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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 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.

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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.
- 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).
- Preliminary studies based on the *Aphidius 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 searching behaviour.
- The results of a series of eight experiments indicated that the provision of additional chemical cues in the form of nepetalactone (a component of the aphid sex pheromone) had the potential to improve the performance of *Aphidius ervi* and *Aphidius colemani* in lettuce crops.
- Risk analysis studies have improved our understanding of crop monitoring procedures and the probability of failing to detect aphid populations with different sample sizes. These studies will continue in 2004, balancing the risk of not detecting infestations against the cost of the monitoring exercise.

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 project and main conclusions

1. Developing suitable parasitoid open rearing systems (ORS) to control the main aphid pests of protected lettuce

The prototype ORS kit that utilises *Aphidius colemani* and *A. ervi* against *Myzus persicae* and *Macrosiphum euphorbiae* respectively was chosen for use in protected lettuce crops. A comparable system was chosen for *Aulacorthum solani* using *Aphilinus abdominalis* (and perhaps *Praon volcare*). All of these parasitoids are available as commercial biocontrol products and can be reared on cereal aphids in ORS units. However a new ORS kit had to be developed against *Nasanovia ribisnigri* with an effective parasitoid.

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.

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.

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. 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.
- When an additional large aphid culture (based on an ORS unit without parasitoids) was placed 5m from the lettuce aphids, *A. colemani* located and parasitised the lettuce aphids. This demonstrated that when the cues were sufficiently strong, *A. colemani* could locate small numbers of lettuce aphids at a distance of at least 5m.
- 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 is required to optimise these effects.

2. To develop cost effective crop monitoring procedures for protected lettuce

A 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 is clear that trying to guarantee detection of aphids at very low threshold levels becomes 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 will continue in 2004, relating the risk of not detecting infestations at various levels to the cost of the monitoring exercise. The studies will also take into account the potential to reduce that risk by adopting the prophylacatic approach to parasitoid release. Industry wide discussions (ie involving growers, marketing groups and retailers) will be instigated in 2004 to explore the possibility of sharing the additional production costs that may be incurred by moving closer to insecticide-free production systems.

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. This project will develop cost effective crop monitoring procedures and 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.

Action points for growers

None at this stage in the project.

Science Section

GENERAL INTRODUCTION

The glasshouse lettuce industry is currently worth around £20m per year at wholesale level and £35m at retail level. The 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 (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 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.
- a number of 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 require 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 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 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) (also known as banker plants) 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.

Overall aim and 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. The specific objectives were to:

1. develop suitable parasitoid ORS units to control the principal aphid pests of protected lettuce.
2. evaluate the parasitoid ORS units in protected lettuce crops.
3. develop cost effective crop monitoring procedures for protected lettuce.
4. prepare a cost benefit analysis of the whole IPM package.
5. promote the new technologies via industry wide discussions (ie involving growers, marketing groups and retailers).

PART 1: PRELIMINARY DEVELOPMENT AND ASSESSMENT OF AN OPEN REARING SYSTEM.

Background

Development of the open rearing system (ORS) for parasitoids of protected lettuce

The preliminary studies were based on Syngenta Bioline's prototype ORS kits that utilise *Aphidius colemani* and *Aphidius ervi* against *Myzus persicae* and *Macrosiphum euphorbiae* respectively. *Aphidius ervi* has also been shown to attack and successfully complete its development in *Aulacorthum solani*, indicating that this ORS could be used against two species of aphids. Both parasitoids are already available as commercial biocontrol products, which simplified further development.

An additional parasitoid was required for use against *Nasanovia 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* Stary. 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. Dr Croft (STC) has secured funds from Defra to investigate important aspects of the biology of both species. Further work is now required to perfect culturing methods and to develop specific ORS rearing units for one or both of these species.

Searching by parasitoids in lettuce crops.

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 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. It is important that they do not abandon this search by choosing to return to the ORS unit or simply dispersing to the glasshouse roof.

Host finding and selection by parasitoids usually involves responses to a hierarchy of stimuli that ultimately bring the parasitoid into contact with its target. These steps to successful parasitisation, which can overlap and are not all obligatory, involve the following behaviours:

1. host habitat location
2. responses to indicators of host presence, often in the form of chemical cues released from the host / plant complex
3. structured searching patterns on aphid host plants
4. host location
5. determining host suitability
6. host acceptance

7. host regulation

A series of empirical preliminary studies used the *A. ervi* ORS to determine whether the parasitoids would locate small populations of *M. euphorbiae* at distances of up to 10m from the ORS unit.

Preliminary practical studies

Materials and method

A single *A. ervi* ORS unit infested with parasitised mummies of *Sitobion avenae* was placed at the eastern side of a 150m^2 glasshouse containing soil-grown lettuce plants. When large numbers of parasitoids were emerging from the mummies, small numbers of *M. euphorbiae* were released on four lettuce plants adjacent to the ORS unit and on four lettuce plants at the furthest point from the unit (*i.e.* 10m distant). After 14 days the numbers of parasitised mummies among the *M. euphorbiae* colonies were recorded. The experiment was replicated in time.

Results and Discussion

Parasitised *M. euphorbiae* were recorded on the lettuce plants adjacent to the ORS unit (mean 20% parasitisation [range 1-50%]) but not among the *M. euphorbiae* at the furthest point from the unit.

The first conclusion to be drawn from these results was that *A. ervi* would readily change their host and attack aphid species that were different to those they had been reared on. This was fundamentally important to the concept of the ORS.

The second conclusion was that *A. ervi* was unable to locate the small numbers of aphids on lettuce plants that were 10m away from the single ORS unit. It would seem probable that the chemical cues produced by that aphid / plant complex were too weak to be detected by the parasitoids; perhaps because there were too few *M. euphorbiae* present or because they were too far away. However, it is also possible that stronger cues from the closer *M. euphorbiae*, or from the ORS unit itself, arrested the parasitoids and prevented them from searching any further.

If the ORS was to be successful in lettuce crops, it was clear that additional techniques would be required to modify the parasitoids behaviour and increase their dispersal in the glasshouse.

PART 2: MANIPULATING THE PARASITOIDS BEHAVIOUR

Introduction

The 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 shown to increase searching activity of some species of parasitoid in the field, including *A. ervi* (Powell & Glinwood, 1998). However, the response with distance from the pheromone can vary with parasitoid species (Glinwood *et. al*, 1998).

Although the technique had potential to improve the performance of the ORS in lettuce crops, it had never been tested within the confines of a glasshouse. Furthermore, the responses of the other parasitoids used in this project (*i.e. A. colemani*, *A. hieraciorum* and *Praon volucre*) were unknown.

A series of six experiments were designed to investigate the possibility of improving the performance of the ORS in lettuce crops using nepetalactone to manipulate the searching behaviour of the parasitoids

Experiment 1. Effect of nepetalactone on direction of flight of *A. ervi* and *A. colemani* in the absence of aphid hosts

The aim of this experiment was to determine whether nepetalactone would influence direction of movement of the two parasitoid species, *A. ervi* and *A. colemani*, in a glasshouse crop in the absence of the aphid host. Preliminary studies showed that both species could be trapped with yellow sticky traps (although only a small percentage of those released were caught) and such traps were used to monitor the dispersal of parasitoids within the glasshouse.

