
FINAL REPORT

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Protected lettuce: Towards insecticide free production

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Commercial – In Confidence

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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.

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PRACTICAL SECTION FOR GROWERS

Headlines

- The overall aim of this project was to develop prophylactic biological control techniques, used in conjunction with physical pest control measures (*i.e.* screening glasshouse ventilators and doors) and cost effective crop monitoring, in order to remove 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 attacked by the parasitoids but are 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 subsequently showed that the behaviour of the parasitoids could be manipulated to improve their performance in lettuce crops. Two ORS units, at opposite ends of a commercial glasshouse of up to at least 4200m², 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 even 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*, were collected from the wild and shown to be potentially useful biological control agents for use against *Nasonovia ribisnigri* (currant lettuce aphid). This still has to be developed into an effective ORS.
- Risk analysis studies 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 allowed 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.
- The frequency of sampling based on the incorporation of the two previous concepts into the sampling decision process achieved a significant reduction in sampling frequency and thereby costs.
- The whole system was evaluated in a sequence of four commercial lettuce crops in the final year of the project. Although aphids breached the defences in each of these crops, the level of infestations were relatively small and could be explained in terms of incomplete screening or gaps in the structure of the glasshouse. The results suggested that the use of ORS units should not be necessary during the winter months.

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 procedure has been 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 completed work

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 research Foundation for use in experiments. In parallel to this project, funds were secured 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. hieraciorum* established that this parasitoid had the potential to be an effective control agent of *N. ribisnigri*. Unfortunately, funding for the continuation of this project could not be secured and an ORS for *A. hieraciorum* has not yet been developed.

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 (*Sitobion avenae* and *Rhopalosiphum padi*), 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 (*S. avenae* and *R. 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 4200m², 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.

- relaxation of the spray option at low levels of aphid infestation when conditions were not suitable for aphid development.

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).

The IPM system was evaluated in a commercial crop in the final year of the project. The sample sizes used in the monitoring schedule were based on effective screening being in place on both the ventilators and the doors. However, the grower removed the door screens because they hindered access to the crop, which allowed aphids to gain entry and become established on plants near the doors. The weekly sampling did not detect these aphids and they weren't found until harvest. Only three individuals were found at harvest in the first crop, suggesting they had originated from a very late invasion, perhaps during harvest. More aphids were found on two crops of mixed lettuce varieties, presumably because the varied harvest times resulted in the doors being open more frequently. The results emphasised the importance of intact screening and the need for that screening to be of practical design.

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 overall increase in production costs has been estimated to be £1438 per 1000m per annum and that could add 7-10% to the wholesale price of lettuce. Discussions with retailers (Co-operative Group and Sainsbury Supermarket Ltd) have shown there to be strong interest in the reduction of insecticides on protected lettuce. However, the absorption of the additional cost as a consequence of an IPM programme would be preferred if it were part of a complete reduction in all pesticides (*i.e.* fungicides and insecticides). The protected lettuce industry is currently considering the possibility of developing new production systems in the form of 'floating platform' hydroponics. Within these production systems it can be envisaged that fungicides could also be reduced, thus contributing to the retailers' broader request.

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 in combination with a reduction in fungicide usage (as suggested above in the 'floating platform' hydroponics system), 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.

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 withdrawal 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.
- 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, 2001) 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, 2001). 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. 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 (i.e. involving growers, marketing groups and retailers).

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 Research Foundation. In October 2002, the identification was confirmed by specialists at the Natural History Museum to be *Aphidius hieraciorum*. Only three previous records of *A. hieraciorum* have been found in the scientific literature and all were overseas; the most recent claiming to be the first recording on *Nasonovia ribisnigri* in Spain (Nebreda *et al.* 2005). Since July 2002, a second parasitoid species, *Praon volucre*, a generalist species of parasitoid, was found attacking *N. ribisnigri* and it is also in culture at STC Research Foundation.

