SWEET PEPPERS: BIOLOGICAL CONTROL OF GREEN LEAFHOPPER (*EMPOASCA DECIPIENS*)

FINAL REPORT 31/3/96 ON HDC PROJECT PC 76

FINAL REPORT

PROJECT NUMBER: PC76

PROJECT TITLE: SWEET PEPPERS: BIOLOGICAL CONTROL OF GREEN LEAFHOPPER (*EMPOASCA DECIPIENS*)

PROJECT LEADERS: DRS M.A.JERVIS & N.A.C.KIDD

LOCATION OF PROJECT: SCHOOL OF PURE & APPLIED BIOLOGY, UNIVERSITY OF WALES, CARDIFF

PROJECT CO-ORDINATOR: DR N.DUNGEY, VAN HEYNINGEN BROTHERS, LITTLEHAMPTON

DATE PROJECT COMMENCED: 01/09/92

DATE PROJECT COMPLETED: 22/10/95*

KEY WORDS: SWEET PEPPERS, BIOLOGICAL CONTROL

* Project starting date was 01/09/92, but due to short notice of confirmation of award of contract, post doctoral assistant (PDRA) appointed in mid-October 1992. PDRA left full-time post on 18/10/95 (third anniversary of employment contract). PDRA continued working on part-time casual basis until 22/12/95, analysing leaf samples from small and large glasshouse experiments.

CONTENTS

1.INTRODUCTION

2.RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

2.1 APPLICATION

2.2 SUMMARY

3. EXPERIMENTAL SECTION

3.1 INTRODUCTION

3.2 MATERIALS AND METHODS

3.3 RESULTS

3.4 CONCLUSIONS

3.5 REFERENCES

3.6 APPENDICES

3.7 CONTRACT

1. INTRODUCTION

This report describes a three-year investigation into the potential for biological control of the green leafhopper, *Empoasca decipiens*. The leafhopper, through its feeding activities, produces highly visible blemishes on the skin of the fruit of glasshouse sweet peppers, and in summer and autumn its numbers can be so high as to reduce significantly the proportion of Class I fruit in a crop. The leafhopper has become a pest of sweet peppers as a result of the successful use of biological control against other pests, where negligible chemical inputs are involved. It is therefore likely to become increasingly prominent as a pest, as nurseries convert to biological control as an overall pest management strategy. Control of green leafhopper has to be obtained using methods that do not conflict either with existing biological control methods, with the use of bees in glasshouses to improve pollination, or with the demand from supermarkets for reduced pesticide usage.

The green leafhopper has a broad host plant range, so it has the potential to spread further within the horticultural industry. Overseas, it is known to transmit plant viruses.

The main target of the project was to find one or more biological control agents that will limit green leafhopper populations to non-damaging levels in glasshouse sweet peppers. Since the larval and adult stages of green leafhopper can move very rapidly over plant surfaces, the types of predator presently used against other glasshouse pests are unlikely to prove effective. Studies on natural populations of other leafhopper species (reviewed in Waloff & Jervis, 1987) suggest that parasitoid wasps (parasitoids are insects that develop within or upon, and subsequently destroy other insects) are good candidates for the control of *Empoasca decipiens*, with those attacking the leafhopper's egg stage having top priority. One group of egg parasitoids is the Mymaridae, all species of which carry out most of their life cycle in the host egg and so do not require pupation sites on the glasshouse floor (where they would otherwise be highly vulnerable to physical damage or desiccation). These parasitoid wasps are relatively easy both to rear and to release in large numbers, and they inflict mortality before the leafhopper reaches the fruit-damaging stage in its life cycle.

The approach adopted by the investigators involved traditional techniques coupled with computer simulation modelling. Interactive, 'user-friendly' computer simulation models can greatly facilitate decision-making by growers and advisors with respect to pest management.

2. RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

2.1. APPLICATION

The primary aim of the project was to find one or more parasitoid species that will limit glasshouse populations of the green leafhopper (*Empoasca decipiens*) to non-damaging levels in sweet peppers. One parasitoid species (*Anagrus atomus*) was found which, if mass-released into a leafhopper-infested pepper crop using a simple delivery system, will achieve this result. Employing a user-friendly, interactive computer simulation model, the grower can precisely time the introductions to optimum effect. The most effective parasitoid release strategy involves five mass releases at five-day intervals during August. Use of the model avoids the need for long-term detailed monitoring of leafhopper numbers in growers' glasshouses.

2.2 SUMMARY

2.1.1 Aims and objectives of project

The main target of the project was to find one or more insect parasitoid species that will limit green leafhopper (*Empoasca decipiens* Paoli) populations to non-damaging levels in glasshouse sweet peppers. The leafhopper, through its feeding activities, produces highly visible blemishes on the skin of the pepper fruit, and in summer and autumn its numbers can be so high as to reduce significantly the proportion of Class I fruit in a crop.

The subsidiary targets were :

1. To devise a method for rearing the leafhopper under laboratory conditions;

2. To obtain several candidate parasitoid species from the British fauna;

3. To establish the candidate parasitoid species in laboratory culture and to devise methods for culturing them on a medium scale for cage and small glasshouse experimentation;

4. To measure key life history parameters for each of the candidate parasitoid species;

5. To determine and compare the *per capita* parasitization efficiency (including density responsiveness) of the candidate parasitoid species;

6. To develop a computer simulation model incorporating parameter values, and to use the model in predicting both the likely impact of candidate parasitoids and the optimum strategy for parasitoid release;

7. To determine whether release of selected parasitoid species (selected and released on the basis of the model's predictions) brings about effective control of the leafhopper pest in the large glasshouse environment.

2.1.2 Summary of results

A highly successful medium-scale culturing procedure for the leafhopper and three parasitoid (*Anagrus*) populations was devised and refined to provide: (a) small numbers of insects for laboratory and small glasshouse experimentation, and (b) large numbers of parasitoids for release in large glasshouses.

Using four complementary taxonomic techniques, the three *Anagrus* populations were found to be of the same species (i.e. conspecific), namely *Anagrus atomus* (Linnaeus). Measurements were successfully taken of key life-history parameters of both *Empoasca decipiens* and *Anagrus atomus*, and the parasitization efficiency (including density responsiveness) of the latter was also measured.

A small glasshouse experiment showed a clear dosage-dependent effect of parasitoid numbers upon leafhopper densities. Data from this experiment, together with data from laboratory experiments, were used to calibrate an interactive computer simulation model of the *Empoasca-Anagrus* population interaction.

Release of *Anagrus* in a grower's large glasshouse in 1995 resulted in a significant reduction (53.6%) in leafhopper population compared to the control. The simulation model accurately predicted: (a) within-season changes in leafhopper numbers (both in the presence and absence of parasitoids), and (b) the effectiveness of the parasitoid in reducing leafhopper numbers.

A practicable delivery system for the parasitoid was developed, and employing the user-friendly interactive model, the optimal parasitoid release strategy for large glasshouses was also determined. The latter involves five releases of 30,000 female parasitoids at five-day intervals during August, the precise timing of the releases to be determined in any year by the grower or advisor using the model.

If passed onto a commercial biological control supplier this spring (1996), the delivery system could easily be 'up and running'in time for parasitoid releases to be made in growers' glasshouses this August.

The results of the project are highly relevant to the biological control of: (a) *Empoasca decipiens* on other glasshouse crop plants, pot plants and bedding plants; and (b) other leafhopper pests e.g. *Hauptidia maroccana* (Melichar), a pest of tomatoes in the UK (Jervis *et al.*, 1993).

2.1.3 Action points for growers

Growers need to:

a) monitor the time of arrival of the leafhopper in each glasshouse;

b) collect daily temperature records (maxima and minima) in each glasshouse for entry into the model;

The action threshold and the timing and size of parasitoid releases are to be decided upon using the model.

2.1.4 Anticipated practical and financial benefits

By reducing leafhopper numbers to very low levels, application of insecticides becomes unnecessary, and biological controls used against other pests need not be compromised. As lost revenue across the UK pepper industry, even from moderate yield depression or loss of Class I premium, could amount to as much as £1 million, the new biocontrol technique is likely to result in substantial savings (Jervis *et al.*, 1993).

The methodology developed could easily be adapted to suit the *Anagrus-Hauptidia* (tomato leafhopper) system (see Copland & Soeprapto (1985) for biology).

Empoasca decipiens is well-known for the broad range of plant species it attacks under field conditions; these include chrysanthemums and other pot and bedding plants. Thus, the biological control system described in this report could benefit the glasshouse industry as a whole, not just pepper and tomato producers. Note also that there is a very high likelihood that some glasshouse herbs will eventually attract leafhopper pests (e.g. see information on leafhopper host plant ranges in LeQuesne & Payne (1981) and Ossiannilsson (1981)).

3. EXPERIMENTAL SECTION

3.1 Introduction

The research targets for the project are given above (2.1.1). The research targets for the final year (1994-1995) were as follows:

a) continue measuring key life-history parameters for *Empoasca decipiens* and *Anagrus atomus* (in the laboratory);

b) continue calibrating the simulation model, using data from the laboratory and from a small-scale glasshouse experiment;

c) test and correct the simulation model, using data from both a small-scale glasshouse experiment (conducted at Cardiff) and large-scale glasshouse experiment (conducted at Van Heyningen Brothers, Littlehampton);

3.2 Materials and methods

3.2.1 Preamble

As has been pointed out both in previous reports and in review meetings, a major constraint upon analysing parasitism of leafhopper eggs contained in pepper leaves is the great difficulty with which parasitised and unparasitised eggs can be located and distinguished. By refining our technique for examining leaf samples, we succeeded in miminising this problem, but could not eliminate it altogether. The problem seriously constrained the speed with which laboratory studies (3.2.3), the small-scale glasshouse experiment (3.2.4) and large-scale glasshouse experiments (3.2.6) were carried out.

3.2.2 Culturing and rearing of leafhopper and parasitoid

Culturing and rearing were carried out for two purposes:

a) to provide insect material for laboratory observation and experimentation;

b) to provide leafhoppers and adult parasitoids for glasshouse experimentation.

