natural enemies were present, but not in sufficient numbers to precipitate the population crashes observed in the study: natural enemy levels and activity "appeared to track, rather than cause, the decline in aphid numbers." Changes in host plant quality have also been investigated with regard to the crash and, although found to influence aphid population dynamics, were not found to be adequate in themselves to account for aphid population crashes. ANON (2002) for example, reported a "robust correlation in time between the pattern of aphid population development and decline in the field and developmental shifts in potato phloem sap amino acid composition" and yet admitted that this was only a potential "component" of factors affecting the crash. Production of alates (winged aphids) does increase at the temperatures often associated with the crash (> 20°C), but it is unlikely that the population crash is caused by a mass emigration of aphids because the proportion of alates remains fairly constant (about 50%) at these temperatures.

In aphids, the egg stage is an adaptation to intolerably cold temperature. Hence it occurs in winter and acts as a 'resting' stage that, in the UK, lasts approximately from November to March. It is known that “when winters are consistently mild and the environment uniform and predictable, it is likely that an aphid species will become anholocyclic” (Dixon, 1998) because the protective adaptation is no longer necessary. *Nasonovia ribisnigri* has been reported overwintering without a sexual stage in Australia (personal communication to R. Collier) and in New Zealand (Stufkens & Teulon, 2003), and can also do so in the UK: “in southern Britain mobile stages can survive and slowly reproduce on outdoor lettuce, chicory, hawkweed and speedwell throughout mild winters” (Rothamsted Insect Survey, 2007). It is because outdoor lettuce is only grown in the UK between about April and October that the anholocyclic potential of *N. ribisnigri* cannot be realized on lettuce, but instead relies on these alternative weed hosts. An assessment of the suitability of these hosts is therefore particularly relevant to considerations of lettuce crop infestation and maintenance of nearby populations in the absence of lettuce.

Day-degree models can be used to model aphid development. Actual day-degrees can be calculated using data from synoptic weather stations such as that at Wellesbourne. Methods make the assumption that developmental rates are proportional to temperature above a base temperature. This is often a false assumption, but even so, it has been found that good predictions can be obtained by judicious choice of 'effective' rather than 'true' bases. Many models lack information about when development after diapause begins and therefore arbitrary dates are selected before which it is assumed diapause is incomplete and/or day degrees above the base are negligible, but mistakes in starting date may be a major source of error.

Once key events are converted to day degrees, these can be used by growers by accessing predictions or calculating day degrees themselves. The important events usually are first egg hatch, first emigration from the primary host or first appearance on the secondary host, peak emigration of alates from the primary host, and peak numbers on the secondary host. A preliminary day-degree forecast for *N. ribisnigri*, albeit based on a small data set, using the simple method in which day-degrees are calculated as the difference between arithmetic mean air temperature and the base temperature, gave 507 ± 111 day-degrees (above a threshold 4.4°C, accumulated from 1 February) for the appearance of the first aphid on lettuce and 935±165 for peak numbers on lettuce (Collier & Harrington, 2001).

Aims and objectives of this project

More detailed knowledge of the population parameters of *N. ribisnigri* will allow more accurate forecasts of its migration into, and numbers on, lettuce crops. Though the timing of life cycle events is dependent on ambient temperature and photoperiod (Dixon, 1998), little has been published on this relationship for *N. ribisnigri*. With these considerations in mind, the overall aim of this research is to quantify aspects of the life-cycle of *N. ribisnigri*.

The objectives of the project are:

1. To establish a technique for quantification of the rate of *N. ribisnigri* parthenogenetic reproduction on lettuce at constant temperatures.
2. To enumerate *N. ribisnigri* and aphid predator numbers and *N. ribisnigri* cadavers and apparent cause of death on lettuce throughout the growing season.
3. To quantify the temperature and / or photoperiod requirements for egg production and egg hatch.
4. To determine whether *N. ribisnigri* is able to overwinter in its parthenogenetic form on secondary hosts and to identify which secondary host plants might serve as good overwintering sites.

Data produced in this research will add to the incomplete knowledge of the life-cycle of *N. ribisnigri* and provide a base for further research aimed at constructing a forecasting model for the spring migration of *N. ribisnigri* into lettuce crops.

Materials and Methods

A laboratory culture of *N. ribisnigri* is maintained at Warwick HRI, Wellesbourne and this was used to provide material for laboratory, and some of the field, experiments.

