FINAL REPORT

Project Title:	Integrated pest management of leaf miner pests of protected tomato crops.
Project Number:	PC 108
LINK Project:	CSA 2921
Project Leader	R J Jacobson
Principal Experimental Workers:	Dr P Croft, Mr J Fenlon, Ms C Sampson, Dr D Skirvin
Project Co-ordinator:	Dr N Dungey
Consortium members:	Science based partners Horticulture Research International Industry-based partners Biological Crop Protection Ltd English Village Salads Ltd Horticultural Development Council Microbio Ltd Hazlewood VHB Ltd
Location:	Horticulture Research International Stockbridge House, Cawood, Selby, North Yorkshire, YO8 3TZ Tel: 01757 268275 Fax: 01757 268996
Date Project Commenced:	September 1995
Duration of Project:	48 months
Date Project Completed:	February 2000
Keywords:	*Liriomyza bryoniae, Diglyphus isaea*, tomato, IPM, simulation model, *Steinernema feltiae*

Authentication

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

Signature.......... R Jacobson ....

R J Jacobson
Project Leader
Horticulture Research International
Stockbridge House
Cawood
Selby
North Yorkshire
YO8 3TZ

Tel. 01757 268275
Fax. 01757 268996

Date 8/3/00

Report authorised by .... G M Tatchell ....
(signature)

M. Tatchell
Head of Entomological Sciences Department
Horticulture Research International
Wellesbourne
Warwickshire
CV35 9EF

Tel. 01789 470382
Fax. 01789 470552

Date 8-3-00

Contents

Page No.

Practical Section for Growers

Background and objectives 1
Results 1-4
Action points for growers 4-5
Research opportunities 5
Practical and financial benefits from study 6

Science Section

General introduction and Objectives 1-3

Part 1: *Liriomyza bryoniae – Diglyphus isaea* simulation model 4-8
1.1 Population-based model
1.2 The stochastic model
1.3 Discussion and conclusions

Part 2: *Liriomyza bryoniae* survival between crops 9-14
2.1 A preliminary examination of factors that affect overwinter in *Liriomyza bryoniae*
2.2 The influence of short days during one generation of *L. bryoniae* on pupal development time and diapause induction
2.3 The influence of short days for two generations of *Liriomyza bryoniae* on pupal development time and diapause induction
2.4 Overall conclusion

Part 3: The biology of *Liriomyza bryoniae* 15-26
3.1 Biological comparisons with other populations
3.2 The relationship between leaf and air temperatures
  3.2. (i): Comparison of leaf, air and soil temperature
  3.2. (ii): The effect of *L. bryoniae* damage on leaf temperature
3.3 Development of *Liriomyza bryoniae* under constant and fluctuating temperature regimes
3.4 *Liriomyza bryoniae* infestations on different types and cultivars
3.5 *Liriomyza bryoniae* performance on different cultivars of tomatoes
3.6 The effect of light on *Liriomyza bryoniae* feeding and egg laying
  3.6(i): The effect of the presence of light on *L. bryoniae* activity
  3.6(ii): The effect of daylength of leaf miner activity
  3.6(iii): The effect of light intensity on *L. bryoniae* activity
  3.6(iv): The effect of light on *L. bryoniae* feeding and egg laying

Contents

Page No.

Part 4: Biology of *Diglyphus isaea* 27-38
- 4.1 Development of *Diglyphus isaea* and *Dacnusa sibirica* at constant and fluctuating temperatures
- 4.2 The effect of *Liriomyza bryoniae* density on *Diglyphus isaea* egg laying and adult longevity
  - 4.2.(i): The effect of two *L. bryoniae* densities on *D. isaea*
  - 4.2.(ii): The effect of four low *L. bryoniae* densities on *D. isaea*
  - 4.2.(iii): Longevity of *D. isaea* without *L. bryoniae* hosts
- 4.3 *Diglyphus isaea* searching
  - 4.3(i): Small scale experiments
  - 4.3(ii): Glasshouse experiments
- 4.4 Overall conclusions

Part 5: The impact of *Liriomyza bryoniae* damage on marketable yield of tomato plants 39-48
- 5.1 Impact of leaf reduction on tomato yield
- 5.2 Impact of *Liriomyza bryoniae* damage on tomato yield
- 5.3 Overall conclusion

Part 6: IPM compatible remedial treatments 49-58
- 6.1 Prelimary studies
- 6.2 Control of *Liriomyza bryoniae* in tomato plants with two application rates of *Steinernema feltiae*
- 6.3 The effect of *Steinernema feltiae* on the leaf miner parasitiod, *Diglyphus isaea*
- 6.4 Crop scale evaluation of *Steinernema feltiae* for the control of *Liriomyza bryoniae* in protected tomatoes
  - 6.4.(i): A crop at HRI Stockbridge house
  - 6.4.(ii): A crop at a commercial tomato nursery
- 6.5 Overall conclusion

Part 7: *Liriomyza bryoniae* invasion and population development in commercial tomato crops 59-65

Part 8: Design and evaluation of sampling procedures 66-68

General summary 69-72

Recommendations for further research 73

Technology transfer 74

References 75-76

Appendix: Description of the *Liriomyza bryoniae-Diglyphus isaea* simulation model 77

SCIENCE SECTION

GENERAL INTRODUCTION

Virtually all tomato growers in the U.K. use integrated pest management (IPM) based on biological control agents integrated with target-specific pesticides. Such systems greatly reduce pesticide use and this has clear benefits for horticultural workers, consumers, plants and the environment, as well as providing the opportunity to pollinate crops with bumble bees. The latter provides significant savings in labour compared to hand pollination and increases yield by improving fruit set. Both IPM and biological pollination must be maintained if U.K. growers are to remain competitive with overseas producers.

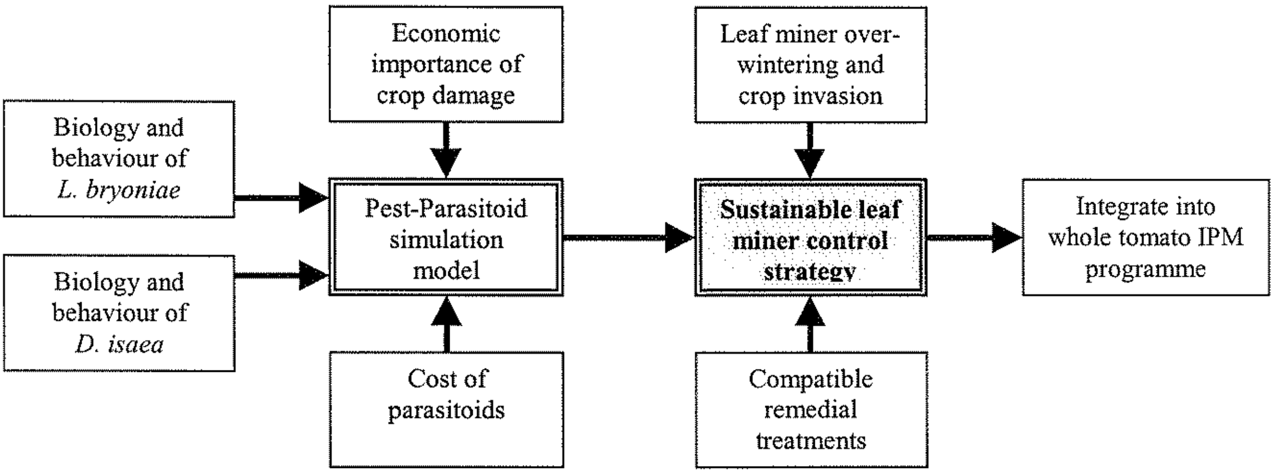
The success of an IPM programme depends on the availability of compatible control measures for use against every pest that attacks the crop. The programmes are more robust if the primary biocontrol agents are supported by other compatible control measures that can be used as a second line of defence at critical times. The use of non-specific pesticides against one pest species can disrupt the entire IPM programme, resulting in further chemical sprays that can mark the end of IPM and biological pollination for the remainder of the season.