Cultures were maintained at 26°C, 18h light: 6h dark photoperiod, 80% humidity and 120μmol m^{-2} s^{-1} light intensity. Leafhoppers were reared on sweet peppers (cv. Mazurka) and broad beans (cv. Sutton). Broad beans were used, in addition to peppers, as they are the most appropriate parasitoid delivery plants (the term 'banker plants', used to describe the latter, is inappropriate) for large glasshouse introduction.

Wooden-framed, fine gauze-sided and fine gauze-roofed culture cages, 80 x 50 x 70cm, were used for culturing and rearing (see 1993/1994 annual report on project PC 76 for details). Twenty-four potted plants were used in each cage. Leafhopper-only and leafhopper/parasitoid cultures were maintained. Each culture was started from c.50-100 leafhopper adults. The number of adult leafhoppers in the leafhopper-parasitoid cages fluctuated around 380 (=15-17 adults per plant)

Cultures of three *Anagrus* populations were maintained:

a) a population derived from one of the large glasshouses (C block) at Holland Nursery, Van Heyningen Brothers (VHB), Littlehampton;

b) a population obtained using trap plants (pepper plants loaded with *Empoasca* eggs) placed outdoors, close to one of the large glasshouses at VHB;

c) a population obtained from English Woodlands Biological Control Ltd, Graffham, Petworth, Sussex, normally supplied for the control of tomato leafhopper (*Hauptidia maroccana*).

Population (c) is known to be *Anagrus atomus* (Linnaeus) (Waloff & Jervis, 1987).

Knowledge of the taxonomic status of natural enemy populations is an essential prerequisite for any biological control work; ignorance of this fact has resulted in several past biological control failures (DeBach & Rosen, 1991). Therefore the aforementioned three *Anagrus* populations were compared using four complementary taxonomic techniques:

a) light microscopy;

b) scanning electron microscopy;

c) morphometric analysis;

d) the random amplified polymorphisms-polymerase chain reaction (RAPDS-PCR) technique of DNA analysis (done at our own expense).

For details of methodologies (a-c), see 1993-1994 annual report on Project PC 76. The RAPDS-PCR technique is described in Williams *et al.* (1990).

For laboratory observation and experimentation, leafhoppers and parasitoids were removed from the cultures as required. Using the above-described culturing technique, around 80 adult (c.40 female) *Anagrus* were produced per plant per week.

For introduction into small glasshouses, leafhopper egg-bearing bean plants were produced. A standardised procedure ensured that egg numbers were as close to equivalent as possible in all the glasshouses: six female and one male hoppers were confined on each of 48 bean plants for 10 days.

For introduction into large glasshouses in 1995, broad bean plants that had been in culture for 4 weeks were confined with leafhopper egg-laden plants (the latter eventually to be used as the parasitoid delivery plants), in a 1:7 ratio. The delivery plants were previously standardised by confining, on each, 5 female and 1 male leafhopper. The combination of culture plants and delivery plants was maintained for four weeks. On average, each delivery plant so maintained would give rise to 130 adult (c.65 female) *Anagrus*.

3.2.3 Measurement of key life-history parameters of leafhopper and parasitoid

The following measurements were taken for *Empoasca* on both broad beans and peppers:

a) time taken from egg laying to hatching of the egg;

b) time spent in each instar;

c) time taken from appearance of adult female to laying of first eggs;

d) time taken from laying of first eggs to laying of last eggs;

e) time taken from laying of last eggs to death of leafhopper;

f) longevity of adults.

For details of protocol, see 1993/1994 annual report on Project PC 76.

The following measurements were taken for *Anagrus*:

a) time taken from egg laying to adult emergence (on both broad beans and peppers);

b) searching efficiency.

For details of protocols, see 1993/1994 annual report on Project PC 76. Data on (b) were obtained from experiments in which varying numbers of adult female hoppers were confined over 24h on single pepper plants, in a 51cm high x 22cm diameter cylindrical mylar cage. This provided different hopper egg densities per leaf for each cage. After removal of adult hoppers, either 1, 3 or 10 female *Anagrus* were introduced into each cage and left for 24h to parasitise the eggs. After removing the parasitoids, the eggs were left to develop until hatching and the numbers of emerging hoppers and parasitoids counted. With the ranges of hopper eggs and searching parasitoid densities used, it was possible to determine:

a) the functional response of *Anagrus* to hopper egg density (Holling, 1966);

b) the degree of mutual interference between searching female parasitoids (Beddington, 1975; Hassell, 1978).

Because the three candidate parasitoid populations were found to be of one species, *Anagrus atomus* (see 3.3.2), measurements were taken of one population only (that derived from the grower's large glasshouses).

3.2.4 Small-scale glasshouse experiment

A small-scale glasshouse experiment was carried out in Cardiff in 1995, with the aims of:

a) determining whether introduction of *Anagrus* can bring about significant reduction of *Empoasca* populations;

b) determining which of three parasitoid introduction (parasitoid density) regimes is the more effective at reducing *Empoasca* populations;

c) calibrating both the parasitoid searching parameters and the leafhopper density dependence in the simulation model.

Eight small glasshouses (2.32m long x 1.74m wide x 1.74m high) at Cardiff were used. Each contained 15 sweet pepper plants (cv. Mazurka, 1m high at the time of their introduction) set in growbags and connected to a watering system. During hot weather, humidity was maintained at a high level by hosing the glasshouse floor (soil). All doors and vents had been fitted with fine gauze barriers to prevent ingress and egress of *Empoasca*, *Anagrus* and other organisms.

A set density of leafhoppers (eggs, nymphs, adults) was introduced into all eight glasshouses in/on broad bean plants in the last week of June. Set densities (10, 50, 150, two replicates each) of mated female *Anagrus* were introduced into the six test glasshouses (two glasshouses served as controls) at a time specified by the simulation model (mid July). Leafhopper numbers were monitored weekly, from mid July (just prior to parasitoid release) to mid October i.e. over three months. A large sample of leaves was taken from each glasshouse at the end of the experiment to determine:

a) the ratio of parasitised to unparasitised leafhopper eggs;

b) whether the controls had been parasitoid-free.

No leaf samples were taken during the experiment because to have done so would have significantly reduced the plant resource available to the leafhoppers.

3.2.5 Development of simulation model

A detailed description of the methodology and rationale involved in the construction of the simulation model is provided in Appendix 1. The later stages of the model's development (e.g. parameter estimation) are intimately involved with the small and large glasshouse experimental programme and are therefore discussed in detail in the relevant sections.

3.2.6 Large-scale glasshouse experiments

Large-scale glasshouse experiments were carried out at Van Heyningen Brothers Ltd. during 1995 with the aims of:

a) determining whether introduction of *Anagrus* can bring about significant reductions in *Empoasca* population numbers;

b) determining which of two parasitoid introduction regimes is the more effective.

c) testing the ability of the simulation model to predict accurately *Empoasca* population development, both in the presence and absence of *Anagrus*.

As was stated in the 1993-1994 annual report on Project PC 76, the optimum parasitoid release strategy was to be determined *not* by trial data *per se* but by the *simulation model*, calibrated and tested as described in subsections 3.3.3, 3.3.4 and 3.3.5 of this report.

Four glasshouses ('blocks') at VHB Holland Nursery were used for the 1995 experiment. In this experiment, there were two treatment/control pairs:

1995	control (no *Anagrus*)	experimental (with *Anagrus*)
Anticipatory regime	Block E	Block C
Reactive regime	Block B	Block A

In the 'anticipatory regime', parasitoids were released using the delivery plant method (see above). Releases were made at monthly intervals starting on 23/03/95 and ending on 23/07/95. With this regime, both the parasitoid and the leafhopper were simultaneously released into the test glasshouse, as there was unlikely to have been 100% parasitism of eggs on the delivery plants. Approximately 2000 female parasitoids were introduced into Block C each month (5 repeated releases of c.65 female parasitoids/plant, 32 plants used each time). E Block was treated with Hostaquick (= Heptenophos) by the grower to reduce leafhopper numbers on 29/7/95, whereupon the population sampling was terminated. In C Block, a further sample was taken in August, shortly before Hostaquick was used there on 18/8/95, 21 days after the control glasshouse treatment.

In the 'reactive regime', a single introduction of c.2000 parasitoids took place on 15/06/95, approximately two months after the first hoppers appeared in Block A. Sampling was initially carried out solely by VHB staff at weekly intervals. It became clear that this provided a poor estimate of population densities, so one major sample was therefore carried out by Cardiff staff on 12/07/95 to compare densities in both control (B) and experimental (A) glasshouses. This was shortly before Hostaquick was applied in both glasshouses on 2/08/95.

At the end of the season in 1995 a sample of 80 leaves was taken from both C and E Blocks (the 'anticipatory' regime), in order to check that parasitism was indeed higher in the experimental compared to the control group (accepting the likelihood that some parasitoids would enter the control block from outside).

3.3 Results

3.3.1 Preamble

This section deals mainly with results obtained in the 1994-1995 season. However, results from previous years are discussed where pertinent.

3.3.2 Culturing and rearing of leafhopper and parasitoid

Because of the immense time and effort put into maintaining them, the insect cultures performed extremely well. Leafhoppers, parasitoids and parasitoid delivery plants were provided precisely when required.

Light microscopy, scanning electron microscopy and morphometric analysis revealed no significant morphological differences between the three *Anagrus* populations (see 1993-1994 annual report on Project PC 76). The RAPDS-PCR technique of DNA analysis employed six different 'primers', and the results obtained from this work provided no evidence to suggest that the three populations are other than one species. Therefore, we are well justified in regarding them as *Anagrus atomus* (Linnaeus).

