Project Title**:**	Protected lettuce: Towards insecticide free production
Project number:	PC 194
Project leader:	Dr R. Jacobson, Stockbridge Technology Centre.
Report:	Year 2 annual report, June 2005
Principal experimental workers:	Dr P. Croft, Stockbridge Technology Centre. Mr J. Fenlon, Warwick University Dr R. GreatRex, Syngenta Bioline
Location of Project:	Stockbridge Technology Centre Cawood Selby North Yorkshire YO8 3TZ Tel: 01757 268275 Fax: 01757268996
Project Co-ordinator:	Mr G Ward OBE
Date Commenced:	1 July 2002
Duration:	3 years and 9 months (*i.e.* to 31 March 2006)
Key words:	Protected lettuce, aphids, IPM, parasitoids, open rearing systems, crop monitoring, nepetalactone, *Nasonovia ribisnigri*, *Myzus persicae*, *Aulacorthum solani*, *Macrosiphum euphorbiae*, *Aphidius colemani*, *Aphidius ervi*, *Aphidius hieraciorum*, *Praon volucre*

The results and conclusions in this report are based on a series of experiments and desk-based studies. The conditions under which the studies were carried out and the results have been reported with detail and 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 the interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

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

Signature…….

Dr P. Croft,
Principal Experimental Worker,
Stockbridge Technology Centre Ltd,
Cawood, Selby, North Yorkshire. YO8 3TZ.
Tel: 01757 268275; Fax: 01757 268996

Date …….

Signature…….

Dr R. J. Jacobson
Project Leader
Stockbridge Technology Centre Ltd,
Cawood, Selby, North Yorkshire. YO8 3TZ.
Tel: 01757 268275; Fax: 01757 268996

Date …….

CONTENTS

GROWER SUMMARY

Headlines

- The overall aim of this project is to develop prophylactic biological control techniques, which could be used in conjunction with physical pest control measures and cost effective crop monitoring to obviate the need for routine applications of insecticides against aphids in protected lettuce.
- A biological control strategy has been formulated based on the prophylactic release of various parasitic wasps using open rearing systems (ORS). The latter are based on cereal plants infested with cereal aphids that are a common host to the parasitoids but not a threat to the lettuce crop.
- Preliminary studies based on the *Aphidius ervi* ORS demonstrated that the parasitoids did not move far from the ORS unit. This was probably because the heavily infested ORS units were more attractive to the parasitoids than small localised colonies of lettuce aphids in the crop.
- The results of a series of experiments have since shown that the behaviour of the parasitoids can be manipulated to improve their performance in lettuce crops. Two ORS units, at opposite ends of a commercial glasshouse of up to at least 4200m^2, provided enough chemical cues to pull *A. ervi* and *A. colemani* across the crop. Both parasitoid species located small colonies of aphids within seven days on at least 50% of occasions. The success rate should be better when the system is used within a full IPM programme.
- When two ORS units were used, the pheromone nepetalactone did not appear to further improve the ability of *A. ervi* and *A. colemani* to locate small colonies of aphids.
- Two parasitic wasps, *Aphidius hieraciorum* and *Praon volucre*, have been collected from the wild and shown to be potentially useful biological control agents for use against *Nasonovia ribisnigri* (currant lettuce aphid).
- Risk analysis studies have improved our understanding of crop monitoring procedures and the probability of failing to detect aphid populations with different sample sizes.
- A simple model of aphid multiplication based on temperature allows the time between samples to be determined according to the cumulated temperature. The model can be driven by actual or forecast temperature, thereby giving the grower more flexibility.
- Prophylactic biological control could halve the rate of growth of aphid populations. This retardation in growth rate allows a longer interval between samples.
- Frequency of sampling based on the incorporation of the two previous concepts into the sampling decision process achieve a significant reduction in sampling frequency and thereby costs.

Background and expected deliverables

Protected lettuce crops are vulnerable to sporadic large invasions of four species of aphids; *Nasonovia ribisnigri* (currant lettuce aphid), *Myzus persicae* (peach potato aphid), *Aulacorthum solani* (glasshouse potato aphid) and *Macrosiphum euphorbiae* (the potato aphid). All these species invade the glasshouse as winged adults, which rapidly produce large populations on the plants.

Consumers are very sensitive to the presence of insects on produce and retailers' standards demand almost total freedom from pests. To achieve such standards, lettuce growers have traditionally depended on routine, and sometimes intensive, applications of insecticides. However, the number of effective aphicides available for use in protected lettuce has become much reduced in recent years and it is now becoming increasingly difficult to control aphids even with intensive insecticide programmes. Furthermore, the FSA and Assured Produce Scheme (APS) have adopted a policy of minimising pesticide residues (particularly multiple residues) and this initiative is being followed by some of the leading food retailers. Although these organisations are urging growers to eliminate (or at least substantially reduce) their dependence on insecticides, reliable alternative aphid control technologies are not yet available.

The HDC funded project, PC132, which was completed in 2001, laid the foundation for a new supervised pest control strategy for protected lettuce. Those studies showed that screening glasshouse ventilators and doors substantially reduced infestation by aphids. However, defences in the screened glasshouses were occasionally breached and crops had to be carefully monitored to determine if / when insecticides were required. A monitoring procedure was developed for use by experienced entomologists in the experimental crops but it was time consuming and considered to be prohibitively expensive for commercial crops. The method is being further developed in this project using risk assessments, coupled with improved knowledge of labour requirements, to provide a cost effective system for glasshouse lettuce crops.

Screening glasshouses reduced invasion by aphids to the point that biologically-based control systems appeared to be feasible. Conventional methods of using parasitoids against aphids involve releasing the adult wasps after the pests are seen, which inevitably allows some pest build up and the presence of unacceptable numbers of "mummified" aphids on the plants.

If biological control is to be successful against aphids on protected lettuce, it must be done prophylactically to prevent populations becoming established on the crop. The authors have previously developed a prophylactic method of controlling *Aphis gossypii* (melon-cotton aphid) on cucumber crops, which used an open rearing system (ORS) for establishing parasitic wasps in the glasshouse. This is based on maize plants infested with cereal aphids, which are a common host to the parasitoids but not a threat to the cucumber crop. The ORS costs little in biocontrol material but does require a significant management / labour input by the grower to maintain insect and plant cultures.

The management / labour input required by growers to maintain the ORS could be much simplified by providing them with ORS kits that require minimal maintenance. Syngenta Bioline, who are partners in this project, have done preliminary development work on ORS kits that could be used by growers for the control of *Myzus persicae* and *Macrosiphum euphorbiae*. The kits utilise two parasitic wasps, *Aphidius colemani* and *Aphidius ervi*, against *M. persicae* and *M. euphorbiae* respectively. Both wasps were already commercially available and this reduced development costs. However, there was no parasitic wasp available for *Nasonovia ribisnigri* and this presented the project team with a potentially insurmountable obstacle.

Summary of work and main conclusions

New parasitoids

Two parasitic wasps, *Aphidius hieraciorum* and *Praon volucre*, were collected from the wild and shown to be potentially useful candidates for the biological control of *N. ribisnigri*. Both have since been kept in culture at STC for use in experiments. In parallel to this project, Dr Croft secured funds from Defra to investigate important aspects of the biology of both species. These complementary studies utilised a new model (developed by Phil Northing at CSL) which predicts the outcome of interactions between a pest and beneficial. In this case, the model was used to determine which of the two parasitoids should be further developed as the control measure against *N. ribisnigri* on protected lettuce. Unfortunately, the initial work with *P. volucre* indicated that it was a relatively weak parasitoid and was unlikely to provide the level of control required in commercial lettuce crops. A similar evaluation of *A. heiraciorum* is underway. If successful, further work will also be required to perfect culturing methods and to develop specific ORS rearing units for this species.