Tomato leaf miner (*Liriomyza bryoniae*) is one of the two most important pests of tomato crops in the U.K. The larvae tunnel in the leaf mesophyll tissue, reducing photosynthetic area and eventually killing the plants if left untreated. Two parasitic wasps have been commercially available in the U.K. for over a decade and both have been included in most IPM programmes; *Dacnusa sibirica* has been most effective in the early part of the season, while *Diglyphus isaea* has performed better during the summer months. Despite their use, many growers have continued to experience difficulties controlling leaf miners during May and June, and this has often led to the application of non-specific insecticides. This project was aimed at developing a leaf miner control strategy that was compatible with the rest of the tomato IPM programme, reliable throughout the season and manageable by non-specialist staff. The studies focussed on *D. isaea* because it was the most effective parasitoid at the critical time of year. The project did not include any of the methods of modifying the initial establishment of *L. bryoniae* that have already been tried by some growers; for example, extensive "clean-up" treatments at the end of the previous crop, encouraging leaf miner survival between crops, picking off mines at the start of the season or use of *Dacnusa sibirica*. The main components of the project are shown in Figure 1.

An important component of the project was the development of a computerised model to predict the response of different parasitoid release strategies. The model was used as an experimental tool, to help determine which aspects of the biology and behaviour of the pests and parasitoids were most important to the success of the biological control system.

At the start of the project, there were important gaps in the knowledge of the biology and behaviour of both *L. bryoniae* and *D. isaea* that were limiting further refinement of the biological control strategy. There had been some debate about the survival of leaf miners between crops and whether they had a complex over-wintering stage (diapause) that could only be broken by a specific combination of conditions. This had to be clarified so that the start of leaf miner monitoring and control programmes could be more accurately timed. It was also important to improve the general understanding of the interaction between leaf miners and parasitoids and to determine why the pest "out-performed" the parasitoids in late spring and early summer.

Figure 1. The main components of the project.



The cost of *D. isaea* has been reduced in recent years due to competition between the main suppliers but it is still a relatively expensive (about £70/1000) product. There seems little scope to further reduce prices, so the parasitoids must be used as efficiently as possible if the biological control system is to be cost-effective. Many of the components of this project contributed towards optimising the frequency and rates of release of *D. isaea*.

No matter how carefully biological control systems are managed, there will always be occasions when control breaks down and it is important to have another compatible control measure in reserve that can be used remedially. The parasitic nematode, *Steinernema feltiae*, was evaluated as an alternative to the non-specific insecticides that had previously been used as a second line of defence.

It was important to know how much leaf miner damage could be tolerated while *D. isaea* were becoming established and when it would become necessary to resort to the second line of defence. Crop scale experiments were therefore designed that would estimate the financial impact of *L. bryoniae* damage by correlating marketable yield to the reduction of photosynthetic area resulting from leaf miner activity.

All pest management decisions are dependent on the availability of accurate information about pest abundance and the establishment of beneficial organisms, and growers must be confident that the data they collect is truly representative of the whole crop. To improve the efficiency of crop monitoring, standardised procedures were designed with statisticians and tested in commercial crops.

OBJECTIVES

The overall objective of the project was to develop an IPM strategy for the control of leaf miners in protected tomato crops that was manageable by non-technical staff, reliable throughout the season and avoided the breakdown in control which commonly disrupted the whole IPM programme and biological pollination during May and June. Specicfic objectives were:

- To investigate the overwintering behaviour of *L. bryoniae* in and around greenhouses and identify the environmental conditions which prompt adult activity.
- To investigate the developmental rate of *L. bryoniae* and its natural enemies under the environmental conditions commonly encountered during May/June.
- To develop methods of improving the efficacy of leaf miner parasitiods between January and May.
- To develop a model to calculate the weekly requirements of biological control material taking into account pest invasion, the dynamics of the leaf miner and beneficial populations within the crop, and the economic damage threshold.
- To investigate the sensitivity of the biological control agents used against leaf miners to commonly used fungicides, adjuvants and flue gases used to enrich carbon dioxide in the aerial environment. (NB - It was subsequently agreed that this objective should be cancelled and resources transferred to more detailed biological studies).
- To develop a satisfactory method of integrating remedial treatments (eg selective use of insecticides, parasitic nematodes) against leaf miners within the whole tomato IPM programme. (NB - It was subsequently agreed that this work should focus on parasitic nematodes rather than chemical insecticides).
- To use the extended knowledge to bring together the range of new techniques to form a robust and sustainable strategy for the control of leaf miners which is totally compatible with the whole IPM programme in tomatoes.

PART 1: *LIRIOMYZA BRYONIAE - DIGLYPHUS ISAEA* SIMULATION MODEL

The main aim of this work has been to develop a model that can aid the development of a suitable programme of biological control for *L. bryoniae* on tomato crops. The model has focussed on using *D. isaea* as the controlling agent. Through the process of developing the model, important areas where further data are needed have been identified and appropriate experimental work has been undertaken.

The initial version was a population-based model, which incorporated equations describing the fundamental biological processes required to describe the population dynamics of *L. bryoniae* and *D. isaea* in tomato crops. This was subsequently replaced by a stochastic version, which was more appropriate because it allowed for the natural variability of the biological processes through the use of randomly sampled distributions, and provided more realistic predictions of the dynamics of the system. There is a detailed description of the simulation model in the Appendix to this report.

1.1. POPULATION-BASED MODEL

Background

The order in which the main processes are simulated in the model are shown in the flow diagram (Figure 1.1.) and a description of the main processes are given below:

Initial distribution of *L. bryoniae* and *D. isaea*: The model uses defined inputs to determine the size of the glasshouse and the number of plants to be simulated. Information regarding the number of mines counted in a set sample size and the proportion of these mines parasitised are used to determine the initial numbers of *L. bryoniae* and *D. isaea* in the simulated system. Temperature data for use in the model are read in from a file. If the data provide weekly temperature data, then these are converted to daily data.

Development: The daily development rates of both *L. bryoniae* and *D. isaea* are calculated from equations fitted to the experimental and literature data that define how the daily temperature affects the development rate of both species. If the accumulated development rates are greater than the longevity of a stage, then it moves into the next stage, or if it is an adult, it is assumed to die.

Reproduction: The number of new mines produced by *L. bryoniae* and *D. isaea* is calculated from an equation based on data in the literature, which incorporates the effect of temperature on the daily reproductive rate. The effect of the age of *L. bryoniae* on reproductive rate is also accounted for as the majority of new mines are produced early in the life of an adult. The sex ratio of the leafminers is assumed to be 1:1.

Host feeding: The number of mines which are killed by host feeding is calculated from a simple functional response equation, which incorporates both the density of *L. bryoniae* and the number of plants that the parasitoids can search. Host feeding is assumed to be a constant proportion of the number of mines parasitised.

Parasitism: The number of mines is calculated from a simple functional response equation as described in the previous section. The sex ratio of the parasites is assumed to be 1:1.

Parasite introductions: This section simulates multiple introductions of the parasitoids, with the numbers being introduced and the timing being read from a file.