1. Developmental rate of parthenogenetic *N. ribisnigri*

A constant temperature experiment was performed in the laboratory at 16 hours light and 8 hours dark (16L: 8D) in every 24 hours, which is approximately equivalent to the photoperiod in the UK in early to mid-summer. To determine the efficacy and reliability of reported methods, the experiment was performed at a single temperature (20°C). Lettuce seeds (cv Saladin) were sown in vermiculite in a seed tray and seedlings were transferred into F1 potting compost (Levington's) in individual pots (plastic, 7 x 7 cm) after one week. An alate was placed on each of twelve of the 3-week old lettuce seedlings and then each pot was covered in a bread-bag (the clear plastic perforated type commonly used for fresh bread), and an elastic band was wrapped around the bag and pot to create an aphid-proof enclosure. The aphids were allowed to reproduce, and then all but one nymph per plant was removed after 24 hours. Daily observations then ensued and a record was made of mortality, ecdysis (moulting), and the number of new-born aphids produced (new-born aphids were removed from the plants immediately).

2. The relationship between natural enemy and *N. ribisnigri* numbers on lettuce

The numbers of *N. ribisnigri* (including cadavers and apparent cause of death) and aphid natural enemies were recorded on lettuce throughout the growing season, and the data were analyzed for correlation and possible causation regarding changes in *N. ribisnigri* numbers. On June 21 2006, 240 4-week old lettuce plants (cv Saladin), raised from seed in a glasshouse, were planted out into the field at Warwick HRI, Wellesbourne in six adjacent blocks of 40 plants. Two blocks were immediately covered with insect-proof netting. The following day, these two blocks were inoculated with *N. ribisnigri*, two of the remaining blocks were inoculated with *N. ribisnigri* and left uncovered, and two were not inoculated and not covered. Inoculation comprised the placement of 5 adult apterae on every plant in the block. This process of plant raising, planting, and inoculation was repeated on 26 July 2006 and 23 August 2006. Subsequent to each planting, aphid populations were assessed weekly via the destructive counts of 2 randomly selected plants per block per week over a period of 10-12 weeks from planting date.

3. Conditions for egg production and hatch

Field observations

In late summer 2006 lettuce plants (cv Saladin) were enclosed in aphid-proof cages, infested with *N. ribisnigri*, and *Ribes* bushes (2-yr. old blackcurrant bushes, variety Ben Alder, supplied by Welsh Fruit Stocks, Hereford, and 2-year old gooseberry bushes, variety not stated, supplied by Charlecote Fruit and Flowers, Warwick) were then placed in plastic pots 19 cm diameter and 17cm deep in the cages. Specifically, on 7 September 2006, fourteen 4-week old lettuce plants were planted inside each of the four 3 m long x 2 m wide x 2 m high walk-in cages and were then inoculated with five *N. ribisnigri* per plant. One week later, on 13 September, a total of 31 potted blackcurrant and 11 potted gooseberry plants were placed inside the four cages; 8 blackcurrant in cages 1, 2, and 4 and 7 blackcurrant in cage 3, and 3 gooseberry in cages 1, 2, and 3 and 2 gooseberry in cage 4. The currant bushes were subsequently monitored weekly for aphids and aphid eggs. On 7 December, the cages were dismantled and the potted currant bushes were removed to a screen-house on site and then, on 29 January, half of these plants were moved to unprotected gravel beds and half were placed in cold storage at 4°C.

Laboratory investigations

Nasonovia ribisnigri eggs that had been laid the previous autumn were induced to hatch by placing them in an incubator (Gallenkamp) at 16L:8D and a constant 16°C. On 29 January 2007 a currant twig infested with approximately 40 eggs was removed from one of the blackcurrant bushes moved to cold storage and from one of those moved to the gravel beds. Each twig was placed separately into a Petri dish sealed with Parafilm M, which was then placed in the incubator. This procedure was repeated weekly until egg hatch was observed on the plants outside on the gravel beds.

4. Overwintering of *N. ribisnigri* in the field and actual / potential host plants

Overwintering of *N. ribisnigri* in the field

When the walk-in cages were dismantled and the potted currant bushes were removed to a screen-house in early December 2006, it was noted that a population of *N. ribisnigri* remained in the deteriorating lettuce. These lettuce plants were therefore left in the ground to monitor this population. New host plants were planted or placed around the original lettuce through December (lettuce, cv Saladin) and January (4-8 week-old lettuce, cv Saladin, and a potential weed host; speedwell found growing around the cages), to provide the aphids with alternative hosts. These potential host species were planted in the soil amongst and close up to the original lettuce throughout the period, as and when neededm, to provide healthy hosts at all times.