In parallel to this project, funds were secured from Defra to investigate important aspects of the biology of both species of parasitoid and the aphid host (HH3119TPC). 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. *Aphidius hieraciorum* was shown to have the greatest potential for this use. Further funding is now required to develop an effective method of releasing the natural enemy in protected lettuce crops.

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 work plan 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. 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 experiments investigated 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. In order to determine the movement of the parasitoids within the glasshouse, the above experiments had involved detection using relatively high densities of host. A further series of experiments looked at the capability of the parasitoids to locate small numbers of aphids whilst moving between ORS units within commercial-scale crops. The conclusions were:

- *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 4200m², 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 monitoring

A desk-based risk analysis examined 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 attempting 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 £1.56k per 0.1ha per annum (adjusted to 2006 prices).

Risk analysis studies continued in 2004, relating the risk of failing to detect infestations at various levels to the cost of the monitoring exercise. The studies also looked at the potential to reduce that risk by adopting the following options:

1. the use of prophylactic bio-control
2. development of a temperature-based growth model for aphids, which allowed a more flexible and focused sampling regime;
3. relaxation of the spray option at low levels of aphid infestation when conditions were not suitable for aphid development.

Conclusions: decisions based on sampling outcomes

In the original recommendation, the crop was sprayed if any aphids were found. In this report we have considered less drastic actions, e.g. watchful waiting – if the incidence level of the pest is low and its prospective rate of growth is slow, then 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 1 presents a simple outcome and decision model in which the decisions are refined.

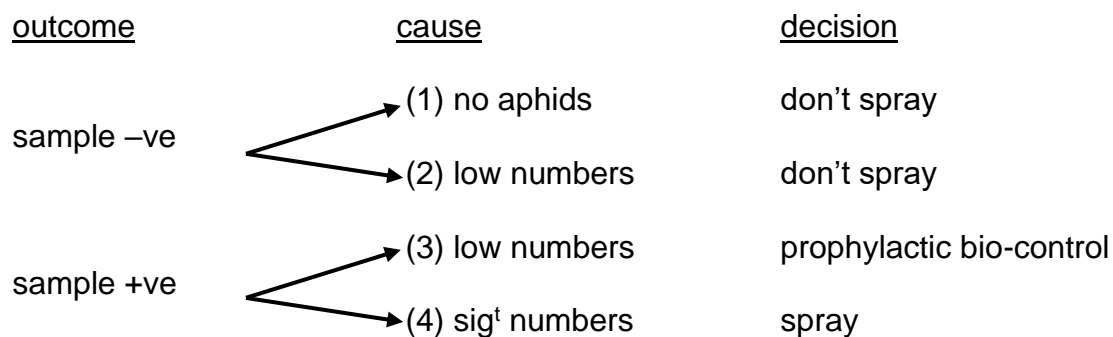
The prediction model can be enhanced in two ways:

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. We also make explicit the idea that, even if no aphids are detected in the sample, it does not negate their presence. The sampling protocol provides a ‘worst-case’ estimate of the numbers of aphids present when none are detected, so that this, too, can be put into the growth model to determine potential population growth.

The use of prophylactic bio-control (ORS) also means that we may be able to delay the need for intervention. Results suggested that at low levels of aphid presence, parasitoids are able to slow down development rates by a factor of two.

Figure 1: Crop sample outcomes and decisions



Cost of different sampling scenarios

Three monitoring schedules have been devised (Table 1). The first schedule is essentially that recommended in the first report (PC132; Jacobson, 2002). Schedules 2 and 3 incorporate the effect of temperature on aphid population growth, and schedule 3 also incorporates the contribution to slowing the aphid population growth that is made by prophylactic biological control (ORS).

Table 2 shows costs based on five crops per annum using the three different inspection schedules. The calculations are based on data from monitored crops in PC132 (Jacobson, 2002). Although costs will vary from year to year, the overall temperature profiles through a full year will be roughly similar, so the figures should provide a reasonably realistic guide.