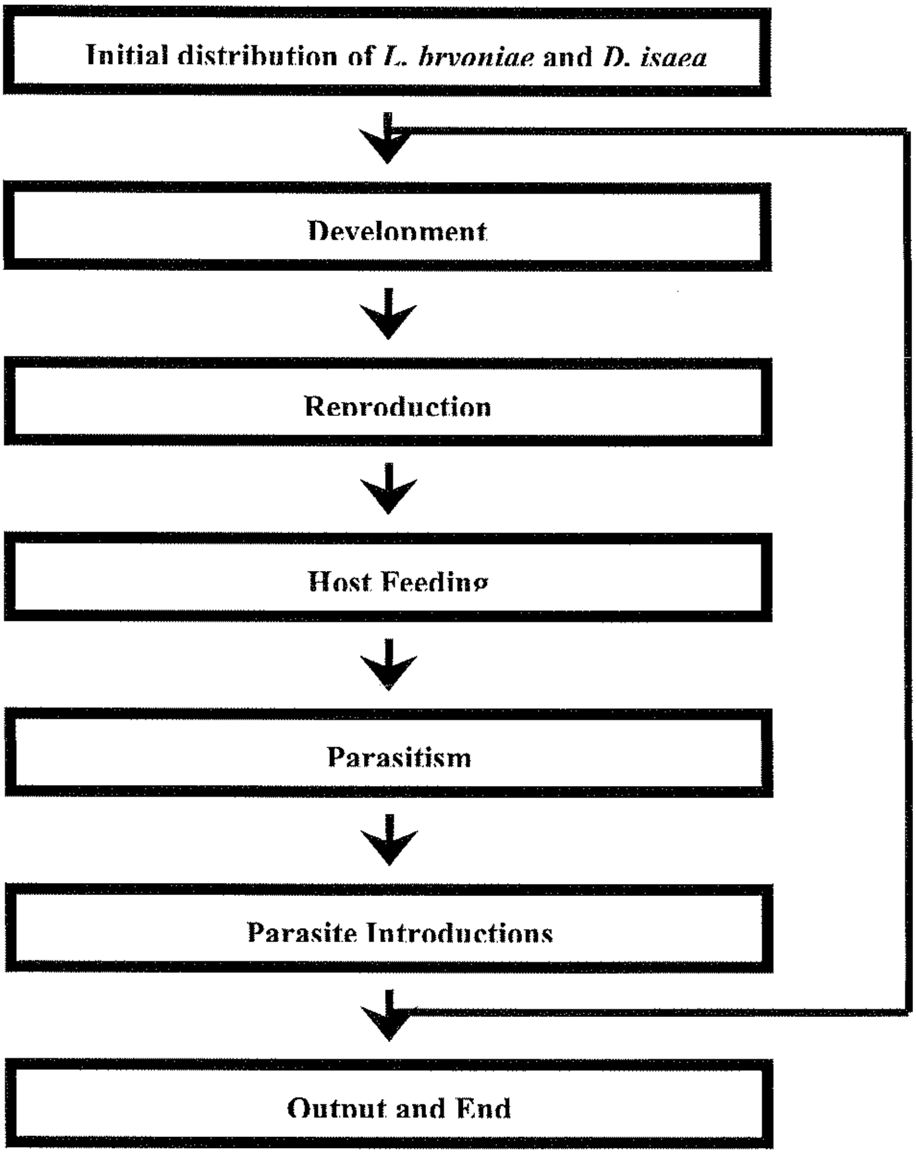


Figure 1.1. Flow diagram outlining the main processes involved in the *L. bryoniae* – *D. isaea* simulation model.

Predictions from the population–based model

Initial predictions from the model were based on constant temperatures, and looked at the effect of the numbers of *D. isaea* released in a single introduction on the degree of control obtained. The model predicted good control of *L. bryoniae*, however, it was realised that the equation used to determine the number of *L. bryoniae* parasitised assumed that all leaf miners were available to be parasitised, which was unrealistic. This equation was modified and a threshold below which no parasitisation occurred was introduced.

The predictions from this modified model were then compared with glasshouse observations in 1997, and the model appeared to fit the data reasonably well. However, when the model was compared with glasshouse observations from 1998, the fit was not as good. Although the model generally predicted the timing of observed increases in *L. bryoniae* well, it was poor at predicting the actual numbers of *L. bryoniae* observed. Also, predictions investigating three different introduction strategies were the converse of what was observed in the field.

Based on this, areas where further information was required to improve the model were identified. These were the survival time of *D. isaea* at a range of host densities and the fecundity of *L. bryoniae*. Further examination of the model also suggested that more information was required about the searching ability of *D. isaea*. It was also felt that the deterministic nature of the population-based model was not suitable for providing good predictions of the population dynamics of the system. It was therefore decided to develop a stochastic version of the model.

1.2. THE STOCHASTIC MODEL

An individual-based version of the model was developed using a modelling framework developed as part of other projects at HRI. This type of model lends itself to the incorporation of stochastic elements as opposed to a population-base model.

This version of the model follows the same process as described for the population-based model, the only difference that the processes are applied to each individual within the model rather than to the population as a whole. Stochastic versions of the equations used in the population-based model were developed. Stochasticity was incorporated by including an error term to the equations, which is based on the standard deviation of the data in the literature. Once the value has been calculated, a deviation is sampled from the appropriate distribution and applied to the value.

In addition to developing the stochastic versions of the equations, additional data concerning the searching behaviour of *D. isaea* were incorporated into the model. The model now adjusts the host feeding and parasitism equations to allow more plants to be searched at lower *L. bryoniae* densities.

Predictions from the stochastic model

With a stochastic model, many runs need to be performed using the same initial conditions. This allows the range of behaviour of the stochastic model for a given set of conditions to be identified. As the model is also individual-based, the runtime of the model is much longer than with a population-based model. Therefore, in order to speed up the process of obtaining data, the model has only been run using constant temperatures of 15°C, 20°C and 25°C. The effect of a range of initial *L. bryoniae* and *D. isaea* numbers on the outcome of the model has been examined. In addition, the effect of multiple parasitoid introductions for a single set of initial numbers has been examined.

The results of the model on investigation of the effect of initial numbers and levels of parasitism, suggest that in the absence of additional introductions, *D. isaea* was unable to control *L. bryoniae* populations. At 15°C *D. isaea* becomes extinct by day 50 even if 90% of the mines are parsitised at the start of the simulation. A similar situation occurs at 20°C. Although the parasitoid population does survive longer with higher initial numbers of mines, it still becomes extinct after 60 days. At 25°C, the parasitoid population survives for less than 30 days at low initial mine densities, and up to 50 days at higher initial mine densities. The reason for the parasitoids going extinct was due to the fact that a threshold is imposed on host feeding and parasitism. These only occur if the total number of larval stages is at a density greater than 1 mine per plant. In all the cases above, the *L. bryoniae* larvae were hardly ever exceeding this density, which meant that the parasitoids were dying of starvation. In all of the above cases, extinction of *D. isaea* occurs after it has reduced the number of *L. bryoniae* larvae below the threshold levels, i.e. after the first peak of *L. bryoniae* activity.

To alleviate this problem, and also because a more up to date searching model had been included, the threshold was removed for host feeding, and the threshold was reduced to 0.01 mines per plant for parasitism. This was done to fit in with the experimental observation that at low host densities only host feeding and not parasitism was observed.

The predictions of the revised model show that *D. iasea* is predicted to be more efficient at locating hosts both for feeding and parasitisation. It is able to control *L. bryoniae* reasonably well after the first peak of activity, and also in later peaks as shown in Figures 1.2 and 1.3. However, the dynamics of the system are less stable than in the population-based model, due to the randomness incorporated into the model. Once the number of *L. bryoniae* has been reduced to a low level, then the random nature of the model can cause either the pest or the parasitoid to die out. The use of multiple introductions of *D. isaea* leads to a more stable situation, as the population of *D. isaea* is enhanced by the introductions, reducing the possibility of it dying out by chance. The predictions, which were done at constant temperatures, suggest that repeated introductions of high numbers (more than 3000 per hectare) of *D. isaea* will lead to greater control of *L. bryoniae* at temperatures of 20°C and 25°C.