Materials and method

The experiment was done in a single 200m^2 glasshouse containing 1m high pepper plants. The environment was maintained at minimum temperatures of 21ºC day and 19ºC night with ventilation at 24ºC.
Two yellow sticky traps were hung above the crop, 12m apart, in easterly and westerly positions. Single commercial units of *Aphidius colemani* and *A. ervi* (*i.e.* approximately 500

and 250 individuals respectively) were released from the product containers in a southerly position, between the two traps and approximately 9m from each. A pheromone lure was attached to one of the traps. Parasitoids were released at weekly intervals. Before each release, the numbers caught on each trap were recorded and the position of the pheromone lure was changed (*i.e.* between east to west traps).

Results and discussion

The mean percentages of parasitoids on traps with or without pheromone lures (expressed as a percentage of the total caught) are shown in Table 1. The percentage recorded on the traps with pheromone was higher but this was not significant ($P<0.05$).

Many insect species demonstrate positive phototaxic responses and it was considered possible that the position of the sun may have had an overriding influence on the parasitoids' direction of flight. This possibility was further explored by comparing the catches of parasitoids on easterly and westerly traps in the absence of pheromone (Table 2). It was clear that the easterly traps were strongly favoured by *A. colemani* with twice as many caught in that position ($P>0.05$). However, the results were not conclusive for *A. ervi* because too few individuals were caught on the traps.

This experiment was done in the absence of aphids and it was possible that the parasitoids would disperse more readily when no insect hosts were present. It was also possible that they would be more likely to respond to alternative stimuli, such as the position of the sun, in the absence of insect hosts. The latter could also be the case at low aphid population densities when the chemical cues from the aphid / plant complex are assumed to be relatively weak. These factors were addressed in subsequent experiments.

Table 1. The mean percentage of parasitoids (*i.e.* from the total caught) on yellow sticky traps with or without pheromone

	A. ervi (28)		*A. colemani* (66)	
	Pheromone	None	Pheromone	None
Mean	70.1	29.9	52.0	48.0
sd	27.6	27.6	33.7	33.7

Table 2. The mean percentage of parasitoids (*i.e.* from the total caught) on traps positioned to the east and west of the point of release

	A. ervi (15)		*A. colemani* (45)	
	East	West	East	West
Mean	72.7	27.3	72.2	27.8
Sd	38.6	38.6	24.1	24.1

Experiment 2. Effect of nepetalactone on distance of flight of *A. ervi* and *A. colemani* in absence of aphid hosts

In the previous experiment, it was impossible to separate the effects of the position of the pheromone and the position of the sun on the direction of parasitoid flight.

The aim of this experiment was to determine whether nepetalactone would influence the direction and distance of flight of *A. ervi* and *A. colemani* in a glasshouse in the absence of aphid hosts. The influence of the position of the sun was reduced by releasing from one side, rather than from the centre, of the glasshouse.

Materials and method

The experiment was done in a single 200m^2 glasshouse containing 1.2m high pepper plants. The environment was maintained at minimum temperatures of 21^{o}C day and 19^{o}C night with ventilation at 24^{o}C.

Fifteen yellow sticky traps were placed in the glasshouse at the top of the crop canopy (Figure 1). Single commercial units of both *A. ervi* and *A. colemani* were released from the centre of the west side of the glasshouse and the pheromone was placed at the centre of the east side of the glasshouse. Four days after release the numbers of parasitoids caught on the yellow traps were recorded. The trial was done twice with pheromone and twice without pheromone.

Results and discussion

For both species, the total numbers caught on traps were similar and independent of whether pheromone was present in the glasshouse (*A. colemani* – 31, *A. ervi* - 7) or not (*A. colemani* - 28, *A. ervi* – 10). The numbers (expressed as a percentage of the total catch) of *A. colemani* and *A. ervi* caught on traps in different positions in the glasshouse, with and without nepetalactone, are shown in Table 3.

Aphidius colemani was more even distributed across the glasshouse when nepetalactone was present, suggesting that the pheromone did increase this parasitoid's mobility and dispersal. This was not the case with *A. ervi*. The latter was surprising because nepetalactone had been previously shown to increase searching activity of *A. ervi* (Powell & Glinwood, 1998). However, it is now known that *A. ervi* is difficult to catch on sticky traps and the poor result may have been at least in part due to the choice of this experimental technique. It is also possible that the pattern of searching cues, in terms of plant and host location, maybe different for this species (Powell & Glinwood, 1998). For example, it is known that *A. ervi* uses honeydew as part of its host location sequence and this was absent in this trial. Subsequent studies were done with aphids in the glasshouse.

Figure 1. The position of fifteen yellow sticky traps used to monitor the dispersal of *A. ervi* and *A. colemani* following their release in a glasshouse containing pepper plants

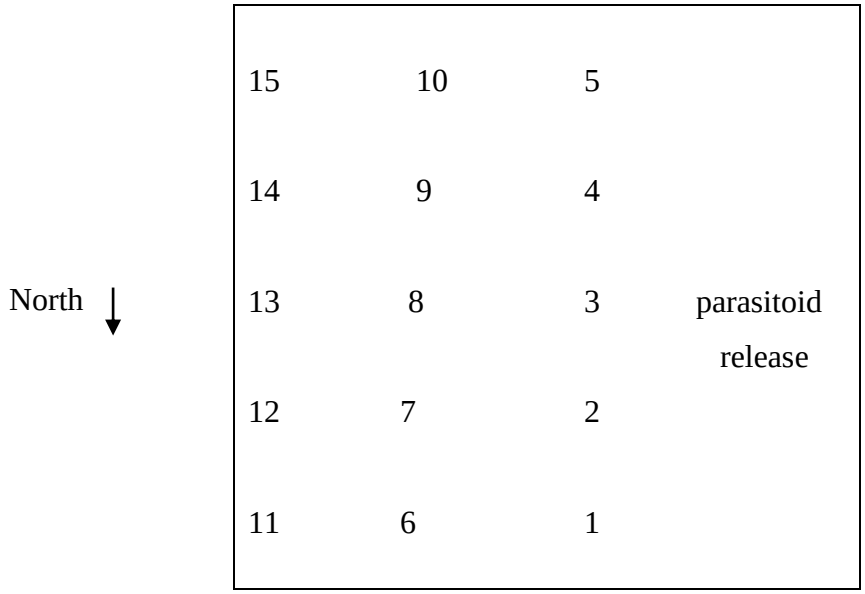


Table 3. The effect of nepetalactone on the mean percentage (±sd) (*i.e.* from the total caught) of *A. colemani* and *A. ervi* on yellow sticky traps

	A. colemani		*A. ervi*	
Traps	Pheromones	No pheromones	Pheromones	No pheromones
1-5	42 (2.7)	83 (7.7)	61 (27.1)	40 (9.8)
6-10	21 (1.3)	4 (5.7)	13 (17.7)	29 (6.3)
11-15	37 (4.0)	14 (2.1)	27 (9.4)	31 (2.8)

Experiment 3. Effect of nepetalactone on the ability of *A. ervi* and *A. colemani* to locate small numbers of aphid hosts in a lettuce crop.