These calculations suggested that schedule 2 (*i.e.* basic temperature monitoring) will provide a 50% saving in sampling costs with a further 10% reduction when using prophylactic bio-control (schedule 3).

Table 1. The number of crop inspections using three different monitoring schedules.

Crop planting & harvesting dates	Crop duration (weeks)	Monitoring schedule (number of weekly inspections)		
		1	2	3
8/3 – 11/5	9	5	2	1
1/6 – 5/7	5	5	3	2
25/7 – 28/8	5	5	4	3
1/9 – 20/10	8	8	4	4
23/10 – 16/2	16	16	1	1

Table 2. The costs of monitoring lettuce crops using three different schedules.

Monitoring schedules	Cost (£) per crop at the following sampling frequencies (plants / 1000m ²)			
	100	200	300	500
1	307	618	930	1556
2	154	308	462	769
3	121	242	363	605

Cautions / additional risk factors

An acknowledged shortcoming of the sampling model is the dearth of appropriate calibration data for specific aphid-parasitoid interactions, as well as intrinsic growth rates of implicated aphid species on modern lettuce varieties. 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.

PART 1: APPLICATION OF THE IPM SYSTEM TO COMMERCIAL LETTUCE CROPS

Introduction

In the final year of HDC project PC 194, the IPM system developed for protected lettuce within this project and HDC project PC 132, was evaluated on a commercial nursery. The principal elements of the IPM system were:

- Physical exclusion of pests by screening ventilators and doors
- A new monitoring system based on risk analysis
- Prophylactic biological control using open parasitoid rearing systems (ORS)

Screening

Much of the work in project PC 132 focused on the development of an integrated control strategy based on screening glasshouse ventilators to exclude aphids and moths from the production glasshouse (Jacobson, 2002). For three seasons, pest establishment was monitored in unsprayed sequentially sown crops in both screened and unscreened experimental glasshouses. Where glasshouse ventilators and doors were screened with Agralan Enviromesh S48, the numbers of aphids on the lettuce crops were substantially reduced. Infestations that did occur in the screened house could usually be traced back to introduction on young plants or entry through damaged screens. Screening the ventilators and doors in experimental glasshouses had no apparent effect on temperature or relative humidity but there was a small effect on accumulated light over the duration of each crop, which was most noticeable during the summer months (*i.e.* 2-5% reduction of accumulated light in crops between December and April, 10-11% reduction between May and July, and 7% reduction between August and October). There was probably less effect of shading from materials on the roof in the winter because the sun was lower in the sky and shone through the glasshouse sidewalls for a greater proportion of the day.

The screening studies were scaled up from experimental glasshouses to commercial production glasshouses in 2000/2001, using Mevalon 0.6mm UV stabilised polyethylene netting on roof ventilators (Figure 2) and PVC strip curtains on doors. The experiment compared a pest control strategy based on screening to reduce pest invasion with a routine spray programme. The studies continued to monitor effects of screening on the glasshouse environment and were extended to determine whether any loss of light affected marketable yield. No aphids were collected from traps in the screened commercial glasshouse but live aphids were found on plants on five occasions between October 2000 and October 2001. On two such occasions, the plants had most probably become infested between the propagation and production glasshouses. On one occasion, small numbers of aphids were thought to have survived on debris in the soil from a previous infestation. On the other two occasions, very small numbers were found either just before or during harvest. It is not known how these aphids gained entry but no action was deemed necessary. By contrast, aphids were collected from water traps in four of the five crops

in the unscreened glasshouse. Despite the routine chemical spray programme in this glasshouse, aphids were also found on plants on seven occasions, with most invasions occurring in late July and August.