Manipulating the parasitoids behaviour

The success of an open rearing system in any crop clearly depends on the parasitoids leaving the ORS unit to search for aphids on the plants. This presents a challenge in protected lettuce crops because the parasitoids must locate and attack the pest aphids while they are still at very low population densities. The chemical cues produced by the large colonies of cereal aphids in the ORS units are almost certainly stronger than those produced by the small colonies of lettuce aphids within the crop, and it is highly probable that the parasitoids will keep returning to the original ORS unit.

Research completed in the first year of the project showed that *A. ervi* and *A. colemani* could be encouraged to leave the ORS unit by providing additional chemical cues in other parts of the glasshouse. These additional cues could be in the form of either nepetalactone pheromone lures or large colonies of cereal aphids, the latter being an alternative host for the parasitoids but not a threat to the crop. It was shown that both parasitoid species would move up to 35m from an ORS unit to locate a bait unit containing large numbers of cereal aphids. Thus, it seemed probable that ORS units placed at opposite sides of the glasshouse would provide sufficient chemical cues to

draw the parasitoids across the crop. However, it was still necessary to show that they would find small colonies of lettuce aphids while moving between the ORS units.

A series of experiments investigated the benefits of strategic positioning of ORS units and pheromone lures on the performance of the parasitoids within commercial-scale lettuce crops. As the introduction of lettuce aphids into the crops presented a high risk to the grower, it was decided to use mobile bait units consisting of small numbers of cereal aphids (*Sitobion avenae* and *Rhopalosiphum padi*) on small trays of cereal plants. Overall, it was concluded that:

- *Aphidius ervi* and *A. colemani* from ORS units can locate small aphid colonies in commercial-scale lettuce crops.
- ORS units provide a constant source of parasitoids and, in addition, provide chemical cues to manipulate the searching behaviour of those parasitoids. Two ORS units, at opposite ends of a commercial glasshouse of up to at least 4200m^2, provide enough chemical cues to pull *A. ervi* and *A. colemani* across the crop.
- Where two ORS units are present in a lettuce crop of this size, both parasitoid species may be expected to locate small colonies of aphids within seven days on at least 50% of occasions.
- There are several reasons why the success rate may be better when the system is used within a real IPM programme in a commercial lettuce crop. In that situation, there would be a continuous supply of parasitoids from ORS units, the invading aphids would not necessarily be at the furthest point from those units and the searching time would not be restricted to seven days.
- When two ORS units were used, the pheromone nepetalactone did not appear to further improve the ability of *A. ervi* and *A. colemani* to locate small colonies of aphids.

Crop sampling and risk analysis

In the previous report, it was stated that trying to guarantee detection of aphids at very low threshold levels could become quite costly. For example, a sample size of 500 plants per 0.1ha would provide an acceptable 1 in 150 probability of failing to detect a 1% level of plant infestation, but it would cost the grower £1.56k per 0.1ha per annum (adjusted to today's prices). However, that model was deterministic, *i.e*. it relied on a very simple rule, which involved monitoring every week in the summer and every fortnight in the winter. The rule was very conservative, and could possibly be relaxed, but it would need to be based on monitoring temperature, which, of course, could be perceived as another cost. Retardation of the growth of aphids by the incorporation of prophylactic biological control allows a further relaxation. More recent risk analysis studies have therefore focused on three areas:

- The incorporation of temperature into the aphid growth model to make the sampling decision model more sensitive to actual or predicted temperature, i.e. the decision to sample is no longer deterministic but depends on the likely growth of the aphids.
- The impact of prophylactic biological control in reducing or retarding aphid colony growth.
- The effect of relaxing the decision to spray, by taking larger samples that guarantee a certain level of protection against aphid numbers.

Three monitoring schedules were investigated. The first was essentially that recommended in the first report, *i.e*. sampling at weekly intervals in the summer and fortnightly during the winter. The second used measured temperatures to drive the aphid growth model to determine the point at which the initial population will have grown five-fold, when sampling recurs. The third schedule allows for a 10-fold growth on the assumption that prophylactic bio-control will hold back the aphid growth rate. In summary, basic temperature monitoring (schedule 2) suggested a 50% saving in sampling costs, with a further 10% reduction using prophylactic bio-control (schedule 3).

Absorbing the cost of IPM

It is inevitable that an IPM programme based on screening ventilators and doors to reduce aphid invasion, combined with improved monitoring for early detection of pests and a prophylactic approach to biological control, will be more expensive than previous strategies based on the routine application of broad spectrum insecticides. The actual increase in production costs will be calculated as the project progresses, but it is not expected to add a prohibitively large premium to the price of lettuces in the shops. Industry wide discussions, involving growers, marketing groups and retailers, have begun to explore the possibility of sharing these additional costs.

Financial benefits to growers

The glasshouse lettuce industry is currently worth around £20m per year at wholesale level and £30m at retail level. Aphids are serious pests of these crops for three-quarters of the year and there are currently only two aphicides available. Some growers are failing to achieve satisfactory control despite routine insecticide application strategies and they commonly abandon cropping for long periods to provide aphid breaks. These difficulties will be exacerbated by the increasing pressure on growers from FSA, APS and some major retailers to further reduce pesticide applications (see Background section).

The development of IPM in protected lettuce is crucial if UK growers are to respond to the decline in the number of pesticides and the requirement to reduce pesticide usage. The adoption of IPM will increase the competitiveness of the UK protected lettuce industry by producing products that satisfy standards sought by consumers and reflected by major food retailers. This will enable them to retain, and perhaps increase, their current share of the UK market.

The adoption of IPM and associated pest monitoring practices could increase production costs, although these will be minimised by the risk analysis studies. This project is developing cost effective crop monitoring procedures and will include a cost benefit analysis of the whole IPM package. Furthermore, industry wide discussions will be instigated to explore the possibility of sharing the additional costs between producers, wholesalers and retailers.

SCIENCE SECTION

GENERAL INTRODUCTION

Background

Glasshouse lettuce crops are vulnerable to sporadic large invasions of four species of aphids; *Nasonovia ribisnigri* (currant lettuce aphid), *Myzus persicae* (peach potato aphid), *Aulacorthum solani* (glasshouse potato aphid) and *Macrosiphum euphorbiae* (the potato aphid). All the species invade the glasshouse as winged adults, which rapidly produce large populations on the plants.

Consumers are very sensitive to the presence of insects on produce and retailers' standards demand almost total freedom from pests. To achieve such standards, lettuce growers have traditionally depended on routine, and sometimes intensive, applications of insecticides. However, the number of effective aphicides available for use in protected lettuce has become much reduced in recent years (due to pest resistance and withdrawl of products) and it is now becoming increasingly difficult to control aphids even with intensive insecticide programmes. Furthermore, the Food Standards Agency (FSA) and Assured Produce Scheme (APS) have adopted a policy of minimising pesticide residues (particularly multiple residues) and this initiative is being followed by some of the leading food retailers. Although these organisations are urging growers to eliminate (or at least substantially reduce) their dependence on insecticides, reliable alternative aphid control technologies are not yet available.

Alternative aphid control systems based on parasitic wasps are widely used in protected salad crops such as tomato, cucumber and peppers. However, it is difficult to achieve the required marketing standards in lettuce when using biological control due to:

- the sporadic nature and size of the aphid invasions
- parasitoids are relatively slow to work and this inevitably allows some build up of aphid numbers before populations are controlled.
- several species of parasitoids are required to control the range of aphids that attack protected lettuce.
- conventional methods of using parasitoids involve releasing the adult wasps after the pests are seen – this inevitably allows some pest build up and the presence of unacceptable numbers of "mummified" aphids on the plants.

Recently completed experimental work in HDC Project PC132 (Jacobson, 2002) showed that screening glasshouse ventilators and doors substantially reduced infestation by aphids. However, defences in the screened glasshouses were occasionally breached and crops had to be carefully monitored to determine if / when insecticides were required. A monitoring procedure was developed for use by experienced entomologists in the experimental crops but it was time consuming and considered to be prohibitively expensive for commercial crops (Jacobson, 2002). Mr John Fenlon, who is a partner in this project, worked with the authors in LINK project

CSA2921 (incorporating HDC Project PC108) to develop a cost effective method for monitoring leaf miners and parasitoid establishment in tomatoes (Jacobson, 2000). These methods required further development using risk assessments, coupled with improved knowledge of labour requirements, to provide a cost effective system for glasshouse lettuce crops.