The preliminary practical studies demonstrated that *A. ervi* and *A. colemani*, reared in ORS units, were unable to locate small numbers of aphids at a distance of 10m in a lettuce crop. Experiment 2 showed that the presence of the pheromone, nepetalactone, increased movement of *A. colemani* across a glasshouse in the absence of aphids. This experiment was designed to determine whether the presence of the pheromone would also improve the parasitoids' ability to locate small numbers of aphids in the crop.

ORS units took up to 4 weeks to become productive, which slowed the rate of experimentation and limited the number of replicates that could be completed within each glasshouse during the growing season. To facilitate more rapid progress, a new technique was introduced which involved releasing parasitoids into aphid infested ORS units, thus mimicking natural emergence. The layout of the trial also reduced the possibility of parasitoid behaviour being influenced by the direction of the sun.

Materials and method

The experiment was done during the summer of 2003 in two identical glasshouses (each 150m^2) containing lettuce crops grown in the soil at ambient temperature.

Two ORS units infested with either *S. avenae* or *R. padi*, but not parasitoids, were placed at the west side of each glasshouse. Approximately 10 late instar / adult *M. persicae* and *M. euphorbiae* were released on separately labelled lettuce plants at the opposite side of each glasshouse (*i.e.* 10m from the ORS units). In addition, one glasshouse contained a pheromone lure positioned 10m from the ORS unit and 1m from the aphid infested lettuce plants.

After 24 hours, single commercial units of *A. ervi* and *A. colemani* were released into the appropriate aphid infested ORS unit. After a further 14-21 days, mummified aphids were recorded in the ORS units and among the *M. persicae* and *M. euphorbiae* colonies. The experiment was replicated in time.

Results and discussion

Parasitised aphids, of both species, were recorded in the ORS units but not among the *M. persicae* and *M. euphorbiae* colonies in either of the glasshouses. The pheromone had not therefore improved the ability of the parasitoids to locate small numbers of aphids at a distance of 10m in the lettuce crop.

It would seem that the parasitoids had not picked up the chemical cues from the *M. persicae* / *M. euphorbiae* / plant complexes; perhaps because there were too few aphids or because they were too distant. Glinwood *et. al* (1998) had reported that the effect of the pheromone was limited to certain distances in some parasitoid species and this may have been the case here. Alternatively, the stronger cues from the aphids in the ORS units may have arrested the parasitoids and stopped them searching over greater distances.

The results suggested that additional chemical cues were required to help the parasitoids to locate small numbers of aphids on the lettuce crop and this prompted two further experiments.

Experiment 4. Introduction of stronger cues to help *A. ervi* and *A. colemani* locate small numbers of aphid hosts in a lettuce crop.

Experiment 2 showed that nepetalactone increased the movement of *A. colemani* in a glasshouse in the absence of aphids. However, in experiment 3 the pheromone did not improve the ability of either *A. ervi* or *A. colemani* to locate small numbers of aphids at a distance of 10m from ORS units in a lettuce crop. In this experiment, stronger cues were applied to draw the parasitoids away from the ORS units.

Materials and methods

The experiment was done during the summer of 2003 in two identical glasshouses (each 150m^2) containing lettuce crops grown in the soil at ambient temperature.

Two ORS units infested with either *S. avenae* or *R. padi*, but not parasitoids, were placed at each side (*i.e.* east and west) of each glasshouse. They were 10m apart from east to west. Approximately 10 late instar / adult *M. persicae* and *M. euphorbiae* were released on separately labelled lettuce plants between the ORS units in the centre of the glasshouse. In addition, one glasshouse contained a pheromone lure positioned just above the crop about 1m from the aphid infested lettuce plants.

After 24 hours, single commercial units of *A. ervi* and *A. colemani* were released into the appropriate aphid infested ORS unit on the west side of the glasshouse. The release tubes were laid horizontally in the ORS unit thus ensuring that the parasitoids encountered the aphids on the cereal plants.

After a further 14 days, mummified aphids were recorded in the ORS units and among the *M. persicae* and *M. euphorbiae* colonies. The experiment was replicated in time.

Results and discussion

Parasitised aphids were found on all the ORS units in both glasshouses. This showed that when the cues were sufficiently strong, both *A. ervi* and *A. colemani* were able to locate aphids at distances of 10m in the glasshouse. In doing so, the parasitoids must have been drawn past the smaller populations of aphids on the lettuce plants in the centre of the glasshouse.

The presence of parasitised *M. persicae* and *M. euphorbiae* on the infested lettuce plants is summarised in Table 4. *Aphidius ervi* only located these small populations of lettuce aphids when assisted by the pheromone. This was broadly consistent with the findings of Glinwood *et. al*. (1998) who suggested that the two different foraging cues (*i.e.* nepetalactone and host / plant complex) provided an additive effect.

Aphidius colemani located the small populations of lettuce aphids in one replicate with and without the presence of nepetalactone. These results confirm that *A. colemani* is easier to manipulate and recover than *A. ervi* (as found in experiment 2), and will readily locate their host with the provision of some additional cues.

Overall, the results suggest that using a combination of stronger chemical cues (*i.e.* the pheromone and host / plant complex) increased parasitoid movement and searching across the crop and this had enabled both *A. ervi* and *A. colemani* to locate small numbers of lettuce aphids at distances of 5m from their point of release. However, that distance is relatively small when considered in the context of a commercial lettuce crop and would probably necessitate the use of too many ORS units. The studies therefore progressed to commercial crop scale (Experiment 6).

Table 4. The effect of the presence of nepetalactone on parasitism of *M. persicae* and *M. euphorbiae* by *A. ervi* and *A. colemani* respectively

Replicate	The presence (✔) of parasitised mummies on lettuce plants			
	Pheromone		No pheromone	
	A. colemani	*A. ervi*	*A. colemani*	*A. ervi*
1	✔	✔	✔	-
2	-	✔	-	-

Experiment 5. Effect of nepetalactone on the ability of *A. ervi* and *A. colemani* to locate small numbers of lettuce aphids in the absence of ORS units

The aim of this experiment was to determine whether the performance of *A. ervi* and *A. colemani* could be enhanced by the presence of nepetalactone so that they became effective against small numbers of aphids without resorting to the use of the ORS. The method also assessed the performance of the parasitoids over time to see if the presence of the pheromone reduced the time taken to locate aphids.

Materials and methods

The experiment was done during the summer of 2003 in three identical glasshouses (each 150m^2) containing lettuce crops grown in the soil at ambient temperature.

Approximately 10 *M. persicae* and 10 *M. euphorbiae* were placed on separate lettuce plants at the eastern end of each crop. After 24 hours, single commercial units of *A. ervi* and *A. colemani* were released on lettuce plants at the western end of each crop (*i.e.* 10 m from the aphids). In one glasshouse, two pheromone lures were placed at even distances between the aphids and the parasitoids (*i.e.* 3.3m apart). The presence of parasitised aphids was recorded 14 and 21 days after parasitoid release.

Results and discussion

The presence of parasitised *M. persicae* and *M. euphorbiae* on lettuce plants at the eastern end of each crop is summarised in Table 5. Both species of parasitoids successfully located their aphid hosts when assisted by the presence of nepetalactone lures at 3.3m intervals. This is broadly consistent with the suggestion by Glinwood *et. al*, (1998) that the response to the pheromone is fairly local for some species of parasitoid.