Figure 2. Screened ventilators on the commercial nursery



Monitoring

The monitoring schedule devised in project PC 132 was deemed effective but costly in terms of labour requirement (Tables 1 and 2, Schedule 1). This project has further developed the monitoring system by incorporating factors to allow for i) temperature effects on the growth of aphid populations and ii) the percentage of the population removed by parasitoids supplied through the ORS. The reduced schedules (Tables 1 and 2, Schedules 2 and 3) have built in provisos and could carry a greater risk if those assumptions are not completely valid. The effectiveness and risk of the reduced monitoring schedules were therefore tested by monitoring weekly throughout the final growing season of the project.

Open rearing system

The development of the ORS for *A. ervi* and *A. colemani* was described in the introductory section of this report (see pages 13-15) (Figure 3). However, further work was required in the final year of the project to monitor the quality of the ORS units (*i.e.* presence of live aphid hosts

and parasitoids) throughout the winter crops because they may have been less effective during periods of low temperature and short daylength.

Figure 3. An ORS unit developed for *A. ervi* and/or *A. colemani* parasitoids



The Defra funded project (HH3119TPC) that ran concurrently with this HDC project, established through small-scale laboratory studies and a predictive population growth model that the parasitoid *A. hieraciorum* had the potential to be an effective biological control agent for *N. ribisnigri*. Unfortunately, that funding ceased before an effective ORS could be developed for *A. hieraciorum* and this left the IPM programme without an ORS component for *N. ribisnigri*. As a contingency, *A. hieraciorum* were reared independently so that they could be released in the crop in large numbers if *N. ribisnigri* were discovered on the plants.

Materials and method

The glasshouse

The commercial glasshouse used in this project was a Venlo structure designed and built by HOK Engineering in 1984/5. It was 36m long with 6m wide bays and was approximately 2.7m

high to the gutters. There were five and a half bays giving a total floor area of approximately 1200m². With 30 plants across each bay and 156 plants along the full length of the glasshouse, there were a total of approximately 25,700 plants.

There were 132 roof ventilators (each 0.73m x 0.83m) with “Belgium-type” vent bar design, and all were individually screened with Mevolon 0.6mm x 0.6mm UV stabilised polyethylene insect netting (Figure 2). The netting was prefabricated for each ventilator and fixed to the inside of the opening with aluminium strips fastened by self-drilling screws. The arms of the ventilator passed through sockets in the netting and were clamped with two stainless steel clamps per vent arm. There were two doors (each 2.9m x 2.4m) in the east wall of the glasshouse. The doors were fitted with clear PVC strip curtains, each consisting of 15 overlapping 0.3m wide strips, which were designed for tractor and forklift traffic.

Monitoring system

Each of the four lettuce crops was monitored weekly for aphids. The number of lettuce plants examined each week was 500 per 1000m² and they were evenly distributed throughout the crop.

ORS units

Separate ORS units for *A. ervi* and *A. colemani* were positioned at both ends of the glasshouse and they were examined weekly for the presence / absence of parasitoids and their aphid hosts (*Rhopalosiphum padi* and *Sitobion avenae*).

Results

Monitoring schedules

Crop 1: 28 April – 13 June 2005

- The first crop was planted with round lettuce plants all of the same age.
- During crop 1, the lettuce plants were assessed for aphids seven times at weekly intervals. No aphids were found during these assessments.
- New ORS units infested with *R. padi* and *S. avenae* and mummified parasitoids were put out every two weeks to provide new food for the existing parasitoids.
- Whilst harvesting the crop (w/c 13 June 2005), the grower found three individual aphids that were identified as *Macrosiphum euphorbiae*.

Crop 2: 30 June – 2 August 2005

- This crop was a mixed planting of cos and round lettuce.
- There were five assessments performed at weekly intervals. No aphids were found on the assessment dates.
- As with crop 1, new ORS units were put out at two week intervals.