If biological control is to be successful against aphids on protected lettuce, it must be done prophylactically to prevent pest populations becoming established on the crop. Two such techniques have been developed in cucumbers to prevent the establishment of *Aphis gossypii* (melon-cotton aphid) (Jacobson and Croft, 1998):

- The first involves regular release of purchased parasitoids throughout the risk period. This is effective but it is expensive in biological control material. A similar approach in lettuce would be even more expensive due to the need to release multiple species of parasitoids.
- The second uses an open rearing system (ORS) for parasitoids in the glasshouse. This is based on maize plants infested with cereal aphids, which are a common host to the parasitoids but not a threat to the cucumber crop. This costs little in biocontrol material but does require a significant management / labour input by the grower to maintain insect and plant cultures.

Since that study was completed, there has been a large increase in the use of ORS overseas in crops that have a very low tolerance for pests. For example, in 2002 it was reported that 8.5ha of French ornamental crops were grown under the protection of various forms of ORS against a number of pests (Maisonneuve, 2002).

The management / labour input required by growers to maintain the ORS could be much simplified by providing them with ORS kits that require minimal maintenance. Syngenta Bioline, who are partners in this project, have done preliminary development work on ORS kits that could be used by growers for the control of *Myzus persicae* and *Macrosiphum euphorbiae* (GreatRex, pers. com.). The kits utilise *Aphidius colemani* reared on *Rhopalosiphum padi* (bird cherry aphid) and *A. ervi* reared on *Sitobion avenae* (grain aphid) against *Myzus persicae* and *Macrosiphum euphorbiae* respectively. However, there was no parasitic wasp available for *Nasonovia ribisnigri* and this presented the project team with a potentially insurmountable obstacle.

Overall aim and specific objectives

The overall aim of this project was to develop prophylactic biological control techniques, which could be used in conjunction with physical pest control measures and cost effective crop monitoring to obviate the need for routine applications of insecticides against aphids in protected lettuce.

At the Project Review Meeting on 21 November 2003, it was agreed that the remaining studies should focus upon the following specific objectives:

1. Further development of an ORS system for *Nasonovia ribisnigri* based on either *A. hieraciorum* or *Praon volucre.*

2. Further experimentation to optimise the effects of additional chemical cues aimed at improving the mobility and performance of *A. ervi*, *A. colemani* and the parasitoid selected to control *N. ribisnigri.*
3. Risk analysis studies, relating the risk of not detecting an infestation level of 1% infested plants to the cost of the monitoring exercise. This will also take into account the potential to reduce that risk by adopting the prophylacatic approach to parasitoid release.
4. Industry wide discussions (ie involving growers, marketing groups and retailers) to explore the possibility of sharing additional production costs that may be incurred in moving closer to insecticide-free production systems.
5. "Whole crop" evaluation of the reduced insecticide input lettuce production systems.

Summary of work completed to date

New parasitoids

An additional parasitoid was required for use against *Nasonovia ribisnigri*. In previous seasons, the authors had found an *Aphidius* species attacking *N. ribisnigri* in lettuce crops in North Yorkshire but it had not been formerly identified. The parasitoid was trapped in July 2002, by baiting crops with lettuce plants infested with *N. ribisnigri*, and it is now in culture at STC. In October 2002, the identification was confirmed by specialists at the Natural History Museum to be *Aphidius hieraciorum*. Only two previous records of *A. hieraciorum* have been found in the scientific literature and both were overseas; the most recent being on a different species of *Nasonovia* in Spain in 1973. Since July 2002, a second parasitoid species, *Praon volucre*, has been found attacking *N. ribisnigri* and it is also in culture at STC.

In parallel to this project, Dr Croft (STC) secured funds from Defra to investigate important aspects of the biology of both species. These complementary studies utilise a new model (developed by Phil Northing at CSL) which predicts the outcome of interactions between a pest and beneficial. In this case, the model is being used to determine which of the two parasitoids should be further developed as the control measure against *N. ribisnigri* on protected lettuce.

Developing the ORS system

The success of an open rearing system in any crop clearly depends on the parasitoids leaving the ORS unit to search for aphids on the plants. In doing this, they are required to change their host from the aphid species upon which they were reared to the pest species on the crop. There is an additional challenge in lettuce crops because the parasitoids must locate and attack the pest aphids while they are still at very low population densities. To find their hosts, parasitoids usually respond to a series of indicators, often in the form of chemical cues released from the insect host / plant complex. It is important that they do not abandon their search by choosing to return to stronger chemical cues from ORS units or simply by dispersing to the glasshouse roof.

Preliminary studies based on the *A. ervi* ORS demonstrated that the parasitoids did not move far from the ORS unit and it was clear that additional techniques would be required to modify their

behaviour. At an interim project review meeting on 18 June 2003, it was agreed that the original workplan should be changed to accommodate the development of such techniques.

The synthetic form of the pheromone nepetalactone (a component of aphid sex pheromone) manufactured from cat mint (*Nepeta cataria*) has been previously shown to increase searching activity of some species of parasitoid in the field (Glinwood *et al.*, 1998; Powell & Glinwood, 1998). The technique had potential to improve the results from the ORS in lettuce crops but it had never been tested within the confines of a glasshouse. A series of eight experiments were planned to investigate the possibility of improving the performance of the ORS in lettuce crops, with particular emphasis on low aphid densities within the glasshouse environment. In summary, the results showed:

- Nepetalactone influenced the direction of movement of *A. colemani* in a glasshouse crop in the absence of aphid hosts, but its influence on *A. ervi* was not so readily detected.
- When released from a single ORS unit, *A. ervi* and *A. colemani* failed to locate small numbers of lettuce aphids at a distance of 10m, regardless of the presence of pheromone lures close to the lettuce aphids. It would seem that the parasitoids had not picked up the chemical cues from the lettuce aphid / plant complexes; perhaps because there were too few aphids or because they were too distant. Alternatively, the stronger cues from the aphids in the ORS units may have arrested the parasitoids and stopped them searching over greater distances.
- The next experiment was set up in a similar way except an additional large aphid culture (based on an ORS unit without parasitoids) replaced the pheromone lure about 5m from the small colony of lettuce aphids. In this case, *A. colemani* located and parasitised the lettuce aphids, thus demonstrating that when the cues were sufficiently strong, *A. colemani* were drawn away from the ORS unit and could locate small numbers of lettuce aphids.
- Similar use of the additional large aphid culture did not provide a sufficiently strong cue to draw *A .ervi* away from the ORS unit. However, this parasitoid did find the small population of lettuce aphids when it was also provided with pheromone lures.
- In the absence of any ORS units, *A. ervi* located small numbers of lettuce aphids when pheromone lures were positioned at frequent intervals between the point of parasitoid release and the lettuce aphids. This approach also reduced the time that *A. colemani* took to find the lettuce aphids.
- In a commercial-scale crop, both species of parasitoids located large aphid cultures at up to 35m with or without pheromone lures positioned at frequent intervals across the glasshouse.

This combination of results indicated that the provision of additional chemical cues, either as additional ORS units or pheromone lures, could improve the performance of *A. ervi* and *A. colemani* in lettuce crops. However, further experimentation was required to optimise these effects.

Crop monitoring

A desk-based risk analysis looked at the sources of risk for aphid ingress, the detection potential of sampling methods and the assumptions behind them, together with some simple models for aphid population growth to determine the impact of failing to detect insects in routine

monitoring. It was clear that trying to guarantee detection of aphids at very low threshold levels would become quite costly. For example, a sample size of 500 plants per 0.1ha would provide an acceptable 1 in 150 probability of failing to detect a 1% level of plant infestation, but it would cost the grower £1.2k per 0.1ha per annum.

Risk analysis studies continued in 2004, relating the risk of not detecting infestations at various levels to the cost of the monitoring exercise. The studies also began to take into account the potential to reduce that risk by adopting the prophylacatic approach to parasitoid release.