1.3. DISCUSSION AND CONCLUSIONS

The model has been a very important tool in understanding the dynamics of the *L. bryoniae -D. isaea* system. It has highlighted the key areas within the system where further information is necessary. These areas are:

1. The searching behaviour and activity patterns of *D. isaea.*
2. The activity patterns of *L. bryoniae.*
3. The effect of environmental factors other than temperature on both *L. bryoniae* and *D. isaea.*
4. The immigration behaviour of *L. bryoniae.*

Although the model is able to predict the population dynamics of the system reasonably well, in order to be useful as a decision support model for growers, information on the key areas mentioned above will be necessary. The model can only ever be as good as the information provided to it, and as the current monitoring data provides little information about adult *L. bryoniae* densities, it is impossible to incorporate the immigration of *L. bryoniae* into glasshouses during the course of the simulation. Since this may be a major factor in causing late season outbreaks, which the model will be unable to predict, information on the movement of adult *L. bryoniae* will be crucial to the development of a robust model.

Because of the stochastic nature of the model, and the use of an individual-based approach, the model does take a long time to produce its output. If the model is to be used in the commercial arena, further work will have to be done to alleviate these problems before either advisors or growers can use it.

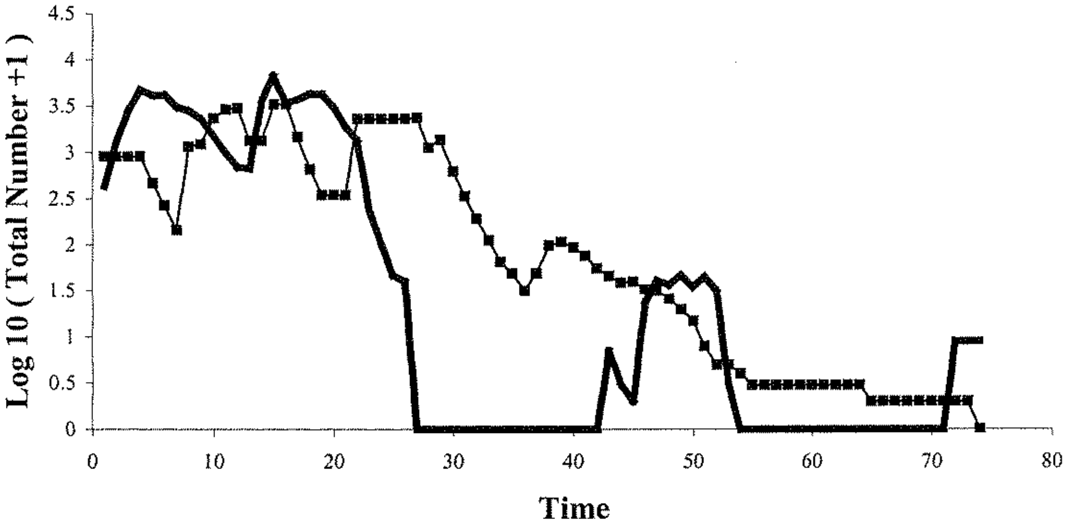


Figure 1.2. The number of new mines per day (solid line) and *D. isaea* adults (solid line plus squares) predicted by the model at 20°C, with three weekly introductions of *D. isaea* at 2000 per hectare.

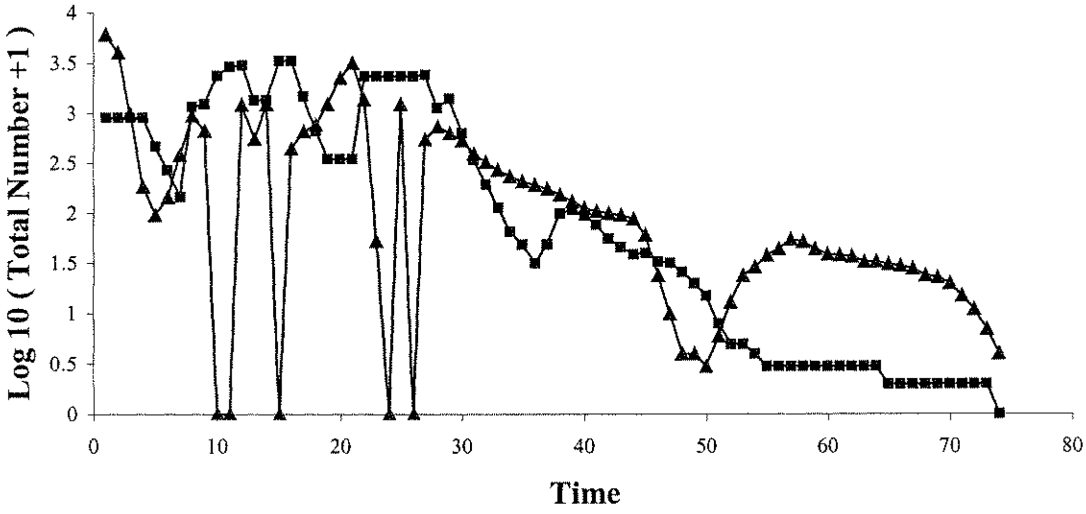


Figure 1.3. The number of *L. bryoniae* larvae (solid line plus trinagles) and *D. isaea* adults (solid line plus squares) predicted by the model at 20°C, with three weekly introductions of *D. isaea* at 2000 per hectare.

PART 2: *LIRIOMYZA BRYONIAE* SURVIVAL BETWEEN CROPS

To determine the most effective time to start crop monitoring and to guide the timing of early parasitoid releases, it was necessary to know when adult *L. bryoniae* were likely to first emerge in tomato crops. It was considered possible that *L. bryoniae* may enter diapause (*i.e.* a state of profound hibernation) in the autumn. This would be induced at a particular phase in the development of the *L. bryoniae* as a response to changes in the environment that suggested that an unfavourable period was approaching. Shortening days and falling temperature would be probable triggers. Diapause would then cease at a specific time after the unfavourable period had passed though this could be dependent on a complex combination of stimuli. If *L. bryoniae* did have a diapause, it was possible that at least some individuals may not emerge from this state until spring. Alternatively *L. bryoniae* may survive between crops in a state of quiescence that was temperature dependent. In this case, they would become inactive as temperature dropped and regain activity as soon as it rose again. The following experiments set out to establish if overwintering in *L. bryoniae* was due to diapause or simply a quiescent phase.

2.1. A PRELIMINARY EXAMINATION OF FACTORS THAT AFFECT OVERWINTERING IN *LIRIOMYZA BRYONIAE*

Objective

To determine the effect of daylength and temperature on pupal colour and the emergence of *L. bryoniae* adults.

Materials and methods

In the first part of this experiment, *L. bryionae* pupae were produced under long day conditions in a constant environment (CE) room (22 ± 2°C, 16:8 L:D) and under natural short day conditions in a heated glasshouse (mean 20°C, approx. 11L:13D). Pupae from each culture were transferred to one of the following environments:

1. Short natural day (c.10L:14D) in unheated glasshouse (<10°C)
2. Short natural day (c.10L:14D) in heated glasshouse (mean 20, range 18-30 °C)
3. Short natural day (c.10L:14D) outside (<8°C)
4. Long day (16L:8D) in CE room (22 ± 2°C)

The time to adult emergence from pupae was recorded. After four weeks in each of the cool environments (*i.e.* treatments 1 and 3) one third of the remaining pupae were moved to the warmer CE room and the subsequent time to adult emergence was recorded. After a further five weeks, half of the remaining pupae in the cool environments were transferred to the CE room and the subsequent time to adult emergence recorded.