5. Potential host plants of *N. ribisnigri*

In the laboratory, selected potential secondary hosts of *N. ribisnigri* were grown from seed to 6 weeks old in small (7x7 cm) plastic pots in an illuminated constant environment room at 20°C, inoculated with the aphid, and incubated at 16L: 8D and 20°C for 4 weeks to assess whether these plants do in fact support the growth and reproduction of the aphid. Once inoculated, the plants were immediately placed together in a single clear plastic container (120 x 45 x 45 cm) in close proximity to one another and various lettuce varieties, allowing an assessment to be made at the end of the experimental period of the relative attraction / suitability of all these plants. The ‘summer’ photoperiod of 16L: 8D was used to inhibit production of sexual forms. The plants used in this experiment were Lettuce Great Lakes, Lettuce Saladin, Lettuce Lobjoits Green Cos, Lettuce Little Gem, Common Speedwell and Chicory, all supplied by Nickys Nursery Ltd., Kent. Four of each plant were placed in the container, producing a total of 24 plants.

Results and Discussion

1. Developmental rate of parthenogenetic *N. ribisnigri*

In the constant temperature experiment performed in the laboratory at 20°C and 16L: 8D, one of the 12 aphids died before producing any offspring. This aphid died two days after inoculation. The mean developmental time of the remaining aphids (time from birth of nymphs to adult) was 8 days, and the mean total number of offspring per aphid was 35.

The developmental time reported in this research was 8 days, comparable with the 8.0 days for apterae and 9.2 days for alatae reported by Diaz and Fereres (2005). The total number of offspring per aphid reported in this experiment was higher, however; 35 compared with 29.5 and 23.6 per aptera and alate, respectively, in Diaz and Fereres' (2005) equivalent experiment on Romaine lettuce. One difference between the experimental design in this research and that of Diaz and Fereres (2005) was the lettuce variety used. The difference in number of offspring produced per aphid may therefore reflect a greater suitability of the Iceberg lettuce variety over the Romaine lettuce variety for reproduction of *N. ribisnigri*. Another potentially important factor, however, was that the aphid clone was different to that used by Diaz and Fereres (2005), and it is possible that this was the reason for differences between outcomes. It should be noted that there were also some technical differences, the most potentially important of which is that Diaz and Fereres (2005) used lettuce leaves, not whole plants, in their experiment. This could also have impacted on experimental outcomes, particularly with regard to the amino acid composition of the sap and its effect on aphid development.

The results of Diaz and Fereres (2005) revealed that less winged forms were produced as temperature declined from 20°C to 16°C and below. It is postulated that this is because, assuming the food source is adequate and uncrowded, continued production of wingless forms is a less risky and more energy-efficient response to sub-optimal summer temperatures. An alternative explanation might be that as the host, lettuce, suffers heat stress at peak summer temperatures and is semi-hardy, surviving temperatures without damage down to 4°C, the lack of alates is a response to feeding on a 'healthy' host. Hence, the formation of alates is mediated via the plant sap, not via temperature. Harrewijn (1978) demonstrated in a study of *Myzus persicae* that the host plant can play a dominating role in wing dimorphism.

In this research, an alate was placed on each potted lettuce seedling and then all but one nymph per plant was removed after 24 hours. This had appeared to be a more straightforward method of achieving a new-born nymph inoculation than that followed by Diaz and Fereres (2005), but it was immediately apparent that great care was needed to ensure that no more than one nymph remained per plant. It is therefore suggested that new-born aphids should be removed to new 3-week old seedlings at 24 hours. To produce an adequate quantity of same-age new-born nymphs, Diaz and Fereres (2005) added 10 alates to a container with a single lettuce leaf. It is proposed that there are risks inherent in this method associated with leaf health, damage, and senescence and the potential effects of these on sap amino acid levels, and that therefore potted seedlings are a more appropriate tool for this and the other parts of this experiment. Hence, it is suggested that an alternative method for obtaining new-born nymphs is to add 5 alates to each of two 3-week old lettuce seedlings contained in aphid-proof bags. It was also noted that exuviae (cast skins) occasionally fell off the plant into the soil, whereupon they were difficult to see. It is important that no exuviae go uncounted as this will lead to errors in determination of the nymphal stage, hence, black paper covers can be cut to the size of the pot and laid on top of the soil.

2. <u>The relationship between natural enemy and *N. ribisnigri* numbers on lettuce</u>

The first planting took place in the week beginning 19 June 2006. However, inoculation with apterous aphids did not lead to their establishment on the lettuce plants. By the time of the second planting, in the week beginning Monday 24 July, it was suspected that the seed used in plantings 1 and 2 might have been treated with insecticide. Some plants from the second planting were therefore inoculated with aphids in the laboratory and colonization was compared with that on other lettuce seedlings, also cv Saladin, but known to be from untreated seed. Once again, aphid populations did not establish on the former set of plants, neither in the field nor in the laboratory. They did establish on the other lettuce plants, leading to the conclusion that the seed used for field trials on 19 June and 24 July was insecticide-treated.