Absorbing the cost of IPM

Although the cost benefit analyses of the crop monitoring procedures are still in progress, it seems inevitable that an IPM programme based on screening ventilators and doors to reduce aphid invasion, combined with improved monitoring for early detection of pests and a prophylactic approach to biological control, will be more expensive than previous strategies based on the routine application of broad spectrum insecticides. The actual increase in production costs will be calculated as the project progresses but it is not expected to add a prohibitively large premium to the price of individual lettuces in the shops. We must therefore ask whether retailers and consumers are prepared to pay a higher price for this premium product. Industry wide discussions (ie involving growers, marketing groups and retailers) have been instigated to explore the possibility of sharing the additional production costs that may be incurred by moving closer to insecticide-free production systems.

PART 1: DETERMINING THE ABILITY OF *A. COLEMANI* AND *A. ERVI* TO FIND APHID HOSTS AT LOW DENSITIES WITHIN A COMMERCIAL GLASSHOUSE

Background

The success of an open rearing system in any crop clearly depends on the parasitoids leaving the ORS unit to search for aphids on the plants. This presents a challenge in protected lettuce crops because the parasitoids must locate and attack the pest aphids while they are still at very low population densities. The chemical cues produced by the large colonies of cereal aphids in the ORS units are almost certainly stronger than those produced by the small colonies of lettuce aphids within the crop, and it is highly probable that the parasitoids will keep returning to the original ORS unit.

Research completed in the first year of the project (Jacobson *et al.*, 2003) showed that *A. ervi* and *A. colemani* could be encouraged to leave the ORS unit by providing additional chemical cues in other parts of the glasshouse. These additional cues could be in the form of either nepetalactone pheromone lures or large colonies of cereal aphids, the latter being an alternative host for the parasitoids but not a threat to the crop.

It was shown that both parasitoid species would move up to 35m from an ORS unit to locate a bait unit containing large numbers of cereal aphids. Thus, it seemed probable that ORS units placed at opposite sides of the glasshouse would provide sufficient chemical cues to draw the parasitoids across the crop. However, it was still necessary to show that they would find small colonies of lettuce aphids while moving between the ORS units.

The previous series of experiments also showed that pheromone lures distributed at frequent intervals throughout the crop would improve the ability of *A. ervi* to locate small numbers of lettuce aphids and reduce the time taken by *A. colemani* to do the same.

The present experiments further investigated the benefits of strategic positioning of ORS units and pheromone lures on the performance of the parasitoids within commercial-scale lettuce crops. As the introduction of lettuce aphids into the crops presented a high risk to the grower, it was decided to use mobile bait units consisting of small numbers of cereal aphids (*S. avenae* and *R. padi*) on small trays of cereal plants.

Materials and methods

(a) Comparison of nepetalactone and ORS units to ORS units alone

Each ORS unit was germinated in a controlled environment room (16L:8D, 22 ± 2^0C) and infested with either *R. padi* or *S. avenae* (units designated XP^1 and XP^2 respectively). Seven days after infestation these ORS units were transferred to a lettuce crop in a commercial glasshouse lettuce (1000m^2). Two ORS units of each type were placed at each end of the glasshouse and parasitoids of the appropriate species (*A. colemani* and *A. ervi* respectively) were released onto them (Figure 1). This method of release, as used in the previous trials,was designed to reduce the time required for ORS units to reach maturity and thus allow more replication "in time" throughout the season.

Four cereal aphid bait units, each containing 10 individuals of either *R. padi* or *S. avenae* (designated B^1 and B^2 respectively) were then placed at the furthest distance from the parasitoid release units (Figure 1).

This method was repeated in a similar adjacent glasshouse with the addition of four nepetalactone lures (designated N) distributed evenly between the ORS units and the cereal aphid bait points (Figure 2).

After seven days, the cereal aphid bait units were removed and placed in a controlled environment (22 ± 2^0C, 16L:8D) and the number of mummies that developed on each unit were recorded. Replication was done over time.

Figure 1. Monitoring the ability of two parasitoid species (*A. colemani* and *A. ervi*), released onto high density aphid infested ORS units (XP^1 and XP^2), to locate low density numbers of aphids on bait units (B^1 and B^2) within a lettuce crop in a commercial glasshouse (1000m^2).

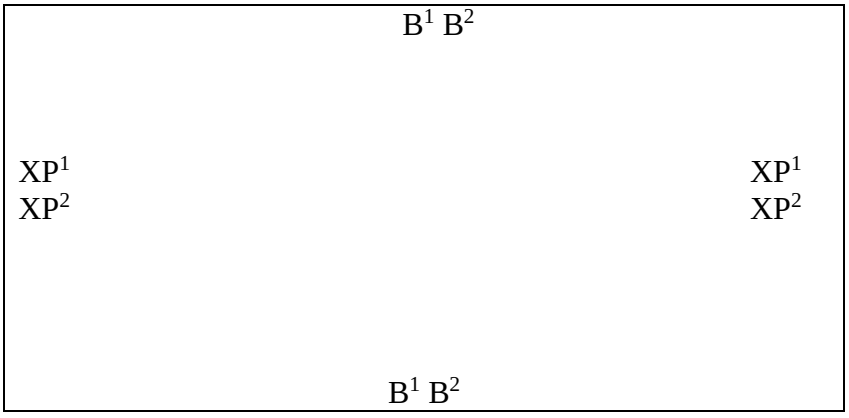
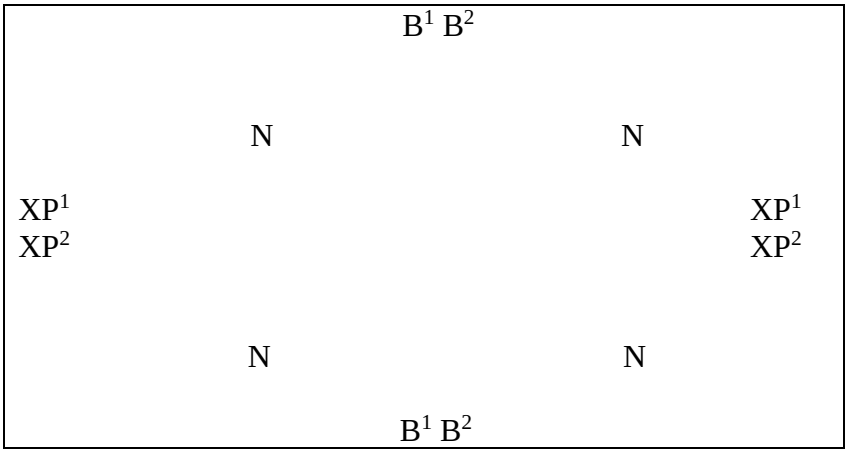


Figure 2. Monitoring the ability of two parasitoid species (*A. colemani* and *A. ervi*), released onto high density aphid infested ORS units (XP^1 and XP^2), to locate low density numbers of aphids on bait units (B^1 and B^2) within a lettuce crop in a commercial glasshouse ($1000m^2$), in the presence of nepetalactone lures (N).



(b) The ability of A. ervi and A. colemani to locate different numbers of low density aphid colonies

ORS units and cereal aphid bait units were prepared and positioned in a $1000m^2$ commercial glasshouse as described above in the method for experiment (a) (Figure 1). Three treatments were tested in series; *i.e.* incorporating one, two and four cereal aphid bait units placed at equal distances from the ORS units.

After seven days, the cereal aphid bait units were removed and placed in a controlled environment (22 ± 2^0C, 16L:8D) and the number of mummies that developed on each unit were recorded. Replication was done over time.

(c) To determine the number of ORS units required in a lettuce crop in a large commercial glasshouse ($4200m^2$)

ORS units and cereal aphid bait units were prepared as described above in the method for experiment (a). There were two treatments in similar adjacent glasshouses; *i.e.* with either two or three *R. padi* and *S. avenae* ORS units. Where two ORS units were used, they were 84m apart (Figure 3), Where three ORS units were used, the third unit was placed between the other two (Figure 4). A single bait unit containing each aphid species was placed at one side of the glasshouse (Figures 3 and 4).