In the second part of the experiment, adult *L. bryoniae* emerging from the culture in the heated glasshouse with short day (mean 20°C, approx. 10L:14D) were either maintained on fresh plants under the same conditions or moved to fresh plants in the CE room. Their offspring were collected daily as they became pupae and then stored in either the heated greenhouse or the CE room. The date of adult emergence, pupal death and pupal colour were recorded.

Results and discussion

The results from the first part of the experiment are given in Table 2.1. Adults emerged in 10-19 days at 22°C in the CE room. Emergence was slightly slower (12-20 days) at 20°C in the heated glasshouse. There was no emergence below 10 °C but when pupae were transferred to the warm environment, their subsequent development was similar to the pupae originally held at that temperature. These results strongly suggest that pupal development is arrested below 10°C. No differences were observed between pupae produced under short and long days.

Table 2.1. The development time (days) in four environments of *L. bryoniae* pupae produced under long and short days. Where pupae were moved from cool conditions to the CE room, the development time was measured from the day of transfer.

No. of weeks before moved to CE room	*L. bryoniae* produced under:							
	long days				short days			
	CE	Heated glasshouse	Outside	Unheated glasshouse	CE	Heated glasshouse	Outside	Unheated glasshouse
0	10-17	13-19	No emergence		10-19	12-20	no emergence	
4	-	-	10-21	6-21	-	-	10-22	6-23
9	-	-	13-20	22-26	-	-	12-22	10-20

The results from the second part of the experiment are shown in Table 2.2. Adult *L. bryoniae* raised under long days produced a significantly higher proportion of pale coloured pupae when they were kept at long days than when they were transferred to short days (L.S.D. = 0.129, $P < 0.05$). Adults raised under short days produced predominantly dark pupae under both long and short day conditions.

These results suggest that short day length triggers the production of more dark coloured pupae. There was no evidence to suggest that pupal colour was associated with a delayed development time or survival under the test conditions, which was broadly consistent with results of previous studies (Helyer & LeDieu, 1990). However, it is speculated that dark pupae have a thicker cuticle and this may be an adaptation to prevent desiccation and aid survival during prolonged periods of low temperature. The latter would not have been detected in this experiment because the test conditions would not promote desiccation.

Table 2.2. The colour of *L. bryoniae* pupae produced under long and short days, from parents that were raised under both long and short day conditions.

Environment in which adult *L. bryoniae* produced	Mean proportion ± sd of pale coloured pupa produced by adults transferred to:		L.S.D
	long day	short day	
Long day	0.62 ± 0.20 (n=21)	0.26 ± 0.24 (n=28)	0.129
Short day	0.36 ± 0.17 (n=10)	0.25 ± 0.31 (n=8)	0.115

2.2. THE INFLUENCE OF SHORT DAYS DURING ONE GENERATION OF *L. BRYONIAE* ON PUPAL DEVELOPMENT TIME AND DIAPAUSE INDUCTION.

Objective

To determine whether subjecting *L. bryoniae* to short days for one generation induced diapause or affected pupal development time.

Introduction

In the preliminary experiment (section 2.1.) development of *L. bryoniae* pupae and emergence of adults appeared to be temperature dependent, suggesting quiescence rather than true diapause. The following experiment was designed to test this observation.

Materials and method

In November 1996, 10 one to three day old adult female *L. bryoniae*s plus a smaller number of males were placed in each of eight cages containing one tomato plant (cv. Spectra) with eight leaves. Four cages were maintained under long day conditions in a CE room (21 ± 2°C, 16:8 L:D) and the other four kept under natural short day conditions in a heated glasshouse (range 13-18°C, approx. 10L:14D). Adults were removed after 72 hours. Newly formed pupae were removed, placed in on damp tissue paper in Petri-dishes and stored in one of the following four environments:

1. Short natural day (c.10L:14D) in unheated glasshouse (<10°C)
2. Short natural day (c.10L:14D) in heated glasshouse (mean 13 °C)
3. Short natural day (c.10L:14D) outside (<8°C)
4. Long day (16L:8D) in CE room (21 ± 2°C)

The time to adult emergence was recorded. After 7 weeks in each of the cool environments (treatments 1 and 3), the remaining pupae were transferred to the CE room and subsequent time to adult emergence was recorded. Analysis was done using ANOVA and L.S.D. on treatment means.

Results and discussion

The results are shown in Table 2.3. Adults emerged in 12-13 days at 21°C in the CE room, which was significantly faster than the heated glasshouse (*P*<0.001). There was no emergence below 10°C but when pupae were transferred to the warm environment, their subsequent development was similar to the pupae originally held at that temperature. It was irrelevant as to whether the pupae or their parents had been subjected to long or short days. The results demonstrate that pupal development and adult emergence time is temperature dependent and not a true diapause.

Table 2.3. The development time (days) in four environments of *L. bryoniae* pupae produced under long and short days. Where pupae were moved from cool conditions to the CE room, the development time was measured from the day of transfer.

Environment		The mean days to adult emergence and total number of pupae produced:			
				Pupae moved to CE room 21°C after 7 weeks	
		Mean	Total pupae produced	Mean	Total pupae produced
Pupae formed at long days and 21°C	CE room 21°C	13.1	143	-	-
	Heated glass 13°C	20.7	132	-	-
	Outside	No emergence	0	11.8	78
	Cold glass	No emergence	0	12.1	83
Pupae formed at short days and 13°C	CE room 21°C	12.4	35	-	-
	Heated glass 13°C	30.0	42	-	-
	Outside	No emergence	0	11.0	19
	Cold glass	No emergence	0	11.1	23
SED (24df)		1.079			

2.3. THE INFLUENCE OF SHORT DAYS FOR TWO GENERATIONS OF *LIRIOMYZA BRYONIAE* ON PUPAL DEVELOPMENT TIME AND DIAPAUSE INDUCTION.

Objective

To determine whether subjecting *L. bryoniae* to short days for two generations induced diapause or affected pupal development time.

Introduction

The previous experiments (sections 2.1.(i) and (ii)), showed that diapause was not induced in *L. bryoniae* following one generation at short days. The following experiment was designed to determine whether diapause would be induced after two generations.

Materials and method

Liriomyza bryoniae adults were reared for two generations under natural short day conditions in a heated glasshouse (range 18-20°C, approx. 10L:14D). Four tomato plants (cv Spectra) were infested with the adults from the second generation. Newly formed pupae were removed, placed on damp tissue paper in Petri-dishes and stored in one of the following environments:

1. Short natural day (c.10L:14D) in heated glasshouse (mean 18-20 °C)
2. Long day (16L:8D) in CE room (21 ± 2°C)

The time to adult emergence was recorded. Analysis was done using ANOVA and treatment means were compared using L.S.D.

Results and discussion

The results are summarised in Table 2.4. Despite *L. bryoniae* having been reared under short days for two generations, the time to adult emergence from pupae was consistent with results from previous experiments where the insects had been kept at long days or at short days for one generation. This further suggests that *L. bryoniae* emergence is temperature dependent and there is no true diapause.

Table 2.4. The development time (days) in two environments of *L. bryoniae* pupae produced after two generations at short days.

Environment		The mean days to adult emergence and total number of pupae produced	
		Mean	Total pupae produced
Two generations under short days at 18-20 °C	CE room 21°C	14.9	43
	Heated glass 18-20°C	17.0	42
SED (6 df)		0.533	

2.4. OVERALL CONCLUSION

The results from these three experiments indicate that *L. bryoniae* will survive in the U.K. in the cold glasshouse between tomato crops as pupae in a quiescent state. Their development will continue when the glasshouse is heated to the normal production temperature and all adults may then be expected to emerge within three to four weeks.