The experiment was not designed to quantify the response of the parasitoids. However observations on the numbers of *M. euphorbiae* / *A. ervi* mummies retrieved, showed that there were considerably more in the pheromone-treated glasshouse than in the non-pheromone house, where only a single mummy was recorded. The results of this experiment suggest that the pheromone, distributed at regular intervals, will assist *A. ervi* to locate small numbers of lettuce aphids.

Mummies of *M. persicae* / *A. colemani* were found in the pheromone house and in the non-pheromone houses, but they were a week later in the latter. Although these results show that *A. colemani* is able to locate aphids in the absence of the pheromone, the pheromone would appear to speed up the process. The latter could be crucial in commercial lettuce crops where the pests must be held at low population densities.

Table 5. The effect of the pheromone nepetalactone on parasitisation of low densities of lettuce aphids

Pheromone present or absent	Replicate	The presence (✔) of parasitised mummies on lettuce plants			
		1st assessment		2nd assessment	
		A. ervi	*A. colemani*	*A. ervi*	*A. colemani*
No pheromone	1		-	-	✔
	2	✔	-	-	✔
Pheromone	1	✔	✔	✔	✔

Experiment 6. Movement of *A. ervi* and *A. colemani* across a commercial lettuce crop with the assistance of ORS units and nepetalactone

Results in experiments 4 and 5 indicated that a strong chemical stimulant from aphids on two ORS units (at opposite ends of a glasshouse) and / or nepetalactone would enhance *A. ervi* and *A. colemani* dispersal and assist them to locate small numbers of aphids in a lettuce crop. However, this work had been done in experimental glasshouses and the parasitoids had only been required to move relatively small distances.

The aim of this series of experiments (6A, 6B and 6C) was to establish the distances parasitoids could be expected to move across a commercial glasshouse and to determine whether this could be enhanced by the presence of nepetalactone. The experiments were also intended to provide some guidance on the number of ORS units that would be required in a commercial glasshouse.
The work was done in commercial lettuce crops (each 1000m^2) at Mr Peter Hardwick's nursery, Snaith, Yorkshire.

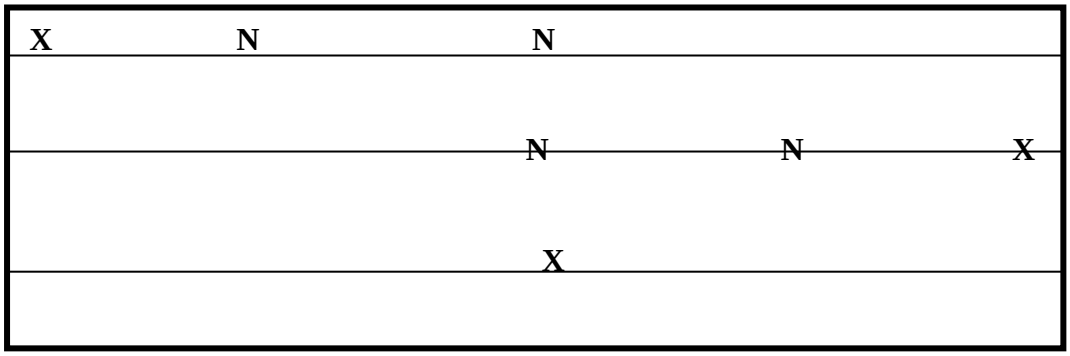
Nepetalactone was not used in experiment 6A because we were waiting for clearance from PSD to use it in a commercial crop.

Materials and methods

Experiment 6A.
This experiment determined whether *A. ervi* and *A. colemani* could locate aphid cultures positioned 17.5m apart. The cultures were based on ORS units infested with *R. padi* and *S. avenae* but without parasitoids. Three cultures were placed along a single path in a lettuce glasshouse at intervals of 17.5m. *Aphidius ervi* and *A. colemani* were released on the unit at one of the end of the path. After 14 days, the presence of mummified aphids of each species was recorded.

Experiment 6B
R. padi and *S. avenae* cultures were prepared as described in Experiment 6A. Three such cultures were placed in each of two glasshouses as shown in Figure 2. The first culture was placed at the end of a path near the centre of the glasshouse. The second and third cultures were positioned 17.5m down the path immediately to the south, and 35m down the path immediately to the north, respectively. *Aphidius ervi* and *A. colemani* were released on the first culture. In addition, in one glasshouse four nepetalactone lures were placed at crop height along the central and northern paths at approximately 11m intervals. After 14 days, the cultures were removed and the presence of parasitised mummies of each species was recorded on each.

Figure 2. The positioning of three aphid infested ORS units and pheromone lures in a commercial glasshouse (Experiment 6B)



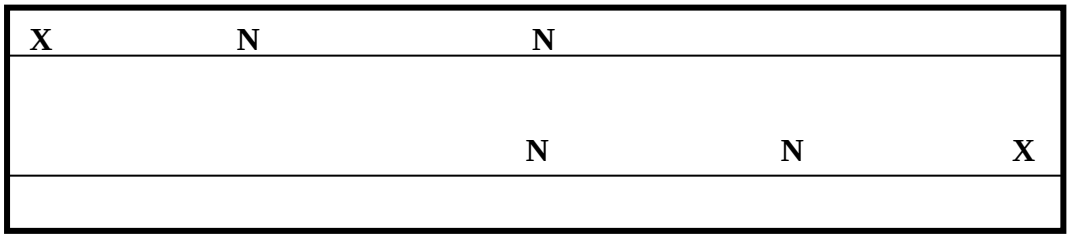
X = ORS unit

N = pheromone lure

Experiment 6C.

R. padi and *S. avenae* cultures were prepared as described in Experiment 6A and two were placed in each of two glasshouses as shown in Figure 3. The first culture was placed at one end of a path near the centre of the glasshouse and the second culture was placed in the adjacent path at the opposite end (35m away). *Aphidius ervi* and *A. colemani* were released on the first culture. In addition, in one glasshouse four nepetalactone lures were placed at crop height along the two paths at approximately 11m intervals. After 14 days, the cultures were removed and the presence of parasitised mummies of each species was recorded on each.

Figure 3. The positioning of two aphid infested ORS units and pheromone lures in a commercial glasshouse (Experiment 6C)



X = ORS unit

N = pheromone lure

Results and discussion

Experiment A

The presence of *A. ervi* and *A. colemani*, as mummified aphids on the aphid cultures, are shown in Table 6. Both species of parasititoids located aphids at a distance of 17.5m but neither reached the aphids at 35m. It is possible that their searching may have been arrested or slowed by the unit at 17.5m.

Experiment 6B and 6C

The presence of *A. ervi* and *A. colemani*, as mummified aphids on the aphid cultures, in experiments 6B and 6C are shown in Tables 7 and 8 respectively. Both *A. ervi* and *A. colemani* were able to locate aphid hosts 17.5m and 35m from the points of release, in the presence or absence of pheromones.

The above trials were not designed to quantify numbers of mummies on the aphid cultures. However, it was observed that the numbers of mummified aphids at 35m in experiment 6C were considerably lower than those observed for other aphid cultures and this was most notable in the glasshouse unit without the pheromone.