After seven days, the cereal aphid bait units were placed in a controlled environment (22 ± 2^0C, 16L:8D) and the number of mummies that developed on each unit were recorded. Replication was done over time.

Figure 3. Monitoring the ability of two parasitoid species (*A. colemani* and *A. ervi*), released onto two high density aphid infested ORS units (XP^1 and XP^2), to locate low density numbers of aphids on bait units (B^1 and B^2) within a lettuce crop in a commercial glasshouse (4200m^2).

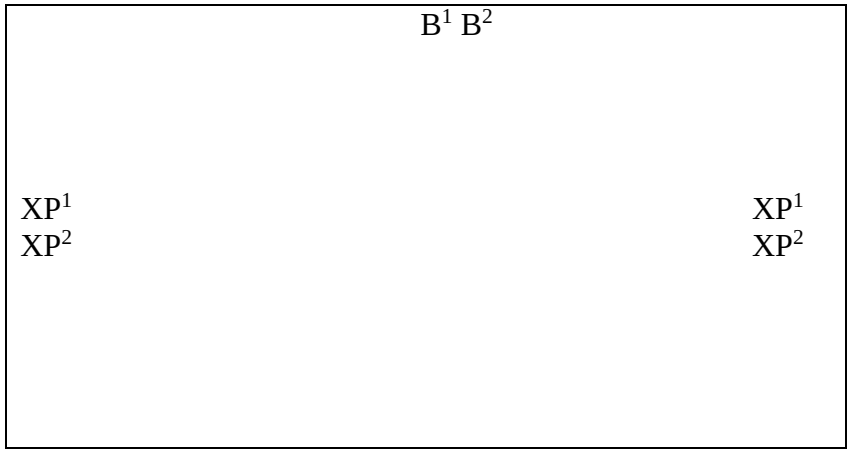
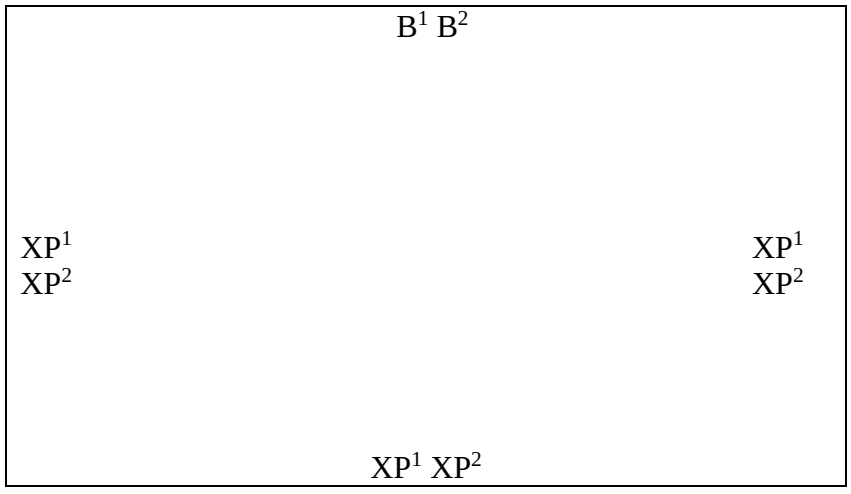


Figure 4. Monitoring the ability of two parasitoid species (*A. colemani* and *A. ervi*), released onto three high density aphid infested ORS units (XP^1 and XP^2), to locate low density numbers of aphids on bait units (B^1 and B^2) within a lettuce crop in a commercial glasshouse (4200m^2).



Results and discussion

(a) Comparison of nepetalactone and ORS units to ORS units alone

The numbers of parasitised cereal aphids recorded in the bait units over a period of seven days, with and without the presence of nepetalactone, are shown in Table 1. Both species of parasitoids found small aphid colonies at both sides of the glasshouse. *Aphidius colemani* located all the colonies offered, while *A. ervi* located seven out of eight colonies. It is probable that the success rate would have been even greater if the parasitoids had been given more time.

The presence of nepetalactone did not increase the ability of the parasitoids to locate the bait units. Furthermore, the number of mummies did not increase on the bait units when the pheromone was present.

The use of two ORS units, positioned at opposite sides of this 1000m^2 glasshouse, appeared to provide sufficient chemical cues to pull both species of parasitoid across the crop and allowed the parasitoids to locate the low numbers of aphids. This was consistent with previous results (Jacobson *et al.*, 2003).

Table 1. A comparison of the use of the nepetalactone to improve location of cereal aphids on two bait units by *A. colemani* and *A. ervi*

Parasitoid	Replicates	No pheromone			Pheromone		
		Total No. of mummies	No. of mummies per bait unit		Total No. of mummies	No. of mummies per bait unit	
A. ervi	1	13	6	7	7	3	4
	2	15	11	4	2	2	0
A. colemani	1	12	7	5	9	7	2
	2	18	11	7	8	7	1

(b) The ability of A. ervi and A. colemani to locate different numbers of low density aphid colonies

The numbers of cereal aphids parasitised by *A. ervi* and *A. colemani* in bait units are shown in Tables 2 and 3 respectively. Both parasitoid species were able to locate the small colonies of cereal aphids when there where 1, 2 or 4 bait units in the glasshouse. However, they did not always find all the colonies within the time available (seven days). When there was only a single bait unit in the glasshouse, both species found it on 66% of occasions. With two bait units present in each replicate, *A. ervi* found 70% and *A. colemani* found 100% of the aphid colonies. The success rate was poorer when four bait units were present in each replicate. In that situation, *A. ervi* found 35% and *A. colemani* found 70% of the aphid colonies. Overall, this indicates a success rate of 50-70% by both species.

There are several reasons why the success rate may be better when the system is used within a real IPM programme in a commercial lettuce crop. In that situation, there would be a continuous supply of parasitoids from ORS units, the invading aphids would not necessarily be at the furthest point from those units (as in these trials) and the searching time would not be restricted to seven days.

Table 2. The ability of *A. ervi* to locate different numbers of ORS bait units in a 1000m^2 commercial glasshouse

No. bait units	Replicates	Total no. of mummies	No. of mummies per bait unit: 1	2	3	4
1	1	6	6	*	*	*
1	2	2	2	*	*	*
1	3	0	0	*	*	*
2	1	13	6	7	*	*
2	2	15	11	4	*	*
2	3	4	3	1	*	*
2	4	2	2	0	*	*
2	5	0	0	0	*	*
4	1	0	0	0	0	0
4	2	0	0	0	0	0
4	3	4	1	1	2	0
4	4	5	4	1	0	0
4	5	6	5	1	0	0

Table 3. The ability of *A. colemani* to locate different numbers of ORS bait units in a 1000m^2 commercial glasshouse

No. bait units	Replicates	Total no. of mummies	No. of mummies per bait unit			
1	1	8	8	*	*	*
1	2	4	4	*	*	*
1	3	0	0	*	*	*
2	1	14	7	7	*	*
2	2	20	13	7	*	*
2	3	3	2	1	*	*
2	4	8	7	1	*	*
2	5	4	1	3	*	*
4	1	0	0	0	0	0
4	2	2	1	1	0	0
4	3	0	0	0	0	0
4	4	0	0	0	0	0
4	5	3	1	1	1	0
4	6	9	8	1	0	0
4	7	11	7	3	1	0

(c) To determine the number of ORS units required in a lettuce crop in a large commercial glasshouse (4200m^2)

The numbers of cereal aphids parasitised by *A. ervi* and *A. colemani* in the relevant bait unit are shown in Tables 2 and 3 respectively. The results showed that both species of parasitoids were able to locate the small colony of aphids on the single bait unit. However, they did not always find the colony within the time available (seven days). The results suggest that there could be a 50% success rate when two ORS units are present in the crop. When three ORS units were present, the result was similar for *A. colemani* but *A. ervi* found all the colonies.