3.3.3 Measurement of key life-history parameters of leafhopper and parasitoid

The key leafhopper and parasitoid life-history information required for the development of the model and listed in the 'Materials and Methods' section above (3.2.3) was presented in detail in the 1993-94 annual report on project PC 76. A summarised version of this is given in Appendix 2. During the current year further work was carried out to:

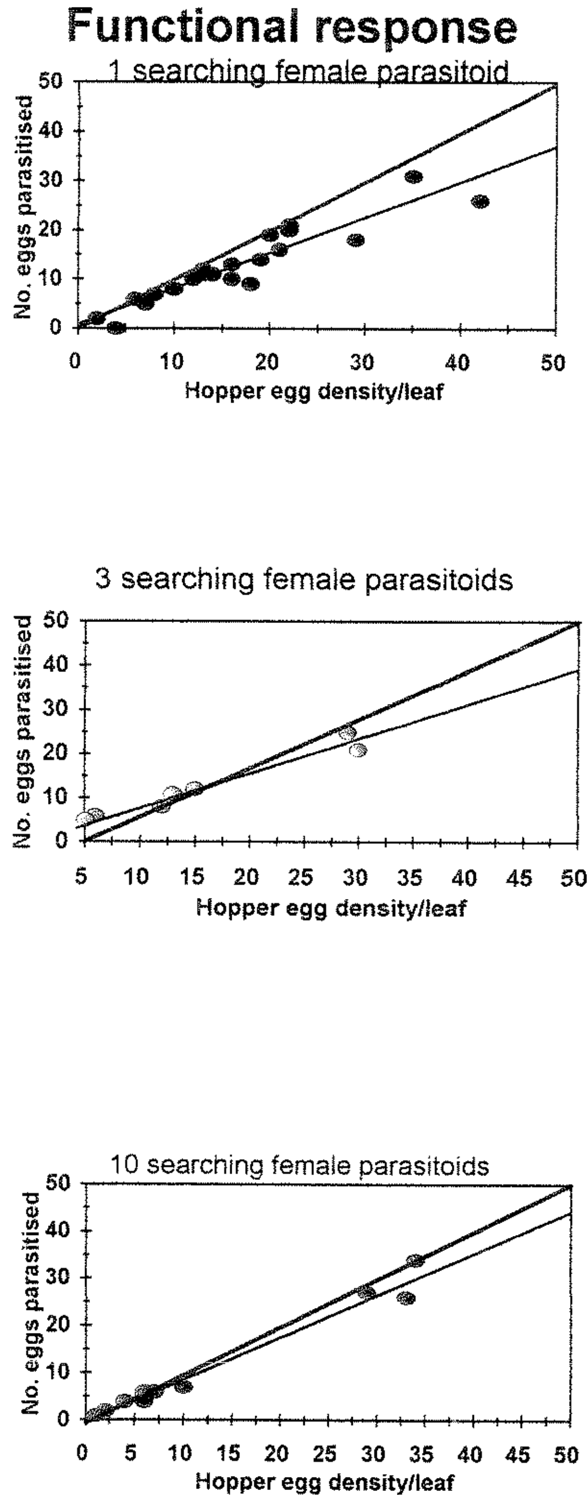
a) establish the most appropriate searching efficiency sub-model for *Anagrus;*

b) determine the correct parameter values for the model chosen.

The searching efficiency sub-model

The response of searching female *Anagrus* to variations in egg density per leaf is shown in Figure 1, for different numbers of searching parasitoids.

Figure 1. *The functional response of* Anagrus atomus *to* Empoasca *egg density (1 parasitoid: Y=0.72X+0.85, R^2=0.87, 19d.f.; 3 parasitoids: Y= 0.73+1.05, R^2=0.96 7.d.f.; 10 parasitoids: Y=0.9X-0.1, R^2=0. 98, 12 d.f.*



The thick 45° lines in each graph represent the maximum possible parasitism levels for each egg density. The thin lines show the calculated regression lines. In all cases the relationships are highly significant and show no indication of any deviation from a

Type I functional response (for definitions and discussion see Holling, 1966; Hassell, 1978; van Alphen & Jervis, 1996; Kidd & Jervis, 1996). In other words, there is no evidence for any *handling time* constraints on searching efficiency.

The regression lines for the three parasitoid densities were not found to be significantly different in either slope or elevation, showing that there is no significant mutual interference (Beddington, 1975, Hassell, 1978) between searching female parasitoids.

A new sub-model of intermediate complexity was developed, so that both handling times and mutual interference could be incorporated into a single, easily parameterised equation and hence into the main *Empoasca* population model. The equation is :

$$N_a = N_t \{ 1\text{-}\exp[-P_t(a_{max} - b.\mathrm{LOG}N_t)P_t^{-c}] \} \ldots\ldots\ldots\ldots(1)$$

where N_a is the number of eggs parasitised, N_t the number of eggs available per leaf, P_t the number of searching female parasitoids, a_{max} the maximum 'area of discovery' (Nicholson & Bailey, 1935). In the absence of handling time and mutual interference, b is the functional response coefficient and c the mutual interference coefficient. In the absence of both handling time and mutual interference (b=0, c=0), the equation reverts to the the standard Nicholson-Bailey format:

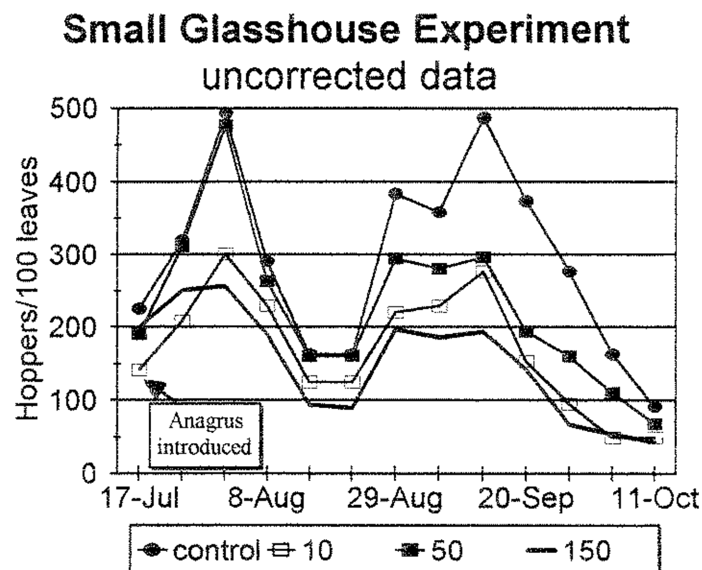
$$N_a = N_t \{ 1\text{-}\exp(-aP_t) \} \ldots\ldots\ldots\ldots(2)$$

Both equations could be used to model the 24h searching behaviour of *Anagrus*, but in the absence of evidence for significant handling time and mutual interference effects, the most appropriate is equation (2). The value of a for this equation was calculated as 1.59 from the functional response data, although, relating to a confined single plant resource, this is likely to be an overestimate and inapplicable to the glasshouse environment (see 3.3.4 below). *The value of this experiment is in testing for the presence of significant handling time constraints and mutual interference, not in calculating the value of 'a'* per se, *which would need to be estimated independently from the glasshouse experiments.*

3.3.4 Small-scale glasshouse experiment

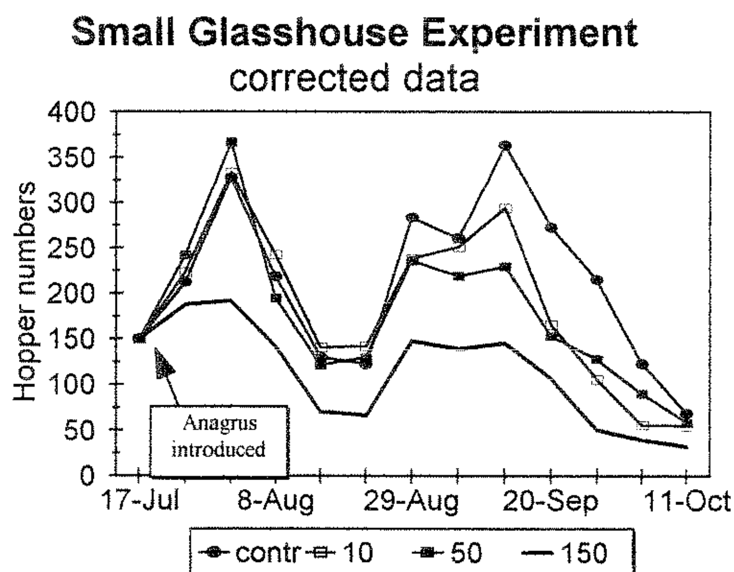
The total number of hoppers (nymphs and adults) counted weekly on 100 leaves in each treatment is shown in Figure 2. The dynamic pattern showed a clear separation into two generations of leafhoppers in all treatments. The control treatment showed the highest densities (around 500 hoppers/100 leaves in both generations) and the 150 parasitoid treatment the lowest. Results for the other treatments were, however, complicated by the effects of variable survival of adult hoppers at the start of the experiment, as a result of differential fecundity and (non-parasitoid related) mortality amongst treatments.

Figure 2 *Density of leafhoppers per 100 leaves sampled at weekly intervals in the small glasshouse experiment (each point is the average of 2 replicates/treatment).*



When corrected for this variation in leafhopper starting densities (*such correction is a standard procedure in population studies*), there is, however, a clear dosage-dependent effect of parasitoid numbers upon leafhopper numbers, particularly in the second generation (Fig. 3). Analysis of variance reveals significant effects of both treatment ($F = 16.3$, $P<0.001$) and date ($F = 15.55$, $P<0.001$).

Figure 3 *Density of leafhoppers per 100 leaves sampled at weekly intervals in the small glasshouse experiment (each point is the average of 2 replicates/treatment).*



Analysis of the leaf samples at the end of the experiment showed a percentage parasitism of less than 1% in the control glasshouses, compared with more than 30% in the 150 parasitoid test glasshouse (Table 1). This:

a) confirms that extremely few parasitoids entered the control glasshouses from the outside;

b) indicates that parasitoids entered those glasshouses very late in the experiment.

Table 1 *Numbers of hoppers and parasitoids emerging from all eggs counted in a 50-leaf sample taken from all treatments at the end of the small glasshouse.*

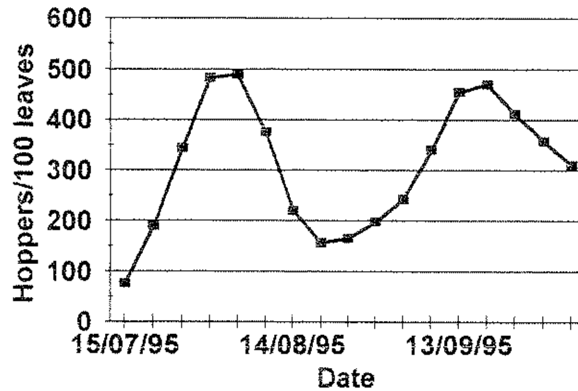
Parasit-oids introduced	**Repl. 1**				**Repl. 2**			
	Total	Hopper	Parasit.	% par	Total	Hopper	Parasit.	% par
control	535	534	1	0.18	396	393	3	0.75
10	450	287	163	36.2	611	533	78	12.7
50	542	348	194	35.7	522	379	143	27.3
150	486	320	166	34.1	442	303	139	31.4

3.3.5 Development and testing of simulation model

Calibration of the model was carried out using data from: (a) the detailed laboratory experiments on parasitoid searching efficiency (3.3.3), and (b) the small-scale glasshouse experiment (3.3.4).