Table 6. Experiment 6A - The presence (✔) or absence (-) of *A. ervi* and *A. colemani* (as mummified aphids) on aphid cultures in a commercial lettuce crop.

Position of ORS unit	Presence(✔) or absence (-) of parasitoids	
	A. ervi	*A. colemani*
0 m (release unit)	✔	✔
17.5m	✔	✔
35.0m	-	-

Table 7. Experiment 6B - The presence (✔) or absence (-) of *A. ervi* and *A. colemani*, as mummified aphids, on aphid cultures at set distances from the parasitoid release point

Position of ORS unit	B			
	Pheromone		No pheromone	
	A. ervi	*A. colemani*	*A. ervi*	*A. colemani*
0.0 m.	✔	✔	✔	✔
17.5m	✔	✔	✔	✔
35.0m.	✔	✔	✔	✔

Table 8. Experiment 6C - The presence (✔) or absence (-) of *A. ervi* and *A. colemani*, as mummified aphids, on aphid cultures at set distances from the parasitoid release point

Position of ORS unit	**C**			
	Pheromone		No pheromone	
	A. ervi	*A. colemani*	*A. ervi*	*A. colemani*
0 m.	✔	✔	✔	✔
35m.	✔	✔	✔	✔

Conclusions from Parts 1 and 2 of the project

- *Aphidius ervi* emerging from a single ORS unit failed to locate small numbers of *Macrosiphum euphorbiae* 10m from that unit. Hence, additional stimulation was required.

- A series of experiments was designed to improve the knowledge of *A. ervi* and *A. colemani* searching behaviour, with particular emphasis on low host densities within the glasshouse environment. A component of the aphid sex pheromone nepetalactone, manufactured from cat mint (*Nepeta cataria*), was incorporated into the experimental programme in an attempt to improve the parasitoids' performance.

- In the early stages of this programme, nepetalactone was found to influence the movement of *A. colemani* in a glasshouse crop in the absence of aphid hosts. However, the influence on the direction of movement of *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.

- When an additional large aphid culture (based on an ORS unit without parasitoids) was placed 5m from the lettuce aphids, *A. colemani* located and parasitised the lettuce aphids. This demonstrated that when the cues were sufficiently strong, *A. colemani* would parasitise small numbers of lettuce aphids at a distance of at least 5m.

- 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 clearly indicated that the provision of additional chemical cues would improve the performance of *A. ervi* and *A. colemani* in lettuce crops. However, further experimentation is required to optimise these effects.

PART 3: RISK ANALYSIS OF APHID CONTAMINATION IN PROTECTED LETTUCE AND DEVELOPMENT OF A COST-EFFECTIVE CROP MONITORING PROCEDURE

Outline:

This section was prepared by John Fenlon, following on from PC132 (Jacobson, 2002), and outlines a risk analysis procedure for preventing aphid contamination and damage of glasshouse lettuce. The examples and methodology follow the sampling methods used in PC132 and are based on the assumptions of 0.1ha screened glasshouse blocks. In this report we will look 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 growth to determine the impact of failing to detect insects in routine monitoring. In this last instance growth will be related to temperature and the impact of temperature on risk will also be considered.

Primary Risks:

1. Risks prior to crop establishment.
Results of aphid sampling / observation from PC132 are summarised in Table 9. Essentially, no aphids were found on the plants prior to transplanting (*i.e.* in the propagation house). In the production houses, no aphids were found in water traps in the screened house, but aphids were found in the unscreened house. Some contamination (small colonies of *M. euphorbiae*) was found in the screened house (at the same location) at the end of Crop 1 and during Crop 2. Significant numbers of *N. ribisnigri* were found in both houses at the first assessment of Crops 3 and 4, and these are believed to have been contaminated in transit between propagation and production houses.

Table 9: Summary of aphid contamination of protected lettuce crops in PC132 (Jacobson, 2002)

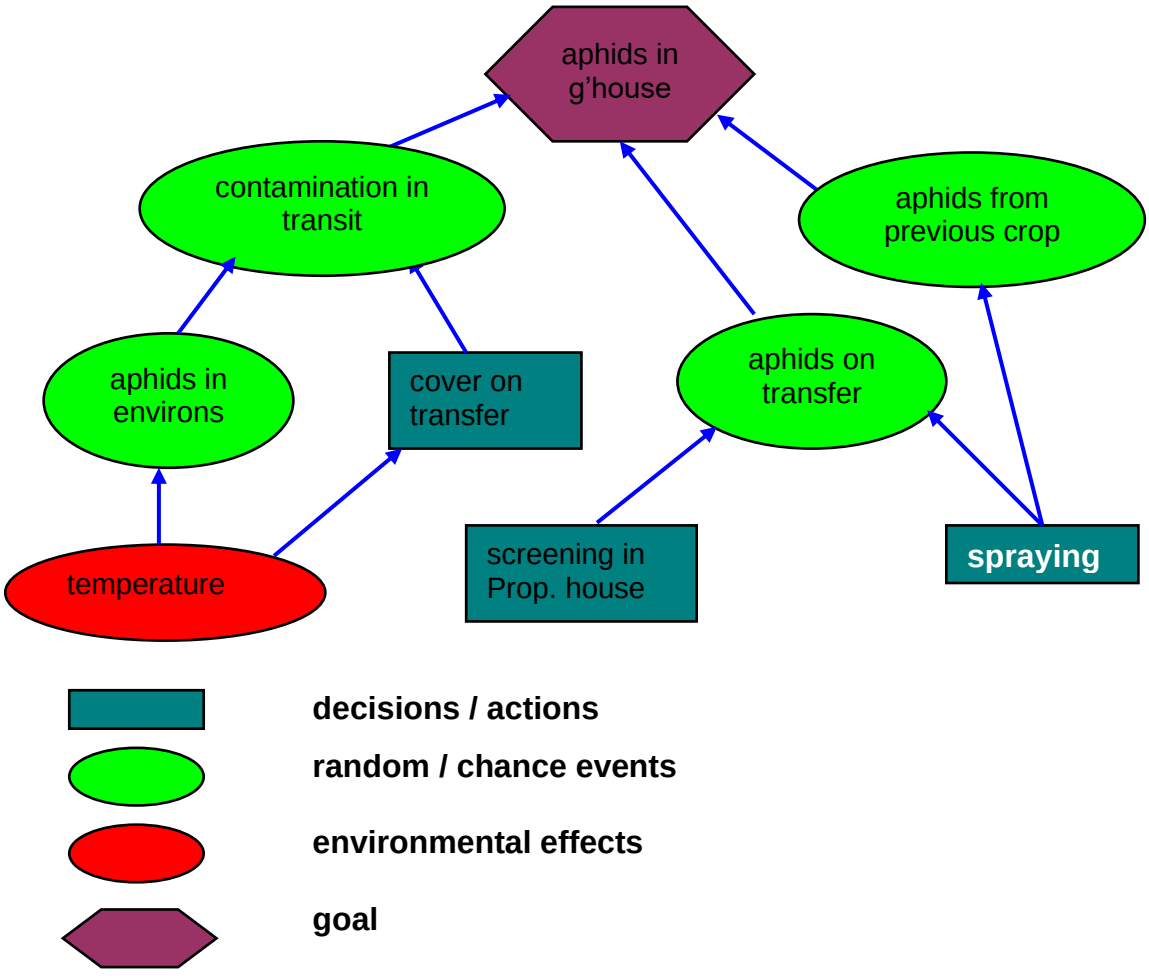
	dates of:			transfer	water traps		on plants		at harvest	
Crop	**planting**	**harvest**	**days**		**scr**	**u/s**	**scr**	**u/s**	**scr**	**u/s**
1	13 Oct	19 Feb	129	X	X	Ns	Me	X	X	X
2	07 Mar	09 May	63	X	X	Me	Me	X	X	X
3	30 May	02 Jul	33	X	X	H.spp	Nr	Nr	√	√
4	20 Jul	28 Aug	39	X	X	As,Nr	Nr	Nr	√	X