As discussed under experiment (b), there are several reasons why the success rate could be better when the system is used within a real IPM programme.

Table 4. The ability of *A. ervi* and *A. colemani* to locate a single bait unit with different numbers of ORS units in a lettuce crop in a large commercial glasshouse (4200m^2)

Parasitoid species	Number of ORS units	Replicate	Number of bait units	Number of mummies
A.ervi	2	1	1	5
	2	2	1	0
	3	1	1	1
	3	2	1	6
A. colemani	2	1	1	12
	2	2	1	0
	3	1	1	4
	3	2	1	0

Conclusions

- *Aphidius ervi* and *A. colemani* from ORS units can locate small aphid colonies in commercial-scale lettuce crops.
- ORS units provide a constant source of parasitoids and, in addition, provide chemical cues to manipulate the searching behaviour of those parasitoids. Two ORS units, at opposite ends of a commercial glasshouse of up to at least 4200m^2, provide enough chemical cues to pull *A. ervi* and *A. colemani* across the crop.
- Where two ORS units are present in a lettuce crop of this size, both parasitoid species may be expected to locate small colonies of aphids within seven days on at least 50% of occasions.
- There are several reasons why the success rate may be better when the system is used within a real IPM programme in a commercial lettuce crop. In that situation, there would be a continuous supply of parasitoids from ORS units, the invading aphids would not necessarily be at the furthest point from those units and the searching time would not be restricted to seven days.
- When two ORS units were used, the pheromone nepetalactone did not appear to further improve the ability of *A. ervi* and *A. colemani* to locate small colonies of aphids.

PART 2: CROP SAMPLING AND APHID CONTAMINATION RISK ANALYSIS IN PROTECTED LETTUCE

Introduction

In the previous report (Jacobson *et al.*, 2003), sampling strategies were considered from a statistical point of view, and simple rules for sample size and sampling frequency given. In this section we look at how modifications to those rules can be developed to provide cost savings whilst attempting to minimise the growers' risk. In particular, we shall look at the following options:

- the use of prophylactic bio-control during times of limited aphid presence;
- development of a temperature-based growth model for aphids, which allows a more flexible and focused sampling regime;
- relaxation of the spray option when any contamination is observed.

Only when such decision-based models are attempted does it become apparent that there are significant areas where little information is available, or where information of one type is not easily convertible into another. Some of these areas are scientific, where, for example, the intrinsic growth rates of an aphid are not directly known, or the behaviour / growth (in extensive terms) of aphid colonies is not predictable. Others relate to more complex decisions such as whether a retailer will accept produce with some contamination, or whether a grower might treat parts of the crop differently. These will be highlighted at the end of the report.

1. Prophylactic bio-control introductions

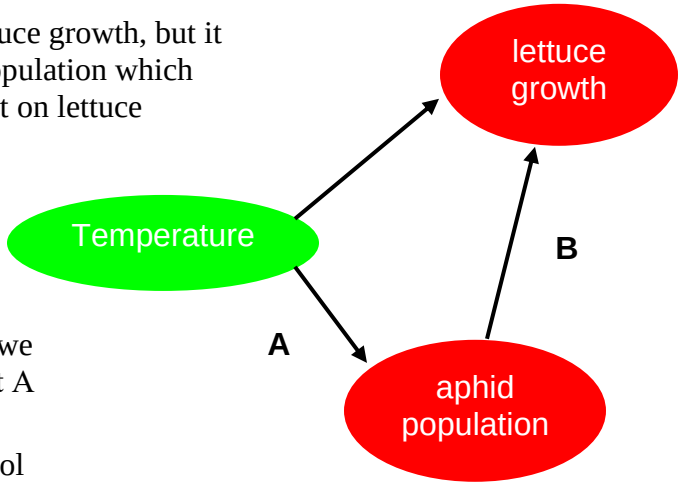
Recent experiments at STC suggest that parasitoids are capable of finding isolated aphid groups, but not totally and consistently. Replicated experiments using *Aphidius ervi* and *A. colmanii* showed that a single isolated community was sought and found by both parasitoids on 60% of occasions. Experiments with two isolated communities were attacked 70% and 100% of the time respectively by the two species, but this dropped to 35% and 50% when there were four communities. Although not comprehensive, this does suggest that between 50 and 70% of the time these parasitoids will seek out and control isolated aphid communities.

2. Development of temperature interactions

Temperature affects both lettuce growth and aphid population growth; the larger the latter then the more it impacts on lettuce wholesomeness. Essentially aphids affect the saleability of lettuce crops in terms of (a) direct damage, and (b) infestation of the saleable commodity. In general terms, both are unacceptable. The basic influences are illustrated by the simple diagram below (Figure 5):

Figure 5: Simple illustration of temperature interactions

Temperature affects lettuce growth, but it also affects the aphid population which itself can have an impact on lettuce growth.



In risk analysis terms we can 'intervene' both at A and at B

A: screens / bio-control
B: sample & control

1. Impact of temperature on crop growth:

If we consider the five crops reported in PC132 (Jacobson, 2002) we can characterise them by the statistics in Table 5.

Table 5: Cumulative temperature input for lettuce crops in PC132

Crop no.	Dates	days	Temp. range	cum. ºC	cum. >2ºC
1	23-Oct to 16-Feb	117	1-12ºC	786	552
2	08-Mar to 11-May	67	5-19ºC	665	531
3	01-Jun to 05-Jul	35	12-25ºC	614	544
4	25-Jul to 28-Aug	35	15-26ºC	625	555
5	19-Sep to 17 Oct	28	12-27ºC	394	338

With the exception of crop 5 this suggests that a very simple model based on cumulative daily temperature above 2ºC is a reasonable descriptor of time to harvest. What this means is that a relatively consistent heat input is required to produce a crop of saleable quality.

2. Effect of temperature on aphid growth

The paper by DeLoach (1974) refers to some earlier empirical equations for aphid growth by Pradhan (1946). A relatively loose interpretation of these equations (i.e. considering the inner part of a logistic equation as linear) provides a simple scheme that sets the approximate intrinsic growth rate (r_m) of aphids as 0.015 per ºC between 5ºC and 25ºC. So, taking the growth rate as 0 at 5ºC, it increases incrementally to 0.30 at 25ºC (see Box 1 for an explanation). Although the rate itself only increases linearly with temperature, this can have a profound impact on the

accumulation of aphids. So, for example, we see from Box 1 that 5 days at 15ºC lead to a 2-fold increase in the number of aphids; if the temperature is 20ºC, then that increase is 2.76-fold, while at 25ºC the increase is 3.71-fold (or effectively twice as fast as at 15ºC). This was the basis for the original proposal to halve the sampling frequency in winter. Note also, that the impact of fluctuating temperatures can be well approximated by considering the corresponding average temperature.

Box 1: Intrinsic growth rate r_m

r_m works just like compound interest, in that, if we start on day 0 with 1 aphid, then we expect (on average) the population to have grown to $(1 + r_m)$ on day 1, $(1 + r_m)^2$ or $(1 + r_m) \times (1 + r_m)$ on day 2, and $(1 + r_m)^k$ by day k.

Example: Suppose the temperature is 15ºC, so that the intrinsic growth rate is 0.15 per day (see above), then in five days the population will have grown $(1.15)^5$-fold, i.e. 2.011 times. By contrast, if the temperature is 25ºC, then in the same period the population will have grown by $(1.30)^5$, or 3.713 times.

Because the growth rate is temperature-dependent, we need to consider what happens when we have consecutive days with different temperatures. Thus, say we have five days with temperatures T_1, T_2, …, T_5, then the overall expected population growth of the aphids will be $(1 + r_{T1}) \times (1 + r_{T2}) \times \ldots \times (1 + r_{T5})$. Note that this product is the same no matter what the order of temperatures.

Example: Suppose the temperature follows the sequence 10ºC, 15ºC, 20ºC, 20ºC, 10ºC, so that the corresponding intrinsic growth rates are 0.075, 0.15, 0.225, 0.225 and 0.075 per day, then the 5-day accumulation will be a 1.994-fold increase. This is very little different from the 5-day growth at the average of these temperatures, 15ºC, as in the example above.