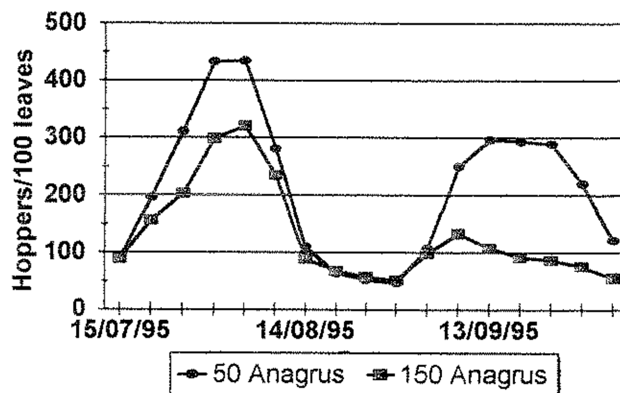
The intraspecific density dependence and density independence parameters constraining the growth rate of the leafhopper population to the plant resources available (calculated as 1125 leaves/glasshouse) were found by iteration of the model (see Kidd & Jervis (1996) for discussion of this technique). The best density dependence function was found to be: $Y = \exp(-0.003*(N-40))$, where N is the number of leafhopper adults, and Y is proportional daily survival for $0>Y>1$. In addition, a 2% proportional mortality was applied each day to the egg stage and a 1% mortality to the nymphal stages. These functions and parameter values provided a satisfactory fit of the leafhopper model to the glasshouse data, not incorporating parasitoids (Fig. 4).

Figure 4 *Model prediction of leafhopper densities per 100 leaves in the small glasshouse experiment with no parasitoids introduced.*



The laboratory experiments on parasitoid searching efficiency (see 3.3.3) revealed neither mutual interference between parasitoids nor significant handling time constraints, suggesting that a simple Nicholson-Bailey model (see Kidd & Jervis (1996)) would be adequate to describe parasitoid searching efficiency. To determine the 'area of discovery' (see Kidd & Jervis (1996)), the model was used iteratively with various parameter values, to determine the best fit to the small glasshouse population data. In practice, a value of $a = 0.011$ proved to give the most effective fit (Fig. 5). As expected, this value of a is very much lower than that calculated for the laboratory experiments on searching efficiency (section 3.3.3).

Figure 5 *Model prediction of leafhopper densities per 100 leaves in the small glasshouse experiments with parasitoids introduced in mid July.*



3.3.6 Large-scale glasshouse experiments

a) Anticipatory regime

Introduction of the parasitoid into C Block (test) in 1995 brought about significantly reduced leafhopper populations compared to E Block (control). Comparing population densities on the last two common sample dates (12/6/95, 21/6/95) before insecticide treatment, two-way analysis of variance showed both treatment differences ($F= 560.1$, $P=0.027$) and dates ($F= 348.4$, $P=0.034$) to be significant, the former accounting for 62% of the variance and the latter 38%. On the 21st June the hopper densities in C Block were 53.6% lower than in E block.

Analysis of the leaf samples at the end of the experiment (Table 2) showed a significantly higher ratio of parasitoids:total host eggs in the experimental glasshouse (C Block) than in the control (E Block) ($\chi^2 = 10.01$, $P<0.01$, 1d.f.). The fact that the control glasshouse had a ratio of 0.28 indicates that *Anagrus* had entered naturally at some point during the summer and established well. Care needs to be exercised in interpreting these ratios, however. Using the model to predict the cumulative total number of eggs laid by hoppers and parasitoids, yields an expected end of season ratio of parasitoid emergence:total hopper eggs of 0.07, considerably lower than that observed. This of course does not take account of destruction of hopper egg shells during the year (from leaf loss, for example), which would raise the expected ratio. This is because most hopper egg shells lost during the early part of the season would have been unparasitised, the highest parasitoid populations occurring towards the end of the season (see Fig. 7 below). Certainly, the ratio predicted by the model is raised considerably if only those hopper and parasitoid eggs laid in the last two months (ratio 0.12) or one month (ratio 0.20) of the season are accumulated. Also, the insecticide treatments carried out in late July and August are likely to have had a considerable effect on the observed ratios.

Table 2 *Numbers of hoppers and parasitoids emerging from all eggs counted in an 80-leaf sample taken from all treatments at the end of the large glasshouse experiment*

E Block	No. hoppers	No. parasitoids	**C Block**	No. hoppers	No. parasitoids
lower leaves	24	10	lower leaves	30	28
mid leaves	22	8	mid leaves	6	15
Total (80)	46	18	Total (80)	36	43
Ratio P/H	0.28		Ratio P/H	0.54	

The 1995 large-scale glasshouse data were used to test the extent to which the model could accurately predict:

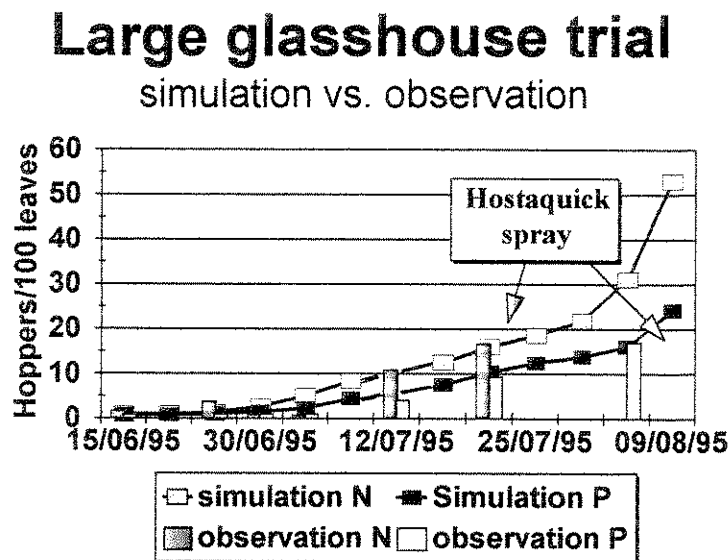
a) within-season changes in leafhopper numbers, given a much larger plant resource availability, in the presence and absence of the parasitoid;

b) the effectiveness of the parasitoid in reducing leafhopper numbers.

The parameter values calculated from the small glasshouse experiment (see 3.3.5) were also used in the large glasshouse version. The only change needed was a density correction factor to take account of the larger number of plants in the large glasshouse.

As can be seen from Figure 6, the model output agreed very well with the observed trends, both with and without parasitoids. Using the I_s/I_v validation index of Kidd (1990), it was found that the model could explain 86.2% of the variation in densities in E block (control) and 98.5% of the variation in C block (with parasitoids).

Figure 6 *Population growth of leafhopper in the 'anticipatory' experiment, both in the presence (P) and absence (N) of* Anagrus



This test of the model thus proved to be an effective validation of its ability to predict leafhopper numbers accurately throughout the season, with and without parasitoids present.

b) Reactive regime

The single set of comparative data from Blocks A and B, to which we can attach some confidence, does not indicate a significant role played by the parasitoid (Table 3). Although the densities are significantly different ($\chi^2 = 9$, $P<0.01$, 1.d.f.), this is in the wrong direction to that expected, if parasitoids are acting to reduce leafhopper numbers. With the simulation model, densities at the time of the sample are found not to be significantly different ($\chi^2 = 0.2$, $P>0.05$, 1.d.f.). Whatever the explanation for

the difference found between Blocks A and B, parasitoids are clearly not significant, which is consistent with the model's predictions.

Table 3 *Observed and predicted hopper densities in A and B Blocks on 12/07/95*

	Introduction of hopper	Introduction of parasitoid	Observed density hoppers/100 leaves (12/07/95)	Predicted density hoppers/100 leaves (12/07/95)
B block (control)	26th April		2.7	9.3
A block (experimental)	11th April	15th June	5.4	8.7

It is clear from both both sets of large glasshouse experiments that a single mass release of 2,000 female parasitoids in the middle of the summer is inadequate to control the leafhopper, but that repeated monthly releases of 2,000 female parasitoids from early spring can significantly reduce pest numbers. These results also go some way to explaining why, in the 1994 experiment, introduction of parasitoids was not more successful in reducing leafhopper numbers (see 1993-1994 annual report on Project PC 76, for details). In that experiment, only 30 female parasitoids were released weekly over 8 weeks, 240 female parasitoids in all, and clearly an inadequate number to achieve significant control.

The 1995 large glasshouse experiments have clearly been successful in:

a) demonstrating that the parasitoid is capable of significantly reducing leafhopper densities;

b) validating the final version of the model.

What they do not provide, however, is an indication of likely hopper population trends in the absence of insecticide intervention. The use of insecticides in the large glasshouse trials was unavoidable, as the growers have to meet commercial targets for fruit production (see below). This, however, is not a problem as we can now use the validated model to explore the leafhopper's likely population dynamics in the absence of insecticide intervention.

3.3.7 Determining optimum biological control strategies

A number of questions can now be set for the model to answer:

a) can the anticipatory regime tested above provide an *adequate control* of leafhopper numbers?

b) are there other introduction/release strategies which would provide better control?

c) is the timing of parasitoid introductions/releases critical?

d) is the number of parasitoids introduced/released important?

Damage thresholds

To answer these questions we first have to define what we mean by 'adequate control'. Is there a damage threshold population level of leafhoppers below which leafhopper numbers should be suppressed? It should be noted that action thresholds for pesticide intervention in glasshouse peppers have so far been based not on prevailing infestation levels of leafhopper, but on a subjective assessment of fruit damage. When too high a proportion of harvested fruit fails to meet specification for Class I peppers, spraying is initiated. In 1995, this action threshold coincided with hopper densities of around 20/100 leaves, giving us an approximate damage threshold criterion with which to assess different possible control strategies using the parasitoid as a biocontrol agent.