It would appear therefore that screening was generally effective in keeping out aphids as evidenced from the water trap sampling. Numbers of aphids found on plants in the screened house was small and appeared to be due to contamination from a previous crop, or infestation in transit between propagation and production houses. The primary risks can therefore be described as follows:

- Ventilators / doors in unprotected glasshouses
- Aphids left over from previous crop
- Aphids brought in from propagation
- Faulty glasshouses
- Aphids carried in on staff or equipment

The impact of screening therefore should be to eliminate primary ingress through doors and vents, and the assumption should be that screened glasshouses would also be defect-free. The risks associated with the stage of production up to and including transfer can be illustrated with the causal diagram in Figure 4:

Fig. 4: An Influence diagram for primary aphid infestation in a glasshouse



Thus, the three primary factors affecting the incidence of aphids in production glasshouses after transfer from propagation are:

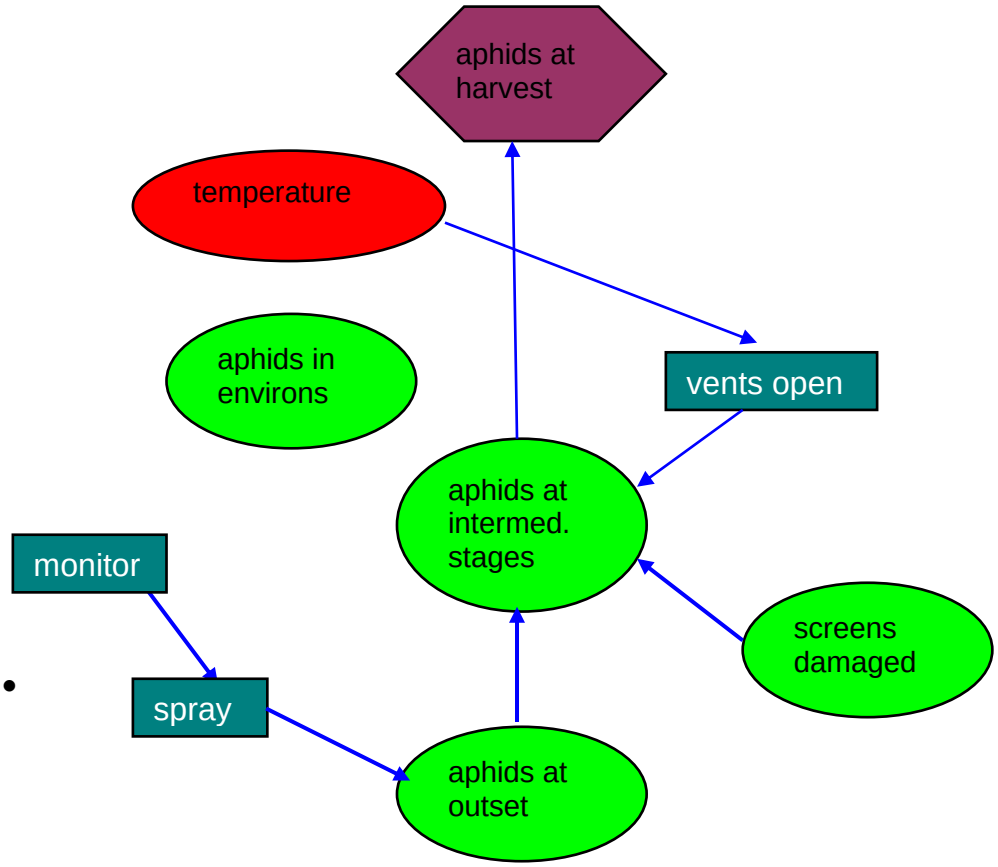
1. aphids in the propagated crop
2. aphids already in the production glasshouse
3. contamination by aphids in transit

The dark green boxes in the diagram show how decision strategies can alleviate or eliminate the risk factors. Indeed the three strategies proposed in Figure 4 show how aphids may be eliminated from the crop on transplanting.

2. Risks during production

Table 9 shows that in the screened glasshouse, apart from contamination of the crop due to residual infestation (in Crops 1 and 2) and aphids brought in on the crop (Crops 3 and 4) there was only a small amount of aphid activity near harvest time in the latter two crops. Obviously there is a strong correlation between time of year and aphid activity, although once established in the crop, aphids will develop according to temperature-based laws (see next Section). However, assuming that there is no physical breach in the screening system of the glasshouse, the only obvious ways in which aphids can establish is from residual populations or by aphids entering the house on staff or equipment. In Figure 5 is presented a further Influence Diagram representing the causes of aphid establishment within the production crop of a screened glasshouse.

Fig. 5: Influence diagram for aphid infestation in a screened glasshouse



Crop sampling

Assuming that all relevant precautions have been taken, and that a production glasshouse has been screened, one could make the assumption that aphid ingress will not occur. At one level this could be regarded as a high-risk – low-probability strategy, but would not be acceptable to many growers who will be risk-averse in a commercial context. Alternative strategies would be:

- routine prophylactic spraying
- decision-based spraying following crop sampling.

In this section we consider the statistical basis of crop sampling in a typical glasshouse, and the impact of different decision rules. In particular, we shall consider the effects of making the wrong decisions, i.e. spraying when not necessary or failing to spray when we should. Obviously these 'errors' have different impacts. Ignoring the ecological impact of unnecessary spraying, the primary impacts are commercial, so that spraying when not necessary incurs a cost related to product cost and implementation; however, failing to spray may result in significant crop damage due to pest activity which is not detected in time. The following development is in two distinct parts: the first relates to the statistical aspects of sampling: assumptions made by different models, and the chances of failing to detect aphids; the second involves the impact of failing to detect aphids at a given sample in terms of potential damage prior to the next sample.

In PC132 the two glasshouses were approximately 0.1ha in size and contained approximately 25,000 plants each. The houses were divided up into simple 'blocks' of approximately 169 plants, a 'block' being the 13 x 13 array of plants bounded by a consecutive pair of posts and a path – there were approximately 150 'blocks' in each house. The number of 'blocks' could be halved by linking pairs on each side of a path. Although there is no reason to assume anything other than random infestation, it seems sensible given the very systematic layout of the crop and the fact that access is fairly uniform, to use the 'blocks' to stratify (and effectively systematise) the sampling.