The third planting (using different seed) took place in the week beginning 21 August. *N. ribisnigri* did establish, but this planting was too late in the year to provide any usable information regarding the aphid crash, which typically occurs in late July or early August. What it did show was that *N. ribisnigri* numbers rose steeply in the covered environment between mid-September and mid-October, and that this rise was not matched in the lettuce plots that were left uncovered (Figure 1).

Figure 1. The numbers of *Nasonovia ribisnigri* on field lettuce at Wellesbourne in an inoculated and net-covered plot, an inoculated and uncovered plot, and an un-inoculated and uncovered plot (untreated control), late summer to autumn 2006.

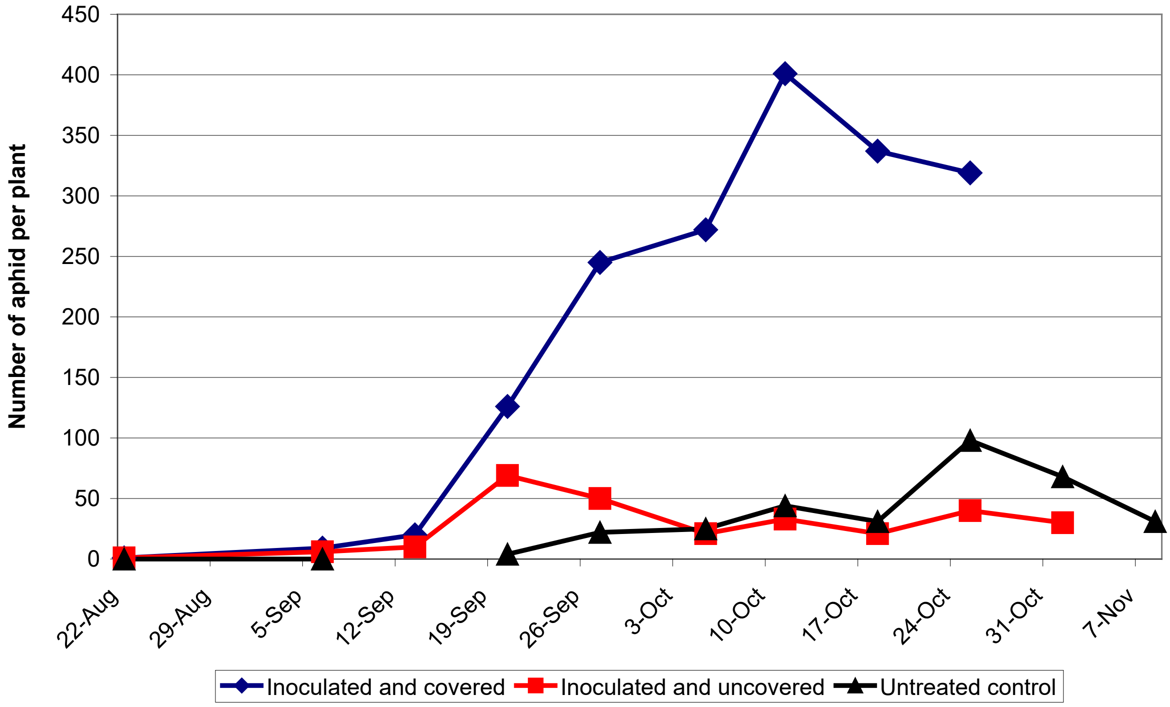

Figure 1 additionally shows an apparent decline in the numbers of aphids in all three plots at the end of the study period, from mid to late October, which allows the conclusion that the autumn peak was observed and occurred on 10 October in the covered plot and on 24 October in the open plots.

The hypothesis on which this experiment was based is that natural enemies cause *N. ribisnigri* numbers to crash in mid-summer, and the presumption in the design of the experiment is that by covering the lettuce, aphid natural enemies are excluded, and hence if higher numbers of *N. ribisnigri* are seen on covered lettuce, this is due to a lack of predation or parasitism. There are other effects of covering a crop with netting that also need consideration, however.

Perhaps the most important effect that needs consideration is that alatae, composing > 50% of the population at a constant 20°C and 24°C (Diaz and Fereres, 2005), are forced to remain in the plot, and hence the reproductive potential of summer migrants remains in the plot, probably adding significantly to the numbers. The issue of accounting for differences in summer migrant reproduction on the open and netted plots might be dealt with partially by noting differences in alate numbers on the lettuce in these plots, and adjusting numbers down on the netted plots to take account of the additional alates' reproductive potential. However, there are multiple potentials for error in such a calculation, connected particularly with unknown levels of emigration and immigration in the open plot and potential, but blocked, emigration and immigration in the netted plot.