Predicted hopper dynamics in the absence of insecticide intervention

Without insecticide intervention, the model predicts that numbers will rise quickly in the control glasshouse during early August to stabilise at a level of around 300 leafhoppers/100 leaves (Fig. 7). With the 'anticipatory' parasitoid release strategy (Block C), numbers rise to a peak of just above 200 leafhoppers/100 leaves (Fig. 8), before crashing to very low levels during late September and early October. Figure 8 also shows the timing of parasitoid introductions (arrowed).

Figure 7. *Model prediction of hopper dynamics in the absence of both parasitoid and insecticide intervention.*

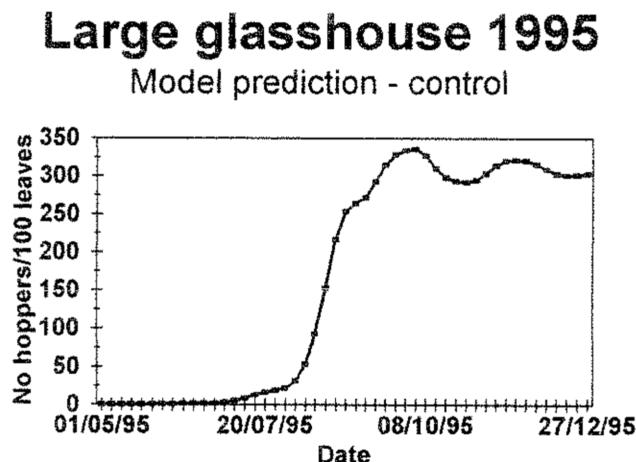
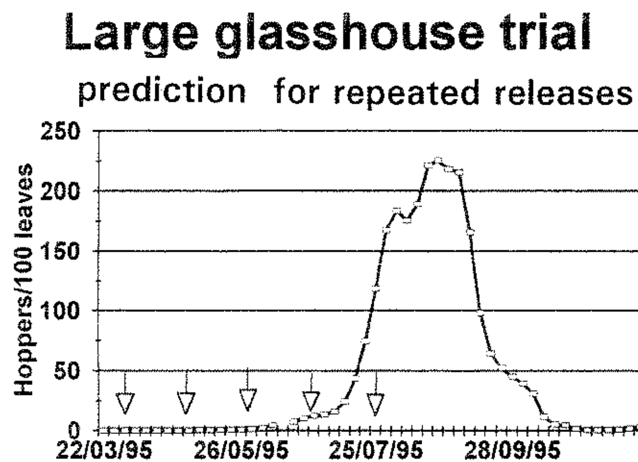


Figure 8. *Model prediction of hopper dynamics in the absence of insecticide intervention, but with repeated monthly release of parasitoids*



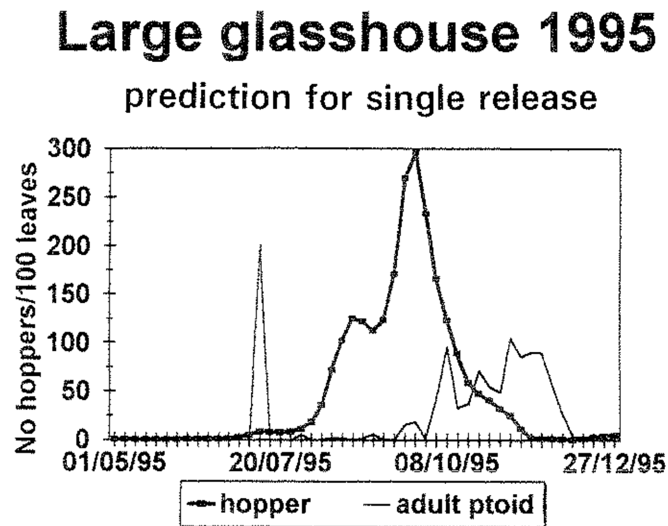
It is clear from Figure 8 that despite reducing peak leafhopper numbers by 40%, the parasitoid introduction regime employed during 1995 was unable to suppress leafhopper numbers to non-damaging levels (c.20 leafhopper/100 leaves, see Fig. 6). Thus, an alternative introduction strategy needed to be sought.

Alternative introduction strategies

i) using a single mass release of parasitoids during early July

Figure 9 shows the result of introducing on 10/7/95 15 times the number of parasitoids used per release in the 1995 trial (30,000 female parasitoids). As can be seen, the strategy is ineffective at reducing leafhopper numbers to non-damaging levels (c. 20 leafhoppers/100 leaves), the peak density remaining at c.300 hoppers/ 100 leaves.

Figure 9. Predicted effect on hopper densities of releasing 30,000 female parasitoids into C block on 10/07/95



ii) using repeated mass releases early in the year

Repeated releases at monthly intervals of 15 times the number of parasitoids used in the 1995 trial also proved ineffective at reducing peak leafhopper numbers.

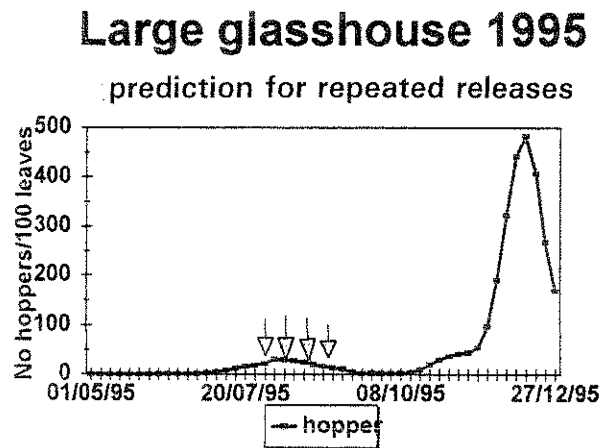
iii) repeated mass releases of parasitoids during August

Figure 10a shows the result of introducing 15 times the number of parasitoids used per release in the 1995 trial (30,000 female parasitoids), at 5-day intervals between 4th and 20th of August. Leafhopper numbers are drastically reduced to an August peak of about 30 leafhoppers /100 leaves (Fig. 10b), only rising again during December, thus obviating the need for pesticide intervention. This encouraging result demonstrates that the strategy of mass releases during August is likely to prove the most useful control option. The rise in leafhopper numbers in December is not important, as by this stage, harvesting of the pepper crop has been completed.

An even better result can be obtained using *five* repeated introductions, rather than four (Fig. 11). Here, mass release of 30,000 female parasitoids on 1/8/95, 5/8/95, 10/8/95, 15/8/95, 20/8/95 reduced the peak to around 20/100 leaves (the damage threshold). As hopper numbers only remain at this peak for a very short time before declining, this strategy would therefore obviate the need for any insecticide intervention.

Figure 10 *Predicted effects on hopper densities of 4 repeated releases of 30,000 female parasitoids at five-day intervals during August*

a.



b.

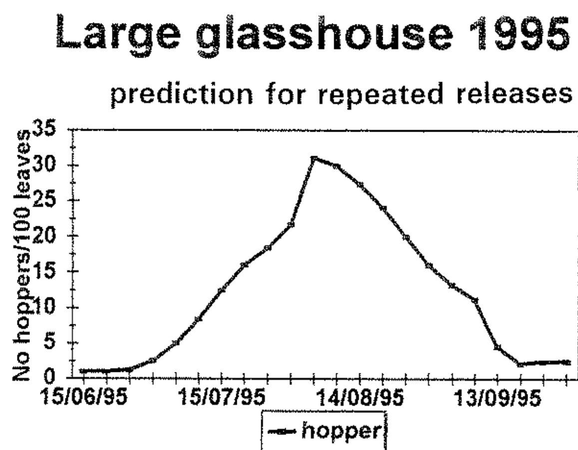
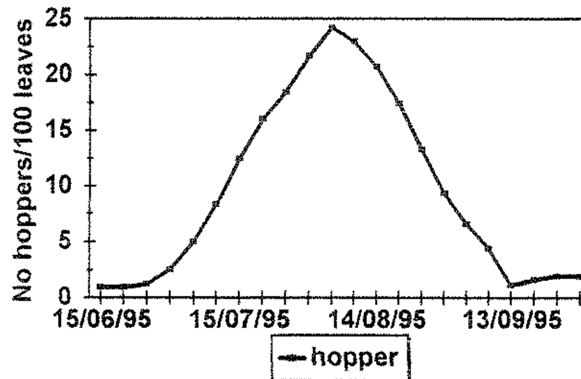


***Figure** 11. Predicted effects on hopper densities of 5 repeated releases of 30,000 female parasitoids at five-day intervals during August*



The large numbers of parasitoids that would be required to implement this strategy would necessitate the involvement of a commercial biological control enterprise with at least medium-scale mass-rearing facilities (estimated numbers of female parasitoids per introduction per glasshouse the size of the experimental one = c.30,000 = 120-150,000 per glasshouse per season). The only feasible delivery system would be the one outlined in the 1993-1994 annual report for Project PC 76, namely the bean plant method (see section 3.2.2).

Note that the specific timing of the aforementioned introductions is temperature-dependent; therefore the model is likely to be a vital tool for determining optimal timing of introductions from year to year. The model has been constructed to be fully interactive and 'user-friendly', to assist the advisor/grower, although some further refinement will be required before it can be released to the user.

3.4 Conclusions

With few changes in the planned programme of work, we have been able to show that the only feasible option for *Empoasca* biocontrol in sweet peppers is through mass-rearing (to be undertaken by a commercial biocontrol operator) and release of *Anagrus atomus*. Employing the fully interactive, user-friendly computer model, the timing and size of parasitoid releases can be decided upon.

The value of the modelling approach we adopted for this project ought to be clear from the conclusions drawn. Using a conventional trials-based research programme, we would never have been able to produce, with the facilities and staff at our disposal, the numbers of parasitoids needed to demonstrate the results shown in Figures 10 and 11. Also, repeated large-scale glasshouse trials with different parasitoid release regimes would have been *too time-consuming* and *expensive* to carry out, *with no guarantee of identifying the optimum parasitoid release strategy*.