Obvious visual damage: Simply walking the crop and looking for any visual damage over the whole area would involve a 200m stroll through each house, unless one had to double-back along each path. This is effectively a survey, and would cover the whole crop. Any suspicion of damage would involve taking appropriate action.

Aphid sampling: The sample size in such situations is usually a compromise between precision and practicality: sampling itself is a cost, both in terms of the time of the sampler, and the cost of lost revenue (if destructive samples are taken). Let us assume that standing on any path the area to right and left between two posts is the natural sampling unit, then we should decide on a sample size in that area before moving on. Using the figures above this gives approximately 75 sampling units per house. If only one lettuce is inspected at random in each sampling unit, and if no aphids are observed, this would be indicative of less than 4% incidence in the whole crop 95% of the time (the probabilities are developed and presented below for different scenarios). Is this sufficient protection? My own inclination would be to take four samples in each area, one (at random) from each of rows 1, 2, 3 and 4 – and, recall, that we are looking at both sides of the path. This pushes the total sample size up to 300, and

means that sighting no aphids means that the 'worst case' is 1% infestation, and that even two aphids means a level below 2%. These are not unreasonable protection levels, particularly if the decision is to spray if any aphid is found.

The above sampling model makes two basic assumptions:

1. That infestation in the crop is totally random;
2. That the four rows closest to the paths are fully representative of all 13 rows in the 'block', i.e. that there is no bias associated with paths or gutters (distant from paths);

The primary reasons for sampling a crop are:

- to determine whether or not a pest is present; and
- if so, to provide information about the extent of the problem.

However, the second reason is not particularly relevant here in that the tolerance level is very low for allowable pest levels, so we will assume that we are only going to consider presence / absence sampling – if we are able to count numbers of pest on infested plants we should have sprayed long ago! So, we can assume that the decision whether or not to treat the crop is essentially guided by the simple presence or absence of aphids in our sample. There is one other decision to be made, which relates to the frequency of sampling – in PC132 sampling occurred every two weeks from November to April, and at weekly intervals for the period May to September.

Table 10: Upper detection limits for a range of sample sizes. The value in each cell is the upper 95% confidence limit for the true infestation level (as a percentage) for a given sample size and an observed number of infested plants.

Sample size	Number of infested plants				
	0	**1**	**2**	**3**	**4**
30	9.5	14.2	18.4	22.3	26.0
50	5.8	8.7	11.3	13.7	16.0
75	3.9	5.9	7.6	9.2	10.8
100	3.0	4.4	5.7	7.0	8.1
125	2.4	3.6	4.6	5.6	6.5
150	2.0	3.0	3.8	4.7	5.5
175	1.7	2.5	3.3	4.0	4.7
200	1.5	2.2	2.9	3.5	4.1
250	1.2	1.8	2.3	2.8	3.3
300	1.0	1.5	1.9	2.3	2.7
400	0.8	1.1	1.5	1.8	2.1
500	0.6	0.9	1.2	1.4	1.7

***The binomial distribution**:* With presence-absence data we usually assume that the number of infested plants follows a binomial distribution. In actual fact this may be a conservative assumption if there is a tendency for infestation to cluster, but it is helpful to use the binomial model as a 'marker'. It is possible to calculate confidence limits for the 'true' infestation level. Presented here are two tables that summarise a couple of the main features associated with binomial sampling. Table 10 is effectively a 'worst case' statement of the potential number of infested plants for a given sample size and observation. We have already seen in the example above (considering the number of 'blocks' to sample) that no aphids in 75 observations ('blocks') would 'guarantee' a worst-case infestation level of 4%, which would decline to approximately 1% if we take 4 samples per block. These values are simply read off from the first column (0 infested plants for a given sample size). In fact, it is only really the first column that is important unless we choose to modify

The second Table (Table 11) shows the probability of observing no infested plants for different sample sizes given a particular infestation threshold. Thus, for example, the risk of not detecting an infestation level of 1% (1 in 100) is 37% (approximately 1 in 3) for a sample size of 100. Doubling the sample size reduces the risk to 13% (closer to 1 in 8).

Table 11: 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

It becomes clear that trying to guarantee detection at very low threshold levels becomes quite costly. From Table 11 one can see an approximate 'rule of thumb' that, for the probability of not observing an infested plant to fall below 5%, then the sample size should be approximately 3 times the inverse of the threshold, e.g. if the threshold is 1% (i.e. 1 in 100) then some 300 samples are necessary to be reasonably sure of finding at least one infested plant.

***The negative binomial distribution**:* The binomial model is an ideal random distribution, whereas in reality, it is much more likely that the distribution is aggregated or clumped. A

negative binomial distribution becomes more appropriate, but, unfortunately, this distribution has an extra parameter to describe aggregation, and it is not easy to produce tables as above. The basic impact is to force the sample size up to achieve the same level of detection. A simple example shows what is happening: suppose there is 4% infestation, then, on a sample size of 75 we would be unlucky not to find an aphid (see Table 10). If, however, infested lettuces were aggregated in simple groups of four, say, we could re-formulate the whole area as plots of four lettuces, and our 'effective' infestation rate is down to 1%, necessitating four times the sample for the same detection level. It is not as bad as that because the stratification of the sample helps to counter this, and, of course, the clustering would never be as regular. Further, on the assumption that the grower is always 'on top of' the aphid problem, new infestations might be anticipated to occur spontaneously, or at random. Nevertheless, the tendency is always to be worse than binomial.

Table 12: Upper detection limits for different levels of aphid aggregation. Columns represent three different distributions: binomial (as in Table 2), and two negative binomial distributions with k^{-1}=0.2 and 1.0. The value in each cell is the upper 95% confidence limit for the true infestation level (as a percentage) when no infested plants are observed for a given sample size.

Sample size	Binomial	NB (1/k=0.2)	NB (1/k=1.0)
25	11	16	76
50	6	8	38
75	4	6	25
100	3	4	19
125	2	3	15
150	2	3	13
175	2	2	11
200	2	2	9
250	1	2	8
300	1	2	6
400	1	1	5
500	1	1	4

Table 12 shows the upper detection limits for different levels of aphid aggregation when no infested plants are observed for a given sample size. For a modest level of aggregation (e.g. k^{-1}=0.2) the protection is poorer than for the simple binomial but not markedly so. Note that as the aggregation increases (k^{-1}=1.0 is quite severe!) even large sample sizes do not provide much protection, but such a distribution would seem unlikely to occur as discussed above.

Aphid population models: The decision model developed in PC132 involved sampling of the crop on a weekly basis between May and the end of September, and taking action (i.e. spraying) if any aphids were found. In this section we consider the justification of this on a more scientific basis, focusing in particular on the impact of temperature on aphid population development, and the risk of a damaging infestation caused by 'missing' an outbreak in the sampling programme.

The final report of PC132 shows the average daily temperatures in the production glasshouses for the four crops – these were the crops as sampled for the research programme. The dates and approximate temperature ranges for the four crops was as follows:

Crop Number	**Dates**	**Temperature range** (ºC)
1	23-Oct to 16-Feb	1-12
2	08-Mar to 11-May	5-19
3	01-Jun to 05-Jul	12-25
4	25-Jul to 28-Aug	15-26

The following table is reproduced from Table 3 of the paper by de Loach (1974):

Population growth models for the increase of cabbage, peach and turnip aphids under various hypothetical conditions after 1, 2 or 3 weeks. The figures represent the number of aphids expected from a population of 1 on day 0.