Also, the micro-climate within a covered plot may at times be more favourable to the aphids. The impact of heavy rain, for example, is dissipated at the surface of the net and hence aphids are not knocked off the plants as they might otherwise be. In a similar fashion, strong winds have less of an impact in a covered plot.

3. Conditions for egg production and hatch

Field observations

Nasonovia ribisnigri was first seen on the currant bushes on 17 October (Figure 2), when both alate and apterous forms were observed. Eggs (Figure 3) were first observed approximately 4 weeks later, on 15 November. By this time, although not counted and recorded, the number of apterous aphids per currant bush was as high as 50, although numbers per plant were very variable, with a small number of plants having < 5 apterae per plant. The variation in the number of eggs per plant was more extreme, with > 100 eggs on some plants and none on others.

Figure 2. *Nasonovia ribisnigri* sexual forms on *Ribes*, autumn 2006

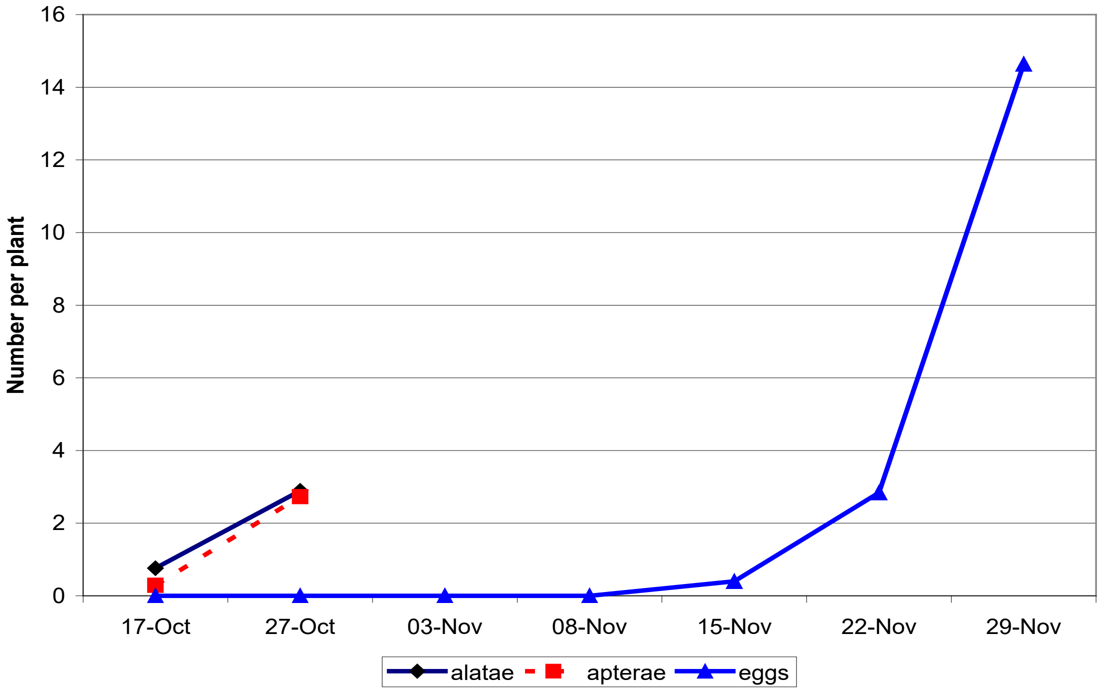

Figure 3. Apterous *N. ribisnigri* and eggs on currant bush on 29 November 2006.

A total of six small, green, almost rectangular nymphs, identified as fundatrices, were seen on two of the 32 currant bushes on 6 March 2007, indicating that first egg hatch was between 27 February (the previous observation) and the observation on 6 March. All the currant bushes were still in bud on this date, and remained so until late-April. Subsequent weekly observations showed that egg-hatch continued through March and April, but populations did not establish on the bushes, presumably because there was no leaf on the plants.

Similar weekly observations of the 18 currant bushes acquired from Norfolk revealed a better synchronization of egg hatch and bud-break. On these bushes, 2 nymphs were observed on a single plant on 21 March, at which time 3 of the 18 plants displayed a single small leaf floret (although not the plant on which the nymphs were seen). Two weeks later, on 2 April, all plants either had buds just about to break or already broken into small leaf florets, and nymphs were observed on 5/18 bushes. On 30 April 10/18 bushes were suffering various degrees of infestation, from a nascent population of 0-10 aphids, to >100 apterae causing local leaf damage.