We are confident that full implementation of the proposed repeated mass release strategy will significantly reduce the *Empoasca decipiens* problem in sweet peppers, particularly when used in conjuction with the simulation model to identify optimum dates for releases. ***Furthermore, it will eliminate the need for insecticide intervention***. A similar control strategy could also become a feasible option for use against the leafhopper, *Hauptidia maroccana*, on glasshouse tomatoes, again using *Anagrus atomus* as the biological control agent.

The parasitoid delivery technique i.e. the use of broad bean plants, is the one we recommend for commercial development. It has the following advantages over other methods:

a) adult parasitoids eclose from the pupa *in the grower's glasshouse*, not in the biocontrol supplier's insectary; adult mortality is therefore minimised;

b) plant tissue desiccation is avoided. Using the cut-leaf system used for the introduction of whitefly would cause massive mortality of parasitoids, as the leafhopper eggs (within which the parasitoids pupate (Waloff & Jervis, 1987) can only survive if leaf tissue remains turgid. Whole broad bean plants can be maintained through the glasshouse hydroponics system.

Broad beans are preferred to glasshouse crop plants as the delivery plant, because:

a) pathogens such as tobacco mosaic virus can be introduced using the latter;

b) much lower densities of leafhoppers (and therefore much smaller numbers of parasitoids) are obtainable on peppers as compared with bean plants.

To ensure that the leafhopper population in an infested glasshouse is not significantly enhanced, the commercial biocontrol operator must ensure that:

a) live leafhopper nymphs and adults are removed from the plants prior to introduction;

b) the percentage of parasitised eggs in the delivery plants is very close to 100.

If passed on to a commercial biological control supplier this spring (1996), the delivery system could easily be 'up and running' in time for parasitoid releases to be made in growers' glasshouses this August. A culture of *Anagrus atomus* is currently maintained by English Woodlands Biological Control Ltd., Petworth, and a culture of *Empoasca decipiens* is currently maintained at Cardiff.

3.5 References

van Alphen, J.J.M. & Jervis, M.A. (1996) Foraging behaviour. pp. 1-62 in M.A.Jervis & N.A.C.Kidd (eds) *Insect natural enemies: practical approaches to their study and evaluation*. Chapman & Hall.

Beddington, J.R. (1975) Mutual interference between parasitoids or predators and its effect on searching efficiency. *Journal of Animal Ecology*, 44,331-340.

Copland, M.J.W. & Soeprapto, N. (1985) Biology of the glasshouse leaf-hopper and its parasite. pp. 58-65, in N.W.Hussey & N.Scopes (eds) *Biological pest control: the glasshouse experience*. Blandford Press.

DeBach, P. & Rosen, D. (1991) *Biological control by natural enemies*. Cambridge University Press.

Hassell, M.P. (1978) *The dynamics of arthropod predator-prey systems*. Princeton University Press., Princeton. N.J.

Holling, C.S. (1966) The functional response of invertebrate predators to prey density. *Memoirs of the Entomological Society of Canada*, 48, 1-86.

Jervis, M.A., Kidd, N.A.C. & Dungey, N. (1993) Green leafhopper: a new pest. *Grower* December: 18-19.

Kidd, N.A.C. (1990) The population dynamics of the large pine aphid, *Cinara pinea* (Mordv.). I. Simulation of laboratory populations. *Researches on Population Ecology*, 32, 189-208.

Kidd, N.A.C. & Jervis, M.A. (1996) Population dynamics. pp. 293-374 in M.A.Jervis & N.A.C.Kidd (eds) *Insect natural enemies: practical approaches to their study and evaluation*. Chapman & Hall.

LeQuesne, W.J & Payne, K.R. (1981) Cicadellidae (Typhlocybinae). *Handbooks for the Identification of British Insects* 2 (2c): 1-95.

Nicholson, A.J. & Bailey, V.A. (1935) The balance of animal populations. *Proceedings of the Zoological Society of London*, 1935, 551-598.

Ossiannilsson, F. (1981). The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark, Part 2. *Fauna Entomologica Scandinavica* 7: 223-593.

Waloff, N. & Jervis, M.A. (1987) Communities of parasitoids associated with leafhoppers and planthoppers in Europe. *Advances in Ecological Research* 17: 281-402.

Williams, J., Kubelik, A., Livak, K., Rafalski, J.A. & Tingly, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18, 6531-6535.

3.6 Appendices

Appendix 1

The Empoasca Model

The model of *Empoasca decipiens* populations was developed using a generalised modelling 'shell' called POPULA devised by Dr N.A.C. Kidd at the University of Wales, Cardiff. The POPULA program is written in TurboBasic and comprises a fully menu-driven, interactive environment for developing customised models for any insect pest species, providing that sufficient biological information is available. A particular feature of the program is the facility to subject model populations to different management options, thus testing their likely efficacy in the real world, prior to any expensive field trials. POPULA has the capability of modelling both hemimetabolous and holometabolous life cycles, and can include the effects of variable temperature on the development of immature stages.

Modelling Rationale

The modelling approach which has been used to develop POPULA (including the customised version for *Empoasca*) is similar to the 'variable life table' format of Gilbert *et al.* (1976) , in which the insect age groups are assigned to elements of an array, each element corresponding to a fixed time period, e.g. one day. Thus, for example,

TOTALEGGS =EGGS(1)+EGGS(2)+EGGS(3)............EGGS(EGGMAX)

where the number of eggs in each age group is variable from day-to-day.

In more conventional mathematical notation this would be

$$E_{total} = \sum_{i=1}^{N} E(i)$$ where E_N is the last age group of eggs.

Similar arrays are dimensioned for all stages from eggs to adults, depending on whether the insect is holometabolous or hemimetabolous, i.e. eggs, nymphs, adults for the latter and eggs, larvae, pupae, adults for the former. The sex ratio of adults is then set.

The reproductive output of each female adult in each daily age group is then assigned to another array of similar length to ADULT, i.e.

ADULT (1), ADULT(2), ADULT(3).........ADULT(N)
REPRO (1), REPRO(2), REPRO(3).........REPRO(N)

Total reproduction for each day is then calculated as:

TOTALREP = ADULT(1)*REPRO(1)+ADULT(2)*REPRO(2)........ADULT(N)*REPRO(N)

To simulate population changes from day-to-day, the computer:

(1) places the initial number in each element of each array (the initial age structure), (2) calculates the total reproduction, (3) ages the population by one day (e.g. ADULT(N)=ADULT(N-1), and (4) places the number reproduced (TOTALREP) into the youngest age group of eggs [EGGS(1)].

The model operates with a time-step of one day and stages 2-4 can be repeated over as many days as the required to simulate population change. This basic model can then be elaborated to include a number of additional components, such as density-dependent mortality acting on each stage, or temperature-dependent development of immatures. In its current form, the POPULA model is purely deterministic in its operation, using fixed parameter values throughout. There is no technical reason why, if so desired, additional stochastic elements cannot also be included.

Temperature-dependent development

Because development rate in arthropods is temperature dependent, individual development and population growth have often been modelled on a physiological time scale of day-degrees (e.g. Hughes, 1962,1963) instead of calendar time. On this time scale, regardless of temperature, development proceeds at a constant rate. Modelling insects on a purely physiological time scale can, however, create problems, especially if it is desirable to incorporate certain components which need to be measured on a calendar time scale. POPULA reconciles these needs by using a basic calendar time scale (usually a time-step of 1 day), but allowing development of the insects to speed up or slow down depending on the prevailing temperatures.

This is done by first assigning thermal constants to the development of each stage, i.e. the number of day-degrees needed to complete development. On a physiological time scale this would be the number of elements in the age group array for each stage, i.e. 1 day-degree for each age group . In practice, the array sizes would become too large for the simulation to run quickly and efficiently, so a convenient interval of, say, 4 day-degrees is used for each age group. This is the interval used by POPULA. Threshold temperatures below which development stops also need to be known. During the simulation the program calculates the thermal accumulation for each day using the average of maximum and minimum temperatures,

i.e. DAYDEG= (MAX+MIN)/2

Then, the development threshold for the stage in question is subtracted:

DAYDEG=(MAX+MIN)/2-THRESH

Without temperature-dependent development, the ageing process is simple. As each day passes, the individuals in one element of an array are moved into the next 'older' one. With temperature affecting development, the number of elements that individuals move becomes variable. The number of elements incremented is determined by :

INCREMENT=INT(DAYDEG/4+0.5)

Thus, where DAYDEG on a particular day is high the number of array elements individual immatures 'move along' (INCREMENT) will also be greater than when DAYDEG is low as on cold days.

Mortality

Each day the individuals in each age group are subjected to mortality from both density-independent sources, e.g. weather factors and also from density-dependent factors, e.g. intra-specific competition The equations determining the action are as follows and act in sequence, i.e. density-independent mortality first:

Stage(i) = Stage(i)*(1-DIMORT/100)...........(1)
Stage(i) = Stage(i)*CONST*EXP(-COEFF/100*Stage(i)) ....(2)

where DIMORT is the percentage density independent mortality, CONST is the density-dependence constant and COEFF the density-dependence coefficient.

Using the Model

The User Interface

The 'shell' within which the programs are run is fully menu-driven, the user responding to prompts and information messages at every stage. The **MAIN MENU** offers a choice of actions **(FILE, EDIT, OPTIONS, PLOT, RUN)**, each of which is accessed by **entering the first letter of the word**.

FILE : This option provides a drop-down menu with a number of file handling routines (**FILE: Load, Save, Write to, Quit**). The **LOAD** option allows the user to retrieve an input file containing the essential information about the population (delimited by the *.**inp** descriptor). On accessing **LOAD**, a list of files in the directory is provided. The choice of file must be typed **exactly as given,** i.e. with the **.inp** descriptor, followed by **<ENTER>. SAVE** and **WRITE TO** can be used to save modified or new data from the edit screen (see below) into the same or new *.inp files (users need to remember to include the ***.inp** descriptor when choosing a new file name with **WRITE TO**! **QUIT** exits the program and returns to the **DOS** prompt).

EDIT : Once a *.inp file has been loaded, choose **EDIT** to check or alter the parameter values or data files. There are two **EDIT** screens, which can be toggled between using **PgUp/PgDn**. The first screen is for the main population, the second for an optional interacting parasitoid population. In both screens use the UpArrow and DnArrow keys to move between the data fields.