PART 3: THE BIOLOGY OF *LIRIOMYZA BRYONIAE*

Predictions for the development of *L. bryoniae* populations used in the model were based on published information on the development time and fecundity of *L. bryoniae* at different temperatures (Minkenberg, 1990). However, the experimental conditions studied by Minkenberg were not always representative of the tomato glasshouse environment. A series of experiments were done to verify Minkenberg's data using *L. bryoniae* populations collected from U.K. glasshouses and using temperature regimes that more accurately reflected environmental conditions in those glasshouses. After the temperature data had been verified, some discrepancies were noted between the numbers of *L. bryoniae* predicted by the model and the actual numbers observed in crops. *Liriomyza bryoniae* numbers were observed to increase more rapidly on some cultivars than others, and during bright sunny weather, and these factors were investigated in another series of experiments.

3.1. BIOLOGICAL COMPARISONS WITH OTHER POPULATIONS

Objective

To compare the development time of *L. bryoniae* reared by HRI to data published in the scientific literature.

Introduction

The specific conditions in insect rearing cultures in different laboratories can put selective pressures on the populations that result in small but significant genetic differences. It was therefore important to compare data from the HRI *L. bryoniae* population to that reported in the scientific literature before such data was incorporated in the model. This experiment measured the development time of *L. bryoniae* at a constant temperature of 22 ± 2°C and compared the time to data published by Minkenberg (1990).

Materials and method

This experiment was completed in September 1995. Twenty five 4-6 day old female *L. bryoniae* were placed in each of four cages containing two tomato plants (cv. Spectra). The cages were kept in a constant temperature room at 22 ± 2°C. After 24h the females were removed. Two marked leaves from each cage were examined daily and the number and stage of development of the *L. bryoniae* progeny were recorded.

Results and discussion

The results in Table 3.1 show that the development time for *L. bryoniae* reared by HRI was consistent with data published by Minkenberg (1990). This provided the confidence to incorporate Minkenberg's data in the model.

Table 3.1. A comparison of the development times of *L. bryoniae* at different constant temperatures

	Development time of leaf miner (days) at four constant temperatures			
	22 ± 2°C**	15 °C *	20 °C *	25 °C *
Mean	24.4	40.6	26.5	17.1
Sd	0.15	0.2	0.1	0.1

* Experiments by Minkenberg (1990)
** Experiments by HRI (1995)

3.2. THE RELATIONSHIP BETWEEN LEAF AND AIR TEMPERATURES

Introduction

The effects of temperature on the development, fecundity and survival of *L. bryoniae* is usually based on equipment that records the temperature of the air around plants. In the early stages of this project, questions arose as to whether leaf temperature could be significantly different to air temperature and whether this could effect the biology of *L. bryoniae*. It was also possible that temperature regulation was impaired in leaves that were damaged by leaf miners, so that larvae were subjected to increasingly high temperatures as the infestation progressed. In addition, leaf miner pupae that fall to the floor may be subjected to quite different temperatures than those that remain on the leaves. The first experiment (3.2.i) was designed to measure differences between leaf, air and soil temperatures. The second experiment (3.2.ii) examined the impact of leaf damage on leaf temperature.

3.2.(i): Comparison of leaf, air and soil temperature.

Objective

To compare leaf, air and soil temperatures in a glasshouse tomato crop.

Materials and method

This experiment was completed during July 1996 in a mature tomato crop grown in rockwool following practices commonly used by the U.K. tomato industry. The temperatures of tomato leaves, exposed soil surface, air in the glasshouse and air outside the glasshouse were recorded using an emissivity meter and calibrated thermometer. Plants were selected from locations in the glasshouse that were either exposed to direct sun or shaded from the sun at the time of assessment. Leaves were selected at the same position and height on all plants and readings were taken three times per day.

Results

The results from plants that were exposed to direct sun and shaded from the sun are shown in Figures 3.1 and 3.2 respectively. In both cases, leaf, air and soil surface temperatures were relatively similar when the plants had an unrestricted water supply. Leaf temperature was usually slightly below that of air. As the readings were not significantly different, it was concluded that measurements based on air temperature were acceptable for use in the model. The situation was different when the water supply to the plants was switched off temporarily. Leaves then appeared to be unable to regulate their temperature and became increasingly warmer than the air.

Figure 3.1. Leaf, air, soil and outside temperatures/solar power on (exposed Plant)

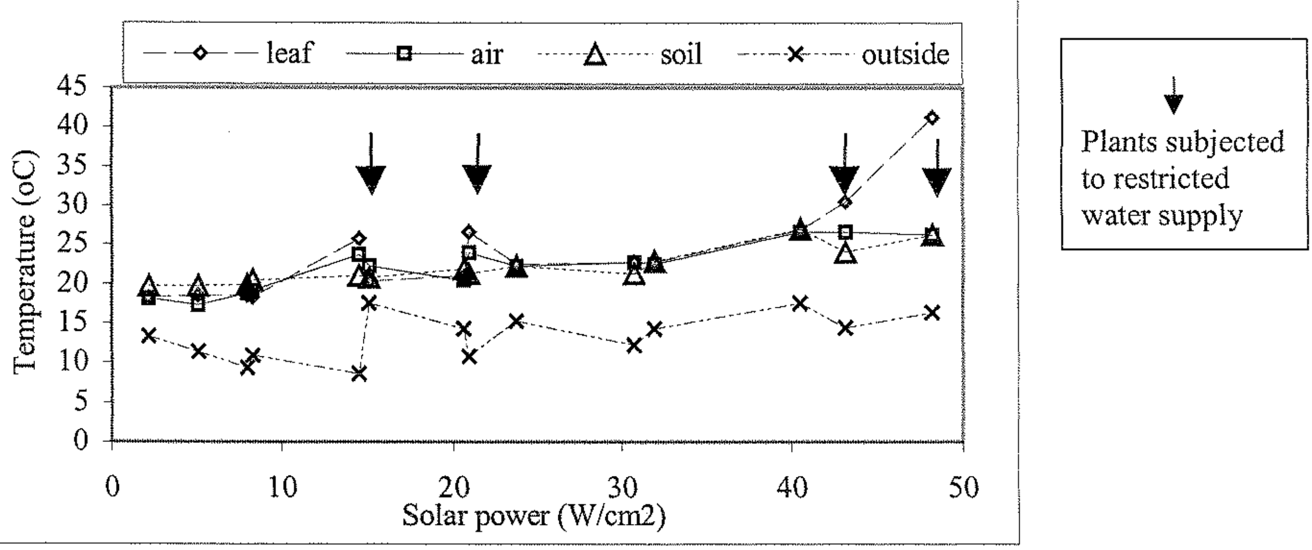
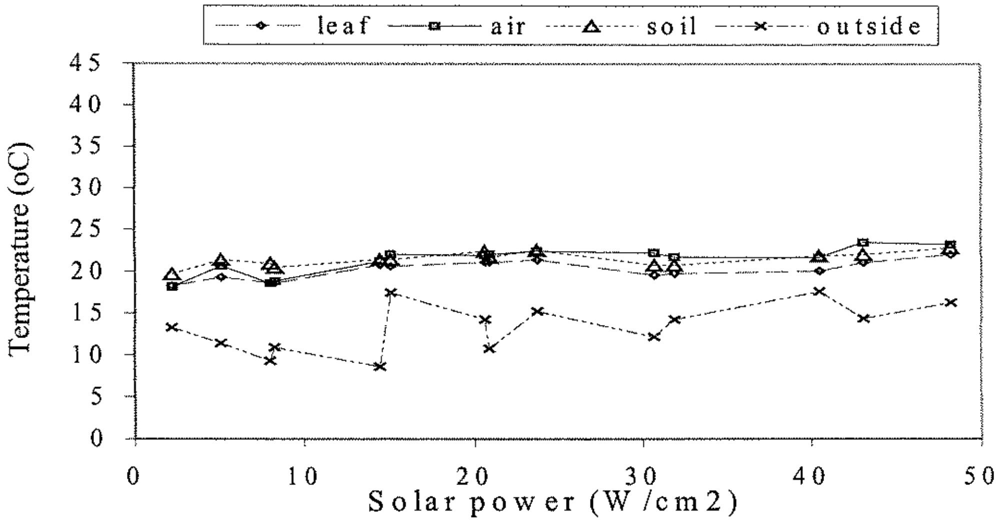


Figure 3.2. Leaf, air, soil and outside temperatures/solar power on (shaded Plant)



3.2.(ii). The effect of *L. bryoniae* damage on leaf temperature.