- The harvesting of the Cos started w/c 21 June 2005.
- The main harvest started w/c 1 August 2005. During the harvest, large numbers of aphids were found in the bay nearest glasshouse door. They were mainly on one variety of round lettuce (cv “Smith”) and were identified as *M. euphorbiae*. In total, 240 lettuces (20 boxes) were affected.
- On examining the lettuce during the harvesting period, parasitoids *A. ervi* and *A. colemani* were found sitting on the affected plants. A sample of aphids (50) was brought back to STC and 2% were found to have been parasitised.

Crop 3: 5 September – 14 October 2005

- The crop was of mixed planting of Little Gem, Cos, round lettuce and curly lettuce.
- The plants were monitored six times for the presence of aphids. No aphids were found during this period.
- As in previous crops, the ORS units were replaced every two weeks.
- Harvesting began on 14 October 2005 and finished 27 October 2005. When the grower harvested the Cos lettuce he found an infestation of *Myzus persicae*, which led to the destruction of 120 lettuces (10 boxes).

Crop 4: 31 October 2005 – 27 February 2006

- Crop 4 was a mixed planting of curly and round lettuce of differing ages.
- The plants were monitored 16 times at weekly intervals over the duration of the crop and no aphids were detected during the assessments.
- Frost protection heating (2°C) was turned on in w/c 5 December 2005. ORS units were only replaced every four weeks, as the barley plants did not deteriorate as quickly under the cooler conditions (see Table 3 for glasshouse temperatures). However, it proved difficult to maintain adequate populations of *R. padi*, *S. avenae*, *A. ervi* and *A. colemani* on the plants and the numbers had to be topped up at two week intervals.
- During harvest on the 3 March 2006, the grower reported three aphids (*M. persicae*) on the bottom leaves of a round lettuce but there was no crop loss.

Table 3. Mean daily temperatures recorded at approximate seven day intervals in the commercial lettuce crop.

Crop	Date	Outdoor temp. (°C)	Glasshouse temp. (°C)
1	28/4/05	12.8	14.7
	5/5/05	12.9	16.8
	12/5/05	10.5	17.9
	20/5/05	16.1	19.3
	26/5/05	17.5	20.3
	2/6/05	17.2	19.5
	9/6/05	17.8	22.7
2	1/7/05	18.7	22.9
	8/7/05	16.6	18.7
	15/7/05	22.3	24.8
	21/7/05	17.7	20.3
	28/7/05	16.8	21.1
3	9/9/05	19.1	21.3
	16/9/05	12.8	14.7
	22/9/05	16.1	19.7
	29/9/05	13.0	15.3
	6/10/05	12.8	15.6
	11/10/05	16.6	20.1
4	3/11/05	14.9	15.4
	11/11/05	13.9	14.2
	18/11/05	3.8	8.0
	24/11/05	7.4	8.4
	2/12/05	7.1	7.5
	8/12/05	7.6	9.6
	15/12/05	9.7	10.0
	21/12/05	8.9	8.7
	6/01/06	2.8	4.4
	12/01/06	4.7	6.4
	20/01/06	7.5	9.3
	27/01/06	4.5	6.5
	3/02/06	3.9	5.2
	10/02/06	3.6	6.7
16/02/06	7.4	10.1	
22/02/06	3.2	5.6	

Results and Discussion

Although monitoring of crops 1-4 (using schedule 1) didn't locate aphids on the plants during production, the pests were found on all four crops at harvest. An assessment of each case revealed the most probable means by which the aphids had breached the glasshouse defences:

Crop 1: Only three aphids (*M. euphorbiae*) were found at harvest. This was in mid-June, which is a peak period of aphid migration, and it would seem most likely that these aphids simply colonised the plants during harvest.

Crop 2: 240 plants close to the doors of the glasshouse were found to be infested with aphids (*M. euphorbiae*) when the crop was harvested at the beginning of August. The protective screens had been removed from these doors because they had been found to impede access of the larger mechanical equipment and this had left the crop vulnerable to attack during the peak period of aphid activity. There would seem little doubt that this breakdown in the defences had allowed invasion during the latter stages of production.