Laboratory investigations

Of the *N. ribisnigri* eggs that were induced to hatch by placing them in an incubator at 16L: 8D and a constant 16°C, the average percentage egg hatch was 11%, with a range of 0-34% (Table 1). The majority of eggs hatched within a week of them being placed in the incubator.

Table 1 Induction of *Nasonovia ribisnigri* egg hatch in an incubator at 16L: 8D and a constant 16°C.

Eggs from	No. of eggs	Hatched 5 Feb	Hatched 12 Feb	Hatched 19 Feb	Hatched 26 Feb	Hatched 5 Mar	Hatched 12 Mar
Outside 29 Jan	48	0	0	0	0	0	0
Cold-store 29 Jan	44	0	0	0	3	0	0
Outside 5 Feb	39	-	4	0	1	0	0
Cold-store 5 Feb	37	-	0	0	0	0	0
Outside 12 Feb	40	-	-	6	0	0	0
Cold-store 12 Feb	44	-	-	7	8	0	0
Outside 19 Feb	36	-	-	-	1	1	0
Cold-store 19 Feb	42	-	-	-	3	1	0

The hypothesis on which the egg production and hatch experiments were based is that photoperiod and temperature determine the timing of the sexual stages of the life-cycle. This hypothesis can be divided into three, more specific, sub-hypotheses.

The first sub-hypothesis is that a long scotophase triggers the switch from parthenogenetic to sexual reproduction, and that there is a correlation between temperature and the percentage of a population that experiences this switch. The migration of *N. ribisnigri* to its winter host observed in this experiment occurred in October / November and this coincides exactly with the literature on this subject. Although aphids were not counted on the currant after 27 October, they were still monitored, as already reported. It was considered that a good indicator of migration to the currant was the number of alates attached to the roof of the cage on a still, sunny day: by mid-November this rough guide to migration was showing that the migration 'peak' had passed. By 7 December no alates had been seen attached to the roof of the cage for one week. By this time also, 33 of the 55 currant bushes had no viable foliage remaining.

Although the winter migration event was complete before December, apterous aphids still populated the lettuce plants in the cage at this time. It is proposed therefore that warmer temperatures may prevent the activation of the sexual life-cycle in *N. ribisnigri* and that the temperature at which this occurs may be different for different individuals (clones).

The second sub-hypothesis is that completion of egg development requires a certain number of day degrees and the timing of egg hatch is therefore temperature-dependent, and that eggs may undergo a period of diapause that is temperature independent. Of the eggs induced to hatch in February 2007, the majority of hatches were recorded in the first week of the eggs being placed in the incubator, indicating that the conditions did appear to have been the cause of egg hatch. The earliest date of hatch inducement was 29 January and this set of eggs was the only one to show no hatches in the first week of incubation. Indeed, there were no hatches until four weeks of incubation, when 3 hatches were noted from the eggs taken from cold storage. This may indicate that diapause was not complete by 29 January (at least in the set of eggs induced to hatch) and that subsequent sets of eggs (those induced to hatch from 5 February onwards) included eggs which had completed diapause. A postulated diapause, completion of which occurred from the beginning of February, also fits with the fact that the first observed egg hatch from the set that remained outside occurred after 27 February. No hatches were observed in the eggs in cold storage throughout the duration of this experiment.

Finally, it is hypothesized that the sexuales and fundatrices are adapted to develop in colder conditions than summer viviparae. No experimentation was used to test this hypothesis in this research, but observations of relevance did not produce any evidence in support of it. Parthenogenic forms in the cages, for example, were extant throughout the time when sexuales were produced and populated the currant plants. It was also observed that, although delayed until early June, a small number of eggs hatched (7 aphids in total) in the 4°C storage conditions in combination with some limited leaf production on the currant bushes. These fundatrices appeared to have an adequate potential food source and yet none of them survived to produce offspring at this temperature.

Observations to date allow the detailed methodology of further laboratory experiments to be proposed. To further test the hypothesis that long scotophase triggers the switch from parthenogenetic to sexual reproduction, and that there is a correlation between temperature and the percentage of a population that experience this switch, viviparae on lettuce could be subjected to different photoperiod and temperature regimes in the laboratory. Specifically, photoperiod could be maintained constant at four different values: 12: 12D (the light to dark ratio two weeks prior to first aphid migration in the field at Wellesbourne); 10L: 14D (the ratio two weeks prior to maximum aphid migration in the field at Wellesbourne); 14L: 10D; 16L: 8D, and a range of constant temperatures could be applied to each photoperiod. Eight-week old lettuce plants, each supporting a colony of approximately 50 aphids, should be enclosed with young uninfested lettuce (replaced weekly) and *Ribes* plants or cuttings, and alatae and apterae on the *Ribes* should be counted at weekly intervals.