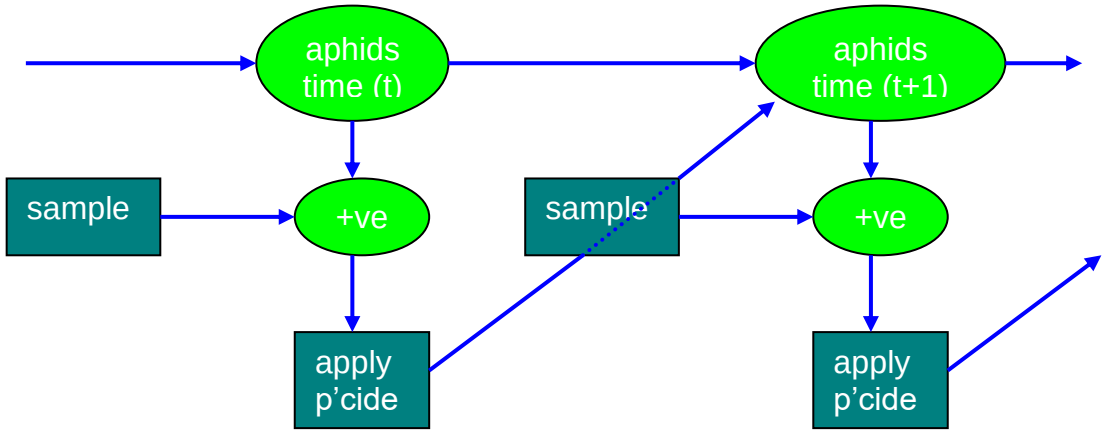
Time period	**Cabbage**	**Green peach**	**Turnip**
		At 15ºC constant temp.	
After 7 days	1.85	3.22	3.04
After 14 days	3.43	10.39	9.23
After 21 days	6.35	33.49	28.02
		At 25ºC constant temp.	
After 7 days	1.99	6.95	13.54
After 14 days	3.95	48.24	183.38
After 21 days	7.84	335.07	2483.22
		Spring-fall model	
After 7 days	1.68	2.95	3.14
After 14 days	2.83	8.69	9.88
After 21 days	4.77	25.64	31.07
		Summer model	
After 7 days	1.93	4.95	9.65
After 14 days	3.71	24.49	93.12
After 21 days	7.13	121.23	898.55

The only other paper with comparable data (Hale & Shorey, 1971) shows an average weekly reproduction rate of close to 5 for 91 distinct samples. A summary of the data shows that the variance is greater than the mean and that therefore a negative binomial distribution would be appropriate to the data. No information is given about temperatures but it can be assumed that

these were measured on summer crops. A complex growth model is probably not deemed appropriate at this stage, and anyway, we have no comparable information for the performance of the different aphid species in the scenario being considered here. However, it would probably be not unreasonable to assume very limited growth for temperatures below 10ºC, a reproduction rate of around 3 at temperatures of 15ºC, with rates of between 5 and 10 at higher temperatures (20 to 25ºC).

In risk terms the implications of this might be considered as follows:

> 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 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. This is encapsulated in the following diagram:



> where we simply include a single repetition of the sequence. The positive actions are sampling and pesticide application – the first is a routine decision, whereas the application of the pesticide is dependent on the outcome of the data collection exercise. The diagram implicitly assumes that the pesticide is not applied if aphids are not found, but also allows for the population dynamics of the aphid population, driven by temperature (not included) and mediated by pesticide application.

Risk-benefit

Significant costs in such a programme as proposed here are the costs of screening and the cost of monitoring. The costs of pesticide usage need also to be considered but this would certainly need to be considered at a 'marginal' level, e.g. in comparison with parallel costs in an unscreened growing system. Further, we have identified that one of the major sources of aphid entry must be via equipment or individuals being brought into the house, so there is an additional 'hygeine' cost that must be included.

In ascertaining and costing the risks, one must look at the game theoretic aspect of crop value and risk. There is very limited tolerance by the retailers of aphid contamination, so that a mistake in sampling leading to contamination can prove very costly, but that, increasingly, 'clean' crops (i.e. pesticide-free, or low pesticide receipt) may attract a premium (or at least be easier to sell). The sampling strategies that have been proposed have been set at quite a high level of producer protection to guard against the costly risk of rejection by the consumer. At the customer (i.e. retailer) level it is not known what their risks (associated with unwanted intruders) are, but that may not be important for the equation if there rules are clear. Other risks which may need further investigation are:

- Sample errors which can lead to the wrong decision (i.e. not to spray), although the sampling strategy is set to high grower protection
- Sample distributions are not strictly known; if the crop is kept clean, however, this should comply with simple distribution theory (e.g. the binomial distribution).
- Biased samples may have an impact if the proximal path area is not representative of the whole crop. Obviously the direct rather than visual sampling, will give greater protection.
- An assumption is made that pesticide sprays are effective, i.e they eliminate the pest.

Cost of Crop Monitoring

Time to monitor 0.1ha of lettuce crop:

The following figures are based on actual crop monitoring. The time varies depending on the growth stage of the crop but for this purpose a mean of 40 secs per plant (including dead time) has been adopted. Labour costs are also variable but have been set at £8/hr (inclusive of employment costs).

Sample size (Plants examined per 0.1ha)	Time (mins) per 0.1ha	Cost (£) per 0.1ha
100	66	8.80
200	133	17.73
300	200	26.66
500	333	44.40

Cost per crop / annum

The following costs are based on five crops per annum (approximate planting dates, harvesting dates and duration are shown in table), with 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	44	88	133	222
1/6 – 5/7	5	5	44	88	133	222
25/7 – 28/8	5	5	44	88	133	222
1/9 – 20/10	8	5	44	88	133	222
23/10 – 16/2	16	8	70	142	213	355
Total per annum	43	28	246	494	745	1243

RECOMMENDATIONS FOR FURTHER WORK

At the Project Review Meeting on 21 November 2003, it was agreed that the continued studies should focus upon:

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

- 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.*

- STC to do more detailed pest monitoring work in Peter Hardwick's commercial crops to allow further experimentation while minimising the risk of crop damage (to be discussed with Mr Hardwick). This may require the provision of additional funding.

- Risk analysis studies will continue in 2004, relating the risk of not detecting an infestation level of 1% infested plants to the cost of the monitoring exercise. The work will also take into account the potential to reduce that risk by adopting the prophylacatic approach to parasitoid release. This will require the provision of additional funding to cover continued input from John Fenlon.

- Industry wide discussions (ie involving growers, marketing groups and retailers) will be instigated to explore the possibility of sharing the additional production costs that may be incurred in moving closer to insecticide-free production systems.

- The project will be extended to 2005 to allow the "whole crop" evaluation of the reduced insecticide input lettuce production systems.

- Promote the new technologies via industry wide discussions.

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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.

Hale and Shorey (1971) – awaiting full reference from JF

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, 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.

de Loach (1974) – awaiting full reference from JF

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.