3. Relationship of sampling to temperature

1. Time between samples and temperature

In the earlier paper we stated that the weekly reproduction rate would be around 3 times for temperatures of around 15ºC, but between 5 and 10 times for temperatures in the 20 to 25ºC range. In risk terms this was interpreted in the following way:

"If the crop is sampled and no aphids are detected, then aphid incidence is low, and no action need be taken. If the sampling has failed to detect aphids, then the aphid incidence should still be low, so that the expected increase in numbers should not be more than 10-fold at the highest temperatures. Such an infestation should be detected at the next time of sampling (presumably one week later at high temperatures). At temperatures of 15ºC and below the same level of

protection is offered by only sampling every two weeks on a compound population increase basis." There is some sleight of hand here inasmuch as we are unable to equate population growth of aphids with the contamination of plants, i.e. we do not know anything about the infestation rate of new plants by growing colonies. Consequently any decision on sampling / spraying has to be based on population increase, a somewhat indirect measure. However, the significance of the above is that we can be more prescriptive about when to sample, simply by monitoring the temperature (as well as using forecast temperatures), so that we can time our sampling to coincide with our expectation of a particular level of aphid development.

2. Cost of missed samples, particularly at low temperature

If we sample the crop, looking at 500 lettuces, and we don't see any aphids, then our 'best' estimate is that there are no aphids – but how reliable a guess is it? If we sample only 50 lettuces, and observe none, then our estimate is still zero but we probably have more 'confidence' in the first estimate than the second, the greater sample size offering more credence to the estimate. In fact, Table 6 shows how this can work. The second column of Table 6 shows the worst infestation rate that is still consistent with observing no aphids for a given sample size. So, Table 6 tells us that if we sample 50 times, then one time out of 20 we could have an infestation rate as high as 5.8%, which could still provide us with no infested samples (because of the random nature of infestation) – obviously, if we had 6% infestation we would expect about 3 infested lettuces; but we could have 2 or 4, 1 or 5, and as we have just seen even none (approximately 5% of the time). By increasing the sample size we can protect against that outcome, and effectively force the 'worst case scenario' down to 0.6% (with 500 samples).

Table 6: Probabilities (expressed as percentages) of observing no infected plants when the infestation level is at a given threshold for a range of sample sizes – equivalent to the risk of not detecting a problem at a given threshold.

Sample size	Infestation threshold			
	5%	**1%**	**0.5%**	**0.1%**
30	21.46	73.97	86.04	97.04
50	7.69	60.50	77.83	95.12
75	2.13	47.06	68.66	92.77
100	0.59	36.60	60.58	90.48
125	0.16	28.47	53.44	88.24
150	0.05	22.14	47.15	86.06
175	0.01	17.22	41.60	83.94
200	0.00	13.40	36.70	81.86
250	0.00	8.11	28.56	77.87
300	0.00	4.90	22.23	74.07
400	0.00	1.80	13.47	67.02
500	0.00	0.66	8.16	60.64

In practice, sampling bears two costs: a fixed cost (essentially a 'call-out' cost) and a time cost which is dependent on the sample size. So, the marginal cost of taking a larger sample is relatively small, but the benefit lies in providing protection against a high infestation rate that might not be spotted. By guaranteeing that the underlying infestation rate is small, we can perhaps moderate the decisions we make.

Table 7: Expected percentage of infected plants for given sample sizes and outcomes (i.e. number of observed number of infested plants). Note that this differs from Table 10 of the previous report (Jacobson, 2002) where we considered a 'worst possible' outcome.

Sample size	No. of infested plants				
	0	**1**	**2**	**3**	**4**
30	0.0	3.3	6.7	10.0	13.3
50	0.0	2.0	4.0	6.0	8.0
75	0.0	1.3	2.7	4.0	5.3
100	0.0	1.0	2.0	3.0	4.0
125	0.0	0.8	1.6	2.4	3.2
150	0.0	0.7	1.3	2.0	2.7
175	0.0	0.6	1.1	1.7	2.3
200	0.0	0.5	1.0	1.5	2.0
250	0.0	0.4	0.8	1.2	1.6
300	0.0	0.3	0.7	1.0	1.3
400	0.0	0.3	0.5	0.8	1.0
500	0.0	0.2	0.4	0.6	0.8

Table 7 shows the 'best' estimate of what the outcome will be if we sample 0, 1, 2, etc. infested lettuces for different sample sizes. Note that if we sample no infested plants then our best estimate is zero regardless of the number sampled, but, as we have already noted, this does not hold for the 'worst case', i.e. column 2 of Table 6**.** So, if we sample more, then we improve our protection against poor outcomes (and wrong decisions!). This also means that we may be able to relax the stringency of the sampling outcome, if we can still be sure that the infestation rate is low. This is spelt out more directly in Box 2.

Box 2: Offsetting spraying with sampling

We saw in Box 1 how the growth rate of aphids can be related to temperature. Another way of thinking of this is in terms of how long it takes for the aphid population to triple. At 15ºC it takes approximately 8 days, at 20ºC 5½ days, and at 25ºC around 4 days.

Thus far we have assumed that we should spray if any infested lettuce is observed. Table 6 shows that if we observe no infestations then our 'best guess' of the proportion infested is zero, although we know that the more we sample the better our guarantee of getting close to that. If, instead we relax the criterion for spraying to some low proportion of infestation we can consider allowing a low number of non-complying lettuces. In the Table below an infestation of 1% allows the number of failures to be proportional to the sample size; the Table also shows the level of protection given by the sample size

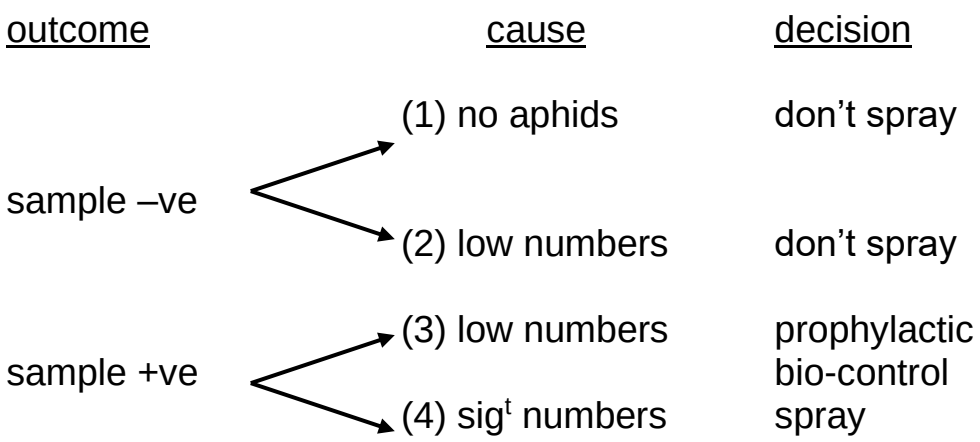
sample size	allowed 'failures'	worst case %age
100	1	4.4
200	2	2.9
300	3	2.3
400	4	2.1
500	5	2.0

Another way of interpreting Table 6 is to look at an individual column. Take, for example, the column for one infested plant. As with the small Table above, one infested lettuce on a sample size of 100, gives an expected infestation rate of 1%. Table 2 shows that the anticipated proportion of infested lettuce can be reduced to one-third by taking three times as many samples. So, if instead of sampling 100 lettuces and allowing one 'failure', we sample 300 and only allow one failure, this is equivalent to a saving of 5½ days at 20ºC – the time it would take the population to treble.

4. Conclusions: decisions based on sampling outcomes

In the original recommendation, if any aphids were found, the crop was sprayed. In this report we have considered less drastic decisions, e.g. watchful waiting, if the incidence level of the pest is low and its prospective rate of growth is slow, delay making a decision. In other words we can refine the original recommendation and make our sampling more dependent on actual (or even forecast) temperatures. Figure 6 presents a simple outcome and decision model where the decisions are refined. Here we also make explicit the idea that, even if no aphids are detected in the sample, it doesn't mean that they are absent.