It is assumed that, although viviparous alatae will visit the *Ribes* plants, they will not settle on them, and that the earliest indicator that the 'switch' has occurred will therefore be a build-up of alatae on *Ribes*. Furthermore, at the temperatures to be used (12°C, 16°C, and 20°C), aphid developmental time will be from 1 to 4 weeks, and it is only after this time that migrants are likely to appear. For each temperature setting, therefore, weekly counts should continue for a total of two multiples of the development time, as reported by Diaz and Fereres (2005) (e.g. at 12°C, development time 15.8 days, counts should be at 7, 14, 21, 28 and 32 days).

The experiment described above also serves to provide a protocol for the production of alate males and gynoparae, necessary for subsequent investigations of egg development. Sexuales can be produced in the laboratory at a photophase and temperature dictated by the results of the experiment referred to above, under the same enclosed set-up as before, in six containers. A single cutting of each of blackcurrant, redcurrant, and gooseberry should then be made available to the aphids for colonization in each container to determine whether *N. ribisnigri* has a preference for any host. The *Ribes* species could also have important effects on population structure, which could also feed in to variation in egg production, hatch, and the fitness of the population to colonise the secondary host. The central thesis that egg hatch is determined by temperature can be tested along with these others by recording the number of alatae, apterae, and eggs on *Ribes* at twice weekly intervals until death of all mobile forms.

The eggs of some aphid species undergo a diapause, in which there is a temperature independent stage and before the completion of which the eggs cannot hatch. Not all species produce diapausing eggs, however, and it is essential to establish whether or not *N. ribisnigri* eggs go through a diapause. A simple experiment to achieve this, linked to and necessarily following on from the previous described experiment, requires that the six sets of eggs are sequentially placed in the conditions already reported here to induce egg hatch, whilst the remainder are held in cold storage. Specifically, one of the six sets of eggs should be incubated at 16L: 8D and constant 16°C immediately after the death of mobile forms, and the remaining five sets removed from cold storage at 2-week intervals, when they too will be incubated at 16L:8D and constant 16°C. Eggs should be monitored twice weekly to watch for emergence of fundatrices. An immediate egg hatch from the first set indicates that there is no diapause and that the eggs are ready to hatch as soon as they are laid. The purpose behind the sequential removal of eggs from cold storage is based on the hypothesis that aphid eggs need a certain period of cold before they will hatch. This hypothesis will be supported if the first set(s) of eggs produce no or lower hatches than later sets.

It has been noted that cold storage at 4°C does not halt the development of either the currant plant or the aphid eggs, but merely slows them. The experiment above is not compromised by this fact, but it is recognized that the cold storage temperature will likely affect outcomes by slowing (at cold storage temperatures <4°C) or hastening (at cold storage temperatures >4°C) the temperature-dependent development of the egg while in cold storage and hence extending or contracting, respectively, the period between first and last recorded egg hatch over the entire experiment.

It would be of great use to determine the optimal temperature for development of oviparae and fundatrices. To determine the optimal temperature for oviparae, sexuales can be produced in the laboratory at a photophase dictated by previous experiments, under the same enclosed set-up as before, using a single *Ribes* species. Once > 20 apterae are seen on every *Ribes* cutting, the lettuce, all alatae, and excess apterae (> 20) should be removed and the temperature set differently for each cutting. Aphid and egg numbers should then be counted twice weekly until the death of all aphids. To determine the optimal temperature for development of fundatrices, an excess of young cuttings of *Ribes* just bursting into leaf can be prepared to coincide with egg hatch. Fundatrices should be transferred to the cuttings, incubated at different temperatures, and their development recorded weekly, along with production of apterae and alatae, and mortality.

4. Overwintering of *N. ribisnigri* in the field and actual / potential host plants

Overwintering of *N. ribisnigri* in the field

The population of *N. ribisnigri* extant in the decaying lettuce in the field in early December 2006 was small, and continued to fall during early 2007. Weekly sampling and the identification of individuals via multiple features (e.g. abdominal pattern, antennal tubercles, 6th antennal segment) showed that the population was still extant on 27 February 2007 (on this day an alate and an aptera were identified), but that by 6 March it had disappeared completely.

Nasonovia ribisnigri established on lettuce in the summer of 2006 was seen to survive into the winter but not to survive through it; an alate and aptera were identified on 27 February 2007, but no *N. ribisnigri* was seen on 6 March.