Objective

To determine whether *L. bryoniae* damage to leaves effects leaf temperature regulation.

Material and method

Leaf and air temperatures were recorded as described in experiment 3.2.(i) from round tomato crops (EVS, Camblesforth and HRI, Stockbridge House) and cherry tomato crops (Bridgeguild, Isle of Wight) with varying degrees of leaf miner damage.

Results

The data in Table 3.2 shows that leaf temperature was usually slightly lower than air temperature, which is consistent with the results obtained in experiment 2.1.(i). There was no evidence to suggest that temperature regulation was impaired in leaves damaged by leaf miners.

Table 3.2. The difference between temperatures of leaves (round tomato plants), with different levels of leaf miner damage and air temperatures.

Leaf miner damage (% loss of leaf area)	Leaf temperature expressed as +/- the following air temperatures:				
	25.1-27 °C	27.1-29 °C	29.1-31 °C	31.1-33 °C	33.1-35 °C
0			-0.5	-3.0	-4.6
1-29	+1.1	-0.1	-2.3	-2.5	
30-49		+0.1		-0.6	
50-69				-1.6	
70-89		0	-2.7	-3.1	-5.1
90+					

Contrasting results were obtained by Dr. Morely in cherry tomato crops at Bridgeguild. In these crops, leaf temperature was found to be 1-3 °C above air temperature in undamaged leaves, 1-2 °C above air temperature where up to 50% leaf area was lost due to leaf miner damage, and 2-6 °C above air temperature where 50-90% leaf area was lost due to leaf miner damage. Subsequent discussions with Dr. Morely suggested that the plants may have been under slight water stress when the readings were taken. This was thought to be due to a combination of factors including the intended growing regime, poor rootstock (cv. Favorita) and the sampling time (mid-morning). If the plants were water stressed, then the results at Bridgeguild were consistent with the observations in experiment 3.2.(i).

3.3. DEVELOPMENT OF *LIRIOMYZA BRYONIAE* UNDER CONSTANT AND FLUCTUATING TEMPERATURE REGIMES

Objective

To measure development time of *L. bryoniae* under a fluctuating temperature regime.

Introduction

An improved understanding of the biology of *L. bryoniae, D. sibirica* and *D. isaea* was required to help explain why control of the leaf miner with these parasitoids often failed during April and May. Minkenberg (1990) reported the development of *L. bryoniae* under three constant and one fluctuating temperature regimes. However, the temperature fluctuations used in his experiments were smaller than those commonly observed in U.K. tomato crops during May and June. The present experiments were designed to examine the development of *L. bryoniae* at fluctuating temperatures that resemble more accurately the conditions at the critical time of year.

Materials and method

In February 1997, single tomato plants (cv. Spectra) were placed in 12 cages housed in three incubators. Four cages were arranged randomly in each incubator and maintained at 18 ± 1 °C for 15h (1800-0900h) and 25 ± 1 °C for 9h (0900-1800h). The light regime was 16L:8D, with the photophase occurring between 0400-2000h. Ten 3-4 days old adult female *L. bryoniae* were placed in each cage and allowed to lay eggs for 24 hours. The females were then removed and the development times of the progeny from egg to adult emergence were recorded. The experiment was replicated three times.

Results and discussion

The 18-25 °C fluctuating temperature regime provided an overall mean of 21 °C. *Liriomyza bryoniae* development rate under this regime was consistent with data produced at fluctuations of 16-22 °C (average 19.5 °C) and constant temperature of 20°C (Table 3.3.). There was no evidence to suggest that leaf miner development time was disproportionately affected by fluctuating temperature. The cause of the difficulty in controlling *L. bryoniae* with parasitoids in April and May still had to be established.

Table 3.3. The development period of *L. bryoniae* at five temperature regimes.

	Temperature (°C)				
	15*	20*	25*	19.5* (16-22)	21** (18-25)
Mean	40.6	26.5	17.1	26.5	25.0
Sd	0.2	0.1	0.1	0.1	1.8

* Experiments by Minkenberg (1990)

** Experiments by HRI, 1997

3.4. *LIRIOMYZA BRYONIAE* INFESTATIONS ON DIFFERENT TYPES AND CULTIVARS OF TOMATOES

Objective

To determine the relative susceptibility to *L. bryoniae* of different types of tomatoes.

Methods

In August 1998, 70 different cultivars of plum, truss-ripened, round and cherry tomatoes were examined in a single glasshouse infested with *L. bryoniae* at Arreton Valley Nursery (AVN), Isle of Wight. Ten plants of each cultivar were chosen at random. One leaf of similar age was taken from each plant and the number of mines and percentage leaf area lost due to leaf miner activity were recorded.

Results and discussion

The average number of mines per leaf and percentage of leaf area lost due to leaf miner activity on the different types of tomatoes are summarised in Table 3.4. Cherry tomatoes were more heavily infested than plum, truss-ripened or round tomatoes; the mean numbers of mines being over 100 per leaf on all cherry tomato cultivars but less than 100 on all the others.

Table 3.4. The relative susceptibility to *L. bryoniae* of different types of tomatoes.

Tomato type	n	Mean of mines per leaf	Mean % leaf area damage
Plum	70	54	27
Truss	20	60	14
Round	300	63	17
Cherry	310	108	39

The differences in amounts of *L. bryoniae* damage may be due to the biochemistry of individual cultivars; for example there appears to be a trend towards more mines in cultivars with a higher sugar content in the fruit (Table 3.5).

Table 3.5. The sugar content of fruit and number of mines per leaf in different cultivars (types) of tomatoes.

Cultivar	Sugar content (%)	Mean no. of mines per leaf
Solairo (round)	4.75	47
Solution (round)	4.75	62
Espero (round)	4.81	87
Campari (cocktail)	5.88	120
Aranca (cocktail)	6.25	100

3.5. *LIRIOMYZA BRYONIAE* PERFORMANCE ON DIFFERENT CULTIVARS OF TOMATOES

Objective

To compare the numbers of eggs laid by *L. bryoniae* on the cherry tomato cultivar, Favorita, and the round tomato cultivar, Solairo.

Introduction

The apparent difference in the size of *L. bryoniae* populations that was observed on round and cherry tomatoes in experiment 3.4 could have been due to differences in the initial attractiveness of the plants or to their subsequent value as a food source. The former would not influence leaf miner performance in a non-choice situation. The latter would be expected to indirectly effect the numbers of eggs laid. A preliminary experiment was set up to separate these two factors by confining adult *L. bryoniae* on leaves of each type of tomato.

Materials and methods

In August 1998, pairs (one male and one female) of *L. bryoniae* were confined in perforated plastic bags on otherwise uninfested leaves positioned tenth from the top of mature tomato plants, cvs Solairo and Favorita. The cultivars were arranged in alternate rows and the plants grown in rockwool following practices commonly used by the U.K. tomato industry. There were eight replicates of each treatment with two replicates per row. After four weeks the infested leaves were removed and the number of mines per leaf counted.