Figure 6: Crop sample outcomes and decisions



So, suppose that we sample the crop and find no aphids – this can mean one of two things: that there are no aphids, or (assuming we have sampled at a sufficiently extensive level) that there are a few aphids which we have been unable to detect. In the second instance the next sample should be taken according to how quickly we might expect that population to grow, and that will depend on temperature. In our earlier proposal we felt that a 10-fold increase (this was a pessimistic assumption of growth rates!) in the potential population would require action – this would occur in one week at high temperatures (20 to 25ºC), but would take two weeks at 15ºC, and even longer, if at all, at very low temperatures. This prediction model can be enhanced in two ways:

1. by 'logging' the actual temperatures and calculating the intrinsic aphid population growth, and then re-sampling when the predicted growth reaches a certain threshold;
2. by using current weather forecasts of temperature (fairly dependable up to 5 – 7 days ahead) to predict when the next sample is due.

This allows us to refine the sampling schedule, from an approximate seasonal-based one to one based on actual temperature patterns.

On the other hand suppose that we find aphids in the crop at a particular sample. If the incidence is very low, we may not want to incur the cost of spraying, perhaps choosing to rely on 'watchful waiting', i.e. monitoring temperature and re-sampling at a point where we feel that the population may have grown to a level where we must intervene. The general principles behind this are described above.

The use of prophylactic bio-control also means that we may be able to delay the need for intervention. Early results suggest that at low levels of aphid presence, parasitoids are able to slow down development rates by a factor of two. Hence, using the aphid growth algorithms we should be able to let the 'nominal' aphid growth increase two-fold, that is, instead of intervening when the aphids have grown 5-fold, we can let them grow 10-fold as we anticipate that the parasitoids will have held them back.

5. Cautions / additional risk factors

As commented earlier it is only when some of these ideas are put together that there is a realisation of how poor some of the information is that we base our decisions on. Some of the areas that impinge on this are outlined below:

1. In the first report we drew attention to the fact that the model we use for sampling is based on what is called the binomial distribution, whereas we have reason (empirical evidence) to assume that aphids do not behave as independent operators. Nevertheless, without information on the level of aggregation (and this is generally quite variable) we are prepared to use the binomial model knowing that it is likely to be conservative

2. Aphid growth rates are based on an old data-set from the literature, using different aphids in different environments. However, it is practically (and economically) impossible to calibrate every glasshouse for every aphid species likely to attack it. So, the information we have acts as a proxy for what we do not know. It could be very useful to monitor how good this model is in practice.

3. Changes in aphid growth rate can not necessarily be equated to lettuce numbers infested. de Courcy Williams (private communication) suggests that the reasons for aphids migrating between plants are complex and not always consistent, so again, we have to rely on aphid growth rate as being a sort of proxy.

In the light of the above qualifications one would venture that the evidence of the behaviour of the sampling plan would be very valuable. Indeed, one could see considerable benefits in eliciting information from growers which would enable a more sophisticated model to be built.

6. Cost of different sampling scenarios

The following costs are based on five crops per annum (approximate planting dates, harvesting dates and duration are shown in Tables 7-9), with different inspection schedules. As in the previous report (Jacobson *et al.*, 2003) we have considered costs based on samples of 100, 200, 300 or 500 plants per 1000m^{2}'.

The first schedule is essentially that recommended in the first report, i.e. sampling at weekly intervals in the summer and fortnightly during the winter. The second uses measured temperatures to drive the aphid growth model to determine the point at which the initial population will have grown five-fold, when sampling recurs. The third schedule allows for a 10-fold growth on the assumption that prophylactic bio-control will hold back the aphid growth rate. The calculations below are based on the monitored data in PC132 (Jacobson, 2002). Costs will obviously vary from year to year, but the overall temperature profiles through a full year will be roughly similar, so the costs should be reasonably realistic.

Table 7: *Schedule 1:* Inspections at two week intervals between October and April (inclusive) and at weekly intervals between May and September (inclusive).

Crop planting & harvesting dates	Duration **(wks)**	**Number of inspections**	**Cost (£) per crop at the following sampling frequencies (plants / 1000m^2):**			
			100	**200**	**300**	**500**
8/3 – 11/5	9	5	55	110	166	278
1/6 – 5/7	5	5	55	110	166	278
25/7 – 28/8	5	5	55	110	166	278
1/9 – 20/10	8	5	55	110	166	278
23/10 – 16/2	16	8	87	178	266	444
Total per annum	43	28	307	618	930	1556

Table 8: *Schedule 2:* Inspections based on cumulated intrinsic growth rates involving 5-fold aphid growth – See Box 1 in text for details

Crop planting & harvesting dates	Duration (wks)	Number of inspections	Cost (£) per crop at the following sampling frequencies (plants / 1000m^2):			
			100	200	300	500
8/3 – 11/5	9	2	22	44	66	110
1/6 – 5/7	5	3	33	66	99	165
25/7 – 28/8	5	4	44	88	132	220
1/9 – 20/10	8	4	44	88	132	220
23/10 – 16/2	16	1	11	22	33	44
Total per annum	43	14	154	308	462	769

Table 9: *Schedule 3:* Inspections as for schedule 2, except that an intrinsic growth rate of 10 is allowed to acknowledge the impact of prophylactic bio-control which effectively halves the aphid growth rate.

Crop planting & harvesting dates	Duration (wks)	Number of inspections	Cost (£) per crop at the following sampling frequencies (plants / 1000m^2):			
			100	200	300	500
8/3 – 11/5	9	1	11	22	33	55
1/6 – 5/7	5	2	22	44	66	110
25/7 – 28/8	5	3	33	66	99	165
1/9 – 20/10	8	4	44	88	132	220
23/10 – 16/2	16	1	11	22	33	55
Total per annum	43	11	121	242	363	605

Schedule 2 (basic temperature monitoring) suggests a 50% saving in sampling costs, with a further 10% reduction using prophylactic bio-control. It is strongly suggested that in the coming year the model is tested by monitoring more frequently than the model would suggest simply to validate the model predictions.

Acknowledgements

The authors are grateful to Peter Hardwick and Ray Blackburn for their continued commitment to this subject and for continuing to allow their glasshouses to be used for the commercial crop trails.

References

Glinwood, R.T., Powell, W. and Tripathi, C.P.M. (1998). Increased parasitization of aphids on trap plants alongside vials releasing synthetic aphid sex pheromone and effective range of the pheromone. *Biocontrol Science and Technology*, 8(4): 607-614.

Jacobson, R. J. (2000). Early season control of tomato leaf miner. *HDC Fact Sheet*, 08/00. 4pp

Jacobson, R.J. (2002). Protected lettuce: An integrated approach to aphid and caterpillar control. *Report of contract work undertaken for HDC (Project PC132)*, March 2002, 44 pp.

Jacobson, R. J. and Croft, P. (1998). Strategies for the control of *Aphis gossypii* Glover (Hom. Aphididae) with *Aphidius colemani* Viereck (Hym. Braconidae) in protected cucumbers. *Biocontrol Science and Technology*, 8 (3), 377-387.

Jacobson, R. J., Croft, P. and Fenlon, J. (2003). Protected lettuce: Towards insecticide free production, *Report of contract work undertaken for HDC (Project PC194)*, December 2003, 39 pp

de Loach C J (1974). Rate of increase of populations of cabbage, gree, .peach and turnip aphids at constant temperatures. *Annals of the Entomological Society of America*, 67, 332-340.

Maisonneuve, J-C. (2002). Biological control in French ornamentals. *IOBC/WPRS Bulletin*, Vol 25 (1), 155-160.

Powell, W. and Glinwood, T. (1998) Aphid sex pheromones to enhance parasitoid efficiency. *HGCA Cereal Project Report*: 155.

Pradhan S (1946). Insect population studies. IV. Dynamics of temperature effect on insect development. *Proceedings of the National Institute of Science, India*, 12**,** 385-404.