Screen 1 :

The first 4 fields allow the inclusion of data on the species being modelled. Simply type in the information you wish to store (up to the character limit of each field). Use **BackSpace** to delete unwanted characters.

The next 2 field contains the filenames of: (a) the data needed to 'seed' the simulation, i.e. the initial numbers entering the population and the times at which they appear, and (b) the fertility profile of the adult female population, i.e. the number of eggs laid by each age group. More information on how to set up these files is given below (see SETTING UP FILES).

The field labelled '**Simulation time**' allows you to set the time limit over which you want the simulation to run. If the time units are days, then 365 would correspond to one year. For phenological simulations of populations running from January 1 - December 31, use a simulation time of 364, setting the 'Initial Data file' (above) so that the first insects (usually adults) appear at a time corresponding to emergence in the field.

'**Sex Ratio**' allows you to set the % of female insects in the emerging adult population.

Below this line we have a table of data which gives information on each of the stages of the population, i.e. eggs, larvae, pupae and adults. The column labelled '**development time**', gives the number of time units (usually days) taken by each stage, from inception to moulting; in the case of the adults, this is corresponds to longevity, i.e. from adult moult to death.

The four columns labelled '**Mortality**' provide the information on stage-specific density-dependent and density-independent mortalities. Density dependence is described by the equation:

$$S=C.\exp\{-b.(N-T)\}$$

where S is the proportion surviving, T is the density threshold above which the mortality begins to take effect. C is the maximum survival (given as a % rather than a proportion in the screen table) , and b the coefficient of density dependence (the larger the value the stronger the density dependence).

Density-independence is given as a % mortality per time unit, e.g. for eggs if the value given is 2, this corresponds to a proportional mortality of 0.002.

Screen 2 :

The data for the parasitoid population are organised in the same way, except that the population is assumed to be all-female, i.e. sex ratio = 100%. No field for altering this is provided. Instead of an initial data file or fertility file, the parameters needed for the parasitoid are concerned with searching efficiency. Three parameter values are

provided for, in case the searching model needs to be altered to a more complex form. At the moment the Nicholson-Bailey parameter ***a*** (the area of discovery) is used and this is given as '**searching parameter 1**'

Development time and mortality data are given as described for screen 1 above.

Use **ESC** to get out of the edit screen. If data has been altered and you wish to save into the same or another *.inp file use **FILE : SAVE** or **FILE : WRITE TO**. Do not forget the ***.inp** descriptor when you choose a new file name.

RUN :
Once the model parameter values and access files have been set in the **EDIT** screen, use the **RUN** option from the main menu to start the simulation. This takes you to a 'fancy screen' which indicates with a moving pointer, how the simulation is progressing. **Do not touch the keys until after the beep, when the Main Menu again appears.**

PLOT :
To plot the data press **'P'** and a small screen will apear asking you to enter the plot interval. Normally this will be 1 (to give daily output), so type 1 <ENTER>. You will then be prompted by a drop-down menu to state whether you want the plot data as arithmetic or log-plot. Use the UpArrow/DnArrow to choose which. Again, a drop-down asks you whether you want the total simulation time plotted or specified periods (for the latter you are asked to input a start time and an end time). Then you are asked whether you want the output on the screen, sent to a printer, or saved as a file.

Screen:
The simulation output is presented initially as a line graph of total numbers (eggs+larvae+pupae+adults) for each time unit. The units are given on the X-axis. As the top line of the screen states, you can also add each stage separately using the first letters as indicated. The age groups are colour coded as shown under the screen title. Use **ESC** to return to the Main Menu.

Print:
Output to a printer simply shows the raw data of numbers in each stage at each time period.

File :
To plot to a file, choose '**FILE**' and a small screen will apear asking you to enter the plot interval. Normally this will be 1 (to give daily output), so type 1 **<ENTER>**. You will then be prompted for a filename. This should be of the form ***.prn** (make sure to add the **.prn** descriptor) if you want to access the file from Quattro, Excel or some other spreadsheet, in order to produce presentation graphics.

OPTIONS :

The **OPTIONS** choice in the Main Menu allows you to add various pest management possibilities to the simulation.

1. '**Parasitism**' gives you the opportunity of adding a set number of parasitoids (with characteristics set by Screen 2 in **EDIT**) at a defined time during the simulation. The stage you want attacked is also specified by a menu. Up to 6 repeated introductions are allowed. To end the input of introduction times before this, simply <**ENTER**> without entering data. This will return you to the drop down menu. **ESC** takes you back to the Main Menu.
2. '**Ovicide**' allows you to kill off a set proportion of eggs (e.g. by an insecticide) during set time periods (up to 6 defined by the user). Enter the % mortality you want and <**ENTER**>. Then enter the time of application (up to 6 repeated values), as for parasitism above.
3. '**Larvicide**' and '**Pheromone**' allow the same kind of mortality to be applied to the larvae and adults respectively.
4. ' **Reset**' cancels all of the OPTIONS settings.
5. '**Exit**' or **ESC** returns you to the Main Menu.

The programme is therefore extremely versatile as a tool for examining both theoretical populations and their interactions with parasitoids, and real insect pests whose population characteristics are known. The pest management options allow some very useful insights into the best strategies for suppressing pest numbers.

References

Gilbert, N., Gutierrez, A.P., Frazer, B.D. and Jones, R.E. (1976) *Ecological Relationships*. Freeman, Reading.

Hughes, R.D (1962) A method for estimating the effects of mortality on aphid populations. *Journal of Animal Ecology*, 31, 389-396.

Hughes, R.D (1963) Population dynamics of the cabbage aphid, *Brevicoryne brassicae* L. *Journal of Animal Ecology*, 32, 393-424.

HOPPER

POPULATION SIMULATION PACKAGE

** by **
*** Neil Kidd ***
* University of Wales *

** HOPPER Modelling Menu **

FILE　EDIT　OPTIONS　PLOT　RUN

[File]
- Load
- New
- Save
- Write to
- Quit

[OPTIONS]
- Parasitism
- Insecticide
- Microbial
- Pheromone
- Reset
- Exit

[PLOT]
- Arithmetic
- Logarithmic

[DATES]
- All Dates
- Select Dates

[DATES]
Start Date (dd/mm/yy) ?

[DATES]
End Date (dd/mm/yy) ?

*** SELECT USING MENU BAR ***

Appendix 2

Summary of life-history information for *Empoasca* and *Anagrus atomus*

1. Development time of *Empoasca descipiens* on pepper.

Stage	22 degrees (days)	26 degrees (days)	Day-degrees	Threshold
egg		9.4	180	9
nymph	19.7	11.2	180	9
adult		36.7		
Total		57.3		

2. Development time of *Anagrus atomus.*

Stage	26 degrees (days)	Day-degrees	threshold
Immature	14.1	252	4
Adult	5		
Total	19.1		

3. Fecundity of *Empoasca descipiens*

Female longevity (d)	Pre-oviposition period (d)	Oviposition period (d)	Total nymphs deposited	Average no. nymphs/day	
36	12	19	37	1.8	Pepper
29	8	19	62	3.3	Bean

(d)= days

3.7 Contract

Contract between the University of Wales College of Cardiff (UWCC) (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT

Contract No: PC/76

SWEET PEPPERS: BIOLOGICAL CONTROL OF GREEN LEAFHOPPER (*EMPOASCA DECIPIENS*)

2. BACKGROUND AND COMMERCIAL OBJECTIVE

The leafhopper *Empoasca decipiens* is a pest of sweet peppers in a large commercial nursery near Littlehampton. It has also been recorded in another nursery nearby, infesting ornamentals. The insect has become a pest as a result of the successful use of biological control against other pests, where negligible chemical inputs are involved. *E.decipiens* is therefore likely to become increasingly prominent as a pest as nurseries convert to biological control. Control of this insect has to be obtained using methods that do not conflict with existing biological methods.

E.decipiens has a wide geographical range that includes Europe and the UK. It has been recorded on fruit trees and potatoes, as well as on a range of wild plants, including stinging nettles. Because of its broad host range, it may have the capacity to spread further within the horticultural industry. Overseas, it is known to transmit plant viruses.

The aim of this project is to find a biological control method for *E.decipiens* that will be compatible with biological techniques used against other pests of sweet peppers. The particular method proposed here involves the use of hymenopteran parasitoids of the egg, nymphal and adult stages. One of us (Dr M Jervis) is the recognised international expert on the biology, ecology and taxonomy of parasitoids of Typhlocybinae - the leafhopper group to which *E.decipiens* belongs (see Jervis 1978a, 1978b, 1979a, 1979b, 1980, 1980b, 1980c, 1982a, 1982b, 1985, 1986, 1992; Waloff & Jervis 1987), and is currently involved in a biocontrol programme launched against closely related leafhopper pests on apples and berryfruits in New Zealand (Jervis, 1991). Dr N Kidd is a recognised expert on insect population dynamics and simulation modelling. Together, we have also published widely on the population biology of parasitoids in general (work which has important implications for biological control (e.g. see Kidd & Jervis, 1989)), and we are currently involved in biological control programmes aimed at non-leafhopper insect pests. At the end of this year, we are to become the editors of the *International Journal of Pest Management*. We are therefore suitably qualified to undertake the proposed work.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

E.decipiens poses a serious problem for the maintenance of biological crop protection in sweet peppers. Control of this insect has required chemical treatments inimical to biocontrol, and the nursery affected has had to revert to chemical control. The collapse of the biocontrol programme could result in financial loss, as a premium may be gained from some retailers on biologically protected produce. The maintenance of biological crop protection programmes and the minimisation of pesticide usage also enhances credibility with consumers generally.

The discovery of natural enemies for use against *E.decipiens* would provide an additional product for sale by the rearing companies.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

The main scientific target is to find one or more insect parasitoids that will limit leafhopper populations to non-damaging levels in sweet peppers. The subsidiary targets are:

1. to devise a method for rearing the green leafhopper in laboratory conditions;

2. to obtain several candidate parasitoid species from the British fauna;

3. to establish the candidate parasitoid species in laboratory culture and to devise methods for culturing them on a medium scale for cage and small glasshouse experimentation.