On 27 February, the lettuce plants were small and mostly providing green leaf only at their very centres, with very little, to no, head development, and it is proposed that the very poor quality of the host plants was the most likely direct cause of the eventual extinction of this aphid population in the following week, as opposed to the cold. This proposition is made with regard to the survival of the population up to 27 February, and that evidence of parthenogenetic reproduction was available up until 13 February, when a nymph was seen in close proximity to an aptera identified as *N. ribisnigri*. Had the plants offered more physical protection and better food quality, the aphid population may have survived into the spring and following summer. With regard to the hypothesis, it is suggested that *N. ribisnigri* is able to survive mild winters in southern Britain in its parthenogenetic form. This echoes the Rothamsted Insect Survey (2007) statement to this effect, but as Wellesbourne must be considered at the northern limits of “southern Britain”, the area of Britain in which *N. ribisnigri* can survive winters may be larger than currently accepted.

5. Potential host plants of *N. ribisnigri*

After four weeks’ incubation, the numbers of *N. ribisnigri* on each plant were counted. The mean number of aphids per plant (given with standard error) was highest on Lettuce Little

Gem (231, 25.9), followed by Lettuce Great Lakes (197, 19.5), Common Speedwell (190, 37.6), Lettuce Saladin (179, 14.7), Lettuce Lobjoits Green Cos (160, 23.9), with Chicory supporting the lowest number of aphids per plant (123, 12.4).

Of the potential host plants of *N. ribisnigri* tested, the largest population after four weeks was recorded on Lettuce Little Gem (231 aphids per plant), and the smallest on Chicory (123 aphids per plant). All plants were within the same container, had their leaves touching adjacent plants, and were well-lit and watered and apparently healthy. It is therefore considered that the differences in aphids per plant were a reflection of aphid preference for the sap of the plant. This 'preference' might be a sensory attraction to volatiles emanating from the plant (sap) attracting more aphids to the plant and / or a superior growth and reproduction rate induced by feeding on the sap causing populations to increase at a faster rate than on other plants. What is perhaps most noteworthy is that Speedwell supported higher populations per plant than two of the lettuce varieties, including Saladin, the variety of lettuce used in the experiments in this research, although the reliability of its placing is put into some doubt due to the relatively high standard error of 37.6. Having ascertained that Speedwell and Chicory do support *N. ribisnigri* populations equal or near equal to those supported by lettuce in rain-free, 'summer' (laboratory) conditions, therefore, the next step would be to assess their suitability in winter temperatures. The plant architecture is likely vitally important during the cold (and wet) of winter: neither Speedwell nor Chicory form a head in the way that lettuce does and therefore the aphids will be exposed fully to the cold and to rain or snow.

Conclusions

Life table parameters reported in this work are mostly similar to those reported in the technically most similar published research (Diaz and Fereres, 2005). Development time was 8 days at 20°C, while total offspring per aphid was, a higher, 35. The difference in the number of offspring produced per aphid may reflect a greater suitability of the Iceberg variety of lettuce for reproduction of *N. ribisnigri* over the Romaine variety used by Diaz and Fereres or be a reflection of differences between different clones of the aphid.

A pronounced rise in *N. ribisnigri* numbers was recorded in the covered environment in the field between mid-September and mid-October (2006). This may have been due lower aphid predator and / or parasite numbers in the covered plot but might just as readily be explained by non-biological factors associated with the netting of the plants. Technical problems and time constraints meant that the likely period of the aphid crash was not monitored in the field trials in this research, and hence an aphid crash was not witnessed.

The migration of *N. ribisnigri* to its winter host observed in this experiment occurred in October / November. Although the winter migration event was complete before December, apterous aphids still populated the lettuce plants in the cage at this time. It is proposed therefore that higher temperatures may prevent the activation of the sexual life-cycle in *N. ribisnigri* and that the temperature at which this occurs may be different for different individuals (clones). Observations did not produce any evidence in support of the hypothesis that the sexuales and fundatrices are adapted to develop in colder conditions than summer viviparae.

Nasonovia ribisnigri established on lettuce in the summer of 2006 was seen to survive into the winter but not to survive through it. Nevertheless, it is concluded that the very poor quality of the host plants was the most likely direct cause of the eventual extinction of this aphid population, as opposed to the cold, and hence that *N. ribisnigri* is able to survive mild winters in southern Britain in its parthenogenetic form. Indeed, the area of Britain in which *N. ribisnigri* can survive winters may be larger than currently accepted.

Speedwell and Chicory support *N. ribisnigri* populations equal or near equal to those supported by lettuce in rain-free, ‘summer’ (laboratory) conditions.

Technology transfer

Half-day seminar at G's Growers 18 January 2007.
Article on *Nasonovia ribisnigri,* published in the 'Vegetable Farmer' March 2007, 36-37.

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