Results and discussion

After four weeks there were an average of 8 (± 4.9) mines per leaf (range 2-17) on Solairo and 16 (± 3.4) per leaf (range 2-47) on Favorita. This suggested that leaf miners do lay more eggs in cherry tomato leaves than round tomato leaves but further replication was required to determine whether the differences were significant. If this assumption is true, a correction factor for different cultivars should be included in the leaf miner prediction model. This aspect of leaf miner performance was not taken further because the collaborative partners agreed to concentrate on standard round tomato cultivars within the remainder of this project.

3.6. THE EFFECT OF LIGHT ON *LIRIOMYZA BRYONIAE* FEEDING AND EGG LAYING

Two sets of experiments were done at HRI Stockbridge House in 1999. One set was done under controlled conditions in incubators during July and August. The second set was done under glasshouse conditions using shading within the glasshouse to alter light levels.

3.6.(i). The effect of the presence of light on *L. bryoniae* activity

Objective

To determine whether light is required for *L. bryoniae* feeding activity and egg laying.

Materials and method

The experiment included two treatments; *i.e.* permanent light (385 J/cm^2) and permanent dark. For each treatment, four caged tomato plants (cv Espero) were placed in an incubator maintained at 19.6°C. Six mated female *L. bryoniae* (5 days old) were released into each of the four cages and the number of feeding punctures and eggs laid were recorded after 24 hours. The experiment was replicated four times.

Results

Liriomyza bryoniae produced feeding punctures and eggs in both light and dark conditions but there were significantly more of each in the light (Table 3.6).

Table 3.6. Mean numbers of eggs laid and feeding marks made per six females per day under constant darkness and constant light conditions (square root transformed data).

Variate	24 hours light	24 hours dark	sed	df	P
Marks / 6 females	41.6	28.5	2.9	3	0.019
Eggs / 6 females	9.2	4.4	1.1	3	0.023

3.6.(ii). The effect of daylength on leaf miner activity

Objective

To determine the effect of daylength on *L. bryoniae* egg laying and feeding activity.

Materials and method

The experimental design, which was similar to that described in experiment 3.6.(i), included the following treatments:

1. 9.5L (152 J/cm^2) : 13.5D (equivalent to February daylength)
2. 16L (256 J/cm^2) : 8D (equivalent to May daylength)
3. 24 hours light (385 J/cm^2)

The experiment was replicated six times.

Results

There was significantly more feeding activity at longer daylengths (Table 3.7). There was a trend towards more eggs laid per female at longer daylengths but the differences were not statistically significant. Fewer eggs were laid in the 24 hour light treatment in this experiment than in experiment 3.6.(i), perhaps because the plants had been grown in hotter weather and the leaves were in poorer condition.

Table 3.7. Mean numbers of eggs laid and feeding marks made per six females per day at different daylengths (square root transformed data).

Variate	9.5 hrs light	16 hrs light	24 hours light	sed	df	P
Marks / 6 females	28.3	32.5	33.5	1.7	9	0.013
Eggs / 6 females	5.1	5.3	5.6	0.4	9	0.3 NS

3.6.(iii). The effect of light intensity on *L. bryoniae* activity

Objective:

To determine the effect of light intensity on *L. bryoniae* activity.

Materials and method

The experimental design, which was similar to that described in experiment 3.6.(i), included the following treatments:

1. 9.5L (11000 lux, 152 J/cm²) : 13.5D
2. 9.5L (3000 lux, 42 J/cm²) : 13.5D

The experiment was replicated six times.

Results

There was no significant difference between egg laying and feeding activity at different light levels (Table 3.8). It should be noted that light levels of above 2000 J/cm² can occur in glasshouses during the summer and even the highest light level in these incubators was equivalent to a relatively dull day.

Table 3.8. Mean numbers of eggs laid and feeding marks made per six females per day under different light intensities, 9.5 hours daylength (square root transformed data).

Variate	152 J/cm²	42 J/cm²	sed	df	P
Marks / 6 females	34.5	33.7	1.7	5	0.7 NS
Eggs / 6 females	6.9	6.5	0.4	5	0.3 NS

3.6.(iv). The effect of light on *L. bryoniae* feeding and egg laying

Objective:

To determine the effect of light intensity on *L. bryoniae* egg laying and feeding activity under glasshouse conditions.

Materials and methods

These experiments were completed in a mature tomato crop (cv Espero) grown in rockwool following practices commonly used by the U.K. tomato industry. Immediately before the start of each experiment, half of the glasshouse was shaded with green netting (rokolene, 40% shade). In each of the shaded and unshaded areas, 20 mated female *L. bryoniae* were confined individually in transparent perforated plastic bags placed on separate leaves that were positioned approximately 14 leaves from the top of the plants. After 24 hours, the number of feeding punctures and eggs laid per female were recorded. Light levels and temperatures were recorded in the shaded and unshaded areas throughout the experiment. The experiment was repeated nine times between 22 March and 19 May, with the shaded area alternating between the east and west sides of the glasshouse. The results from any individual females that died during the experiment were excluded from the analysis.

Results and discussion

Liriomyza bryoniae showed increased feeding activity and egg laying at higher light levels. Overall, females laid 37.5% more eggs ($P<0.001$, 260 df) and made 16% more feeding punctures ($P<0.01$, 258 df) in the unshaded treatments. There was a significant correlation between the numbers of eggs laid against light intensity ($P<0.01$; Figure 3.4.) and against daylength ($P<0.001$; Figure 3.5.). However, light intensity and daylength are also correlated so these effects cannot be separated. There was no significant correlation between egg laying and temperatures (Figure 3.6.) although the difference in average temperatures between the warmest and coldest days was only 3°C.

The glasshouse experiments showed that *L. bryoniae* females laid more eggs and fed more actively under conditions of greater light intensity or duration. Leaf miners laid approximately three times more eggs per day under bright conditions in May compared to dull conditions in March. This information is important as a correction factor for the model, which was only using temperature data to predict oviposition rates throughout the season. This may help to explain why the model was under-predicting increases in *L. bryoniae* populations during May.

Food quality is also known to be an important factor in leaf miner fecundity (Minkenberg and Ottenheim, 1990) and this may explain some of the differences in female performance between experiments. In the incubator experiments females laid between 3 and 22 eggs per day (young plants, cv Espero), fewer eggs being laid on spring-grown plants with thicker leaves. In the glasshouse experiments females laid between 2 and 8 eggs per day on mature plants (cv Espero). Minkenberg and Helderman (1990) reported 15 to 20 eggs per female per day on young plants (cv Moneydor). Further experiments would be required to separate the relative importance of nutrition, light intensity and temperature.

Figure 3.3. Mean number of eggs laid per female per day under different light conditions

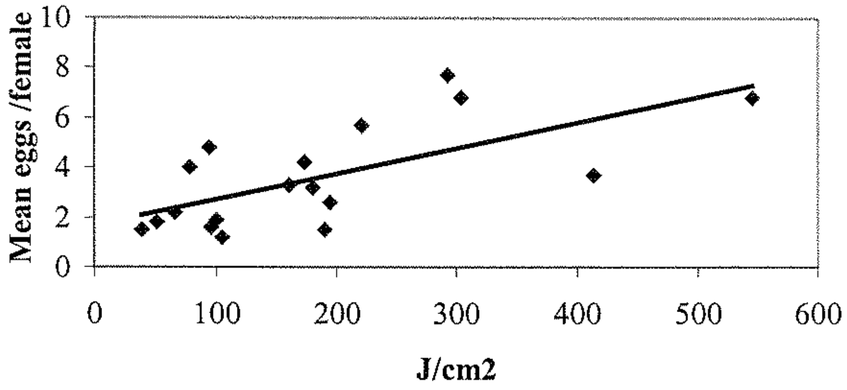


Figure 3.4. Mean numbers of eggs laid per female per day at different daylengths