Crop 3: As in crop 2, a large number (120) of plants close to the doors of the glasshouse were found to be infested with aphids at harvest and this was again attributed to the removal of the door screens. The multiple harvest dates meant the doors were open more frequently and this increased the opportunities for invasion. In addition, the crop wasn't monitored during the last two weeks due to a misunderstanding about harvest dates and it is possible that the infestation would have been detected during this period.

Crop 4: A small number of aphids were found near a gap between the glass and wall of the building.

The risks associated with the monitoring schedules were calculated under the belief that the screens in the glasshouse would be intact and that there would not be any other breakdown in the defences against aphids. Both the removal of the door screens and the gaps that were subsequently found in the structure of the glasshouse provided additional opportunities for the aphids to invade and increased the risk of failure. If these additional risks had been known, the sample size would have been increased and there would have been at least some sampling biased towards the most vulnerable areas.

The design of door screens clearly requires more attention because it is unacceptable that they should impede normal work practices. More complicated structures such as double door systems (eg Figure 4) and outward facing fans (eg Figure 5) should be considered. However, such systems would inevitably add more cost to the IPM system.

When judging the success of this IPM programme, it must be remembered that control programmes based on chemical insecticides are rarely completely successful and it is not uncommon to suffer some wastage due to pests surviving the routine treatments.

Figure 4. Example of a double door system installed at the entrance of a Spanish glasshouse



Figure 5. Example of outward facing fans directed towards the double door entrance of a Spanish glasshouse. These fans switch on automatically when the door begins to open and continue to run until it is closed.



The activity of the parasitoids and their aphid hosts in the ORS units was monitored during crop 4 to assess their efficacy during the less than favourable winter conditions. The percentages of occasions upon which *R. padi*, *S. avenae*, *A. colemani* and *A. ervi* were found on the dwarf barley plants are shown in Table 4. It was clear that the neither their aphids nor their parasitoids thrived at this time of year and the ORS units are unlikely to have provided any protection against invading aphids. However, this should not be important because the risk assessment did not incorporate any contribution from the prophylactic biological control component at this time of year.

Table 4. Activity of the two aphid species (*R. padi* and *S. avenae*) and their parasitoids (*A. colemani* and *A. ervi*) during crop 4.

Species	Presence recorded during crop 4 (expressed as the percentage of total number of assessments)
<i>A. colemani</i>	16.7
<i>A. ervi</i>	33.3
<i>R. padi</i>	66.7
<i>S. avenae</i>	100

PART 2: ABSORBING THE COST OF IPM

The development of IPM in protected lettuce is crucial if UK growers are to respond to the decline in the number of insecticides 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.

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 initial estimate of the cost of monitoring the IPM system, which was produced in HDC project PC132 (Jacobson, 2002), has been reduced within this project by i) incorporating a monitoring schedule that takes into account the effect of temperature on the growth of the aphid populations, and ii) allowing for the contribution prophylactic biological control can make to reducing the risk of failure of the IPM system.

The overall increase in production costs has now been estimated to be £1438 per 1000m per annum, which could still add 7-10% to the wholesale price of lettuce. Discussions with retailers (Co-operative Group and Sainsbury Supermarket Ltd) have shown there to be strong interest in the reduction in use of insecticides on protected lettuce. However, the absorption of the additional cost would be preferred if it were part of a complete reduction in all pesticides (*i.e.* fungicides and insecticides). The protected lettuce industry is currently considering the possibility of developing new production systems in the form of 'floating platform' hydroponics. Within these production systems it can be envisaged that fungicides could also be reduced, thus contributing to the retailers' broader request.

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References

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.

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

Nebreda, M., Michelena, J.M. and Fereres, A. (2005). Seasonal abundance of aphid species on lettuce crops in central Spain and identification of their main parasitoids. *Journal of Plant Diseases and Protection*, 112 (4), 405-415.