4. to measure key life-history parameters for the leafhopper and each of the candidate parasitoids;

5. to determine and compare the *per capita* parasitisation efficiency (including density responsiveness) of the candidate parasitoid species;

6. to develop a computer simulation model, incorporating parameter values obtained from (4) and (5) above, and to use the model in predicting both the likely impact of candidate parasitoids and the optimum strategy for parasitoid release;

7. to determine whether release of selected parasitoid species (selected and released on the basis of the model's predictions) brings about effective control of the leafhopper pest in the large glasshouse environment.

Even before objective (7) has been completed, the biological material and information on rearing systems could be passed to a biological control agent supply

company for further development. The same material and information would also be made available to ADAS entomologists (at the discretion of the HDC).

5. CLOSELY RELATED WORK

The egg parasitoid *Anagrus atomus* (Mymaridae) is currently used successfully against a leafhopper pest (*Hauptidia maroccana*) of other glasshouse crops. *Anagrus* species, together with *Aphelopus* species (Dryinidae) are also being considered for use against leafhopper pests (*Edwardsiana crataegi, Ribautiana tenerrima*) of apples and berryfruits in New Zealand (Jervis, 1991). A biological control programme (in which MAJ has been involved), launched by the US Department of Agriculture, has concentrated on parasitoids as potential control agents for *Empoasca fabae* (see Jervis (1992) for taxonomy of the dipteran parasitoids of *E.fabae*).

The above programmes, together with the large body of information on parasitoid biology gathered by Jervis (see references and also Ph.D. thesis by L.Munroe (1991) his student), provide a good platform of knowledge on which the proposed project may be built. In particular, they suggest *Anagrus* species and *Aphelopus* species as prime candidates for use in biocontrol of *E.decipiens*.

Note also that in Cardiff techniques have been successfully developed for continuous culturing of close relatives of *E.decipiens*, *Eupteryx* species (Stiling, 1979; Munroe, 1991).

6. DESCRIPTION OF THE WORK

a) Establishment of *E.decipiens* laboratory populations and collection of parasitoids (September 1992-May 1993)

E.decipiens will be obtained from growers' glasshouses and allowed to oviposit in one of its main natural host plants (Le Quesne & Payne, 1981)), stinging nettles (*Urtica dioica*) grown in pots under glasshouse conditions. The nettle plants, loaded with eggs, will then be placed in the field in a variety of habitats as "trap" plants for any *Anagrus* species present. The plants will be returned to the laboratory in October/November and will then be subjected to different temperature/photoperiod regimes in an attempt to accelerate the breaking of diapause in both the leafhopper eggs and the parasitoids (hatching of *E.decipiens* eggs is normally expected in March/April). Typhlocybine leafhoppers of a wide range of species will also be collected in September and October, and *Aphelopus* species (parasitoids of the nymphal and adult stages) reared from them. The overwintering stages (prepupae) of the parasitoids will be kept in an outdoor insectary.

Further explorations for parasitoids will also take place

during subsequent stages of the project.

b) Culturing of *E.decipiens* and parasitoids (March 1993-Sept 1995)

The leafhopper and its parasitoids will be cultured in the laboratory, on both nettles and sweet peppers. Techniques for handling the leafhopper and parasitoids will be developed and refined. Identification and morphological characterisation of parasitoids will be carried out (it is important that the different *Anagrus* species, which may possibly be undescribed, be distinguished and characterised).

Cultures of parasitoid species considered as 'best' for biocontrol (see (d) below) to be expanded, for large glasshouse trial in March 1995.

c) Measurement of key life-history parameters of leafhopper, measurement and comparison of key life-history parameters and *per capita* parasitisation efficiency of candidate parasitoid species (March 1993-December 1994)

Development rates of insects, at temperatures comparable with those in glasshouses, to be measured. Parasitisation efficiency of parasitoids to be measured by means of functional response experiments (see Sahragard, Jervis & Kidd (1991), for details of experimental design). Measures of 'overall' parasitisation efficiency to be obtained from functional response and aggregative response experiments, both types of experiment being designed to test density responsiveness of parasitoids on density scales comparable with those normally occurring under glasshouse conditions (determined by monitoring of populations in growers' glasshouses). Evidence for competition between different *Anagrus* species sought.

d) Development and testing of simulation model (October 1993-August 1995)

Controlled release of parasitoids in infested glasshouse(s). Host-parasitoid simulation model to be constructed, incorporating key life-history parameters of host and parasitoids. Potential of different parasitoid species to control and regulate host populations, and also to interfere with/complement each other, investigated using model. Initial predictions of likely impact of candidate parasitoid species, and choice of 'best' species to be made by March 1994.

e) small-scale glasshouse trials involving selected ('best') parasitoids (April-September 1994)

Testing of predictions of simulation model, using data obtained from regular monitoring of host populations (host densities, numbers/proportions of hosts parasitised).

f) Validation/correction of simulation model (October-December 1994).

Further laboratory experiments on parasitoids to refine the model for final use.

g) Large glasshouse trial (February-September 1995)

Large-scale glasshouse trials of parasitoid species selected and implemented on the basis of laboratory and smaller-scale glasshouse rearing experience and model's predictions.

7. COMMENCEMENT DATE AND DURATION

Start date 01.09.92; duration 3 years.

The project will commence on September 1st, 1992, however the placing of "trap plants" and collection of parasitoids will commence before this date.

The appointment of both the postdoctoral RA and the technician would commence from September 1992.

Because the biologies of *E.decipiens* and *Anagrus* species are poorly known, it is essential that two entire field seasons be allowed for the research into the life cycles of leafhopper and parasitoids before final field trials are carried out.

8. STAFF RESPONSIBILITIES

Supervision of Project: Drs M.A.Jervis & N.A.C.Kidd (10% each)

Project officer: Postdoctoral RA (to be appointed) (100%)

Technician: to be appointed, concerned with rearing of insects and assisting with experiments)(100%).

The RA will be ideally qualified, after the three years, to take up employment in an institute such as HRI, working on biological control.

9. LOCATION

All the work, except for the final large glasshouse trials and some preliminary investigation of *E.decipiens* populations (see 6(c) above), to be carried out in the laboratories and glasshouse facilities of the School of Pure & Applied Biology, UWCC. Parasitoids will be collected from habitats (a) close to glasshouses at Van Heyningen Brothers Ltd, Littlehampton, and (b) within approximately a 10 mile radius of Cardiff.

APPENDIX REFERENCES

Jervis, M.A., 1978a. Homopteran bugs. In: *A Dipterist's Handbook* (Eds. A.Stubbs & P.J.Chandler). Hanworth: Amateur Entomologist's Society, pp. 173-176.

Jervis, M.A. 1978b. Ecological studies on the entomophagous parasites of Typhlocybine leafhoppers. Unpublished Ph.D. thesis, University of Wales.

Jervis, M.A., 1979a. Parasitism of *Aphelopus* species (Hymenoptera: Dryinidae) by *Ismarus dorsiger* (Curtis) (Hymenoptera: Diapriidae). *Entomologist's Gazette* 30: 127-129.

Jervis, M.A., 1979b. Courtship, mating and 'swarming' in *Aphelopus melaleucus* (Dalman) (Hymenoptera: Dryinidae). *Entomologist's Gazette* 30: 191-193.

Jervis, M.A., 1980a. Studies on oviposition behaviour and larval development in species of *Chalarus* (Diptera:Pipunculidae), parasites of typhlocybine leafhoppers (Homoptera: Cicadellidae). *Journal of Natural History* 14: 759-768.

Jervis, M.A., 1980b. Life history studies of *Aphelopus* species (Hymenoptera: Dryinidae) and *Chalarus* species (Diptera: Pipunculidae), primary parasites of typhlocybine leafhoppers (Homoptera: Cicadellidae). *Journal of Natural History* 14: 769-780.

Jervis, M.A., 1980c. Ecological studies on the parasite complex associated with typhlocybine leafhoppers (Homoptera: Cicadellidae). *Ecological Entomology* 5: 123-136.

Jervis, M.A., 1982a. Taxonomic studies on *Chalarus* species (Diptera, Pipunculidae), using the scanning electron microscope. *Acta Entomologica Fennica* 38: 25.

Jervis, M.A., 1982b. Cover feature. *Bulletin of The Royal Entomological Society of London* 6: 245.

Jervis, M.A., 1985. Two new species of *Chalarus* Walker (Diptera, Pipunculidae) from Burma. *Schweizerische Entomologische Gesellschaft Mitteilungen* 5: 435-440.

Jervis, M.A., 1986. New host records for *Aphelopus* (Hymenoptera: Dryinidae). *Entomologist's Gazette* 37: 37-38.

Jervis, M.A., 1991. Biological control of leafhoppers in New Zealand - a feasibility study. *Bulletin of the British Ecological Society* 22: 35-38.

Jervis, M.A., 1992. A taxonomic revision of the pipunculid fly genus *Chalarus* Walker, with particular reference to the European fauna. *Zoological Journal of the Linnean Society* 104, in press.

Kidd, N.A.C. & Jervis, M.A., 1989. The effects of host-feeding behaviour on the dynamics of parasitoid-host interactions, and the implication for biological control. *Researches on Population Ecology* 31: 235-274.

Le Quesne, W.J. & Payne, K.R., 1981. Cicadellidae (Typhlocybinae). *Handbooks for the Identification of British Insects* 2 (2c):1-95.

Munroe, L.M., 1991. Biological studies on nymphal-adult parasitoids of Eupteryx leafhoppers, with particular reference to *Aphelopus atratus* (Dalman) (Hymenoptera: Dryinidae). Unpublished Ph.D.thesis, University of Wales.

Sahragard, A., Jervis, M.A. & Kidd, N.A.C., 1991. Influence of host availability on rates of oviposition and host-feeding, and on longevity in *Dicondylus indianus* Olmi (Hym., Dryinidae), a parasitoid of the Rice Brown Planthopper, *Nilaparvata lugens* (Stal) (Hem., Delphacidae). *Journal of Applied Entomology* 112: 153-162.

Stiling, P.D., 1979. Ecological studies on leafhoppers occurring on stinging nettles, *Urtica dioica* L.. Unpublished Ph.D. thesis, University of Wales.

Waloff, N. & Jervis, M.A., 1987. Communities of parasitoids associated with leafhoppers and planthoppers in Europe. *Advances in Ecological Research* 17:281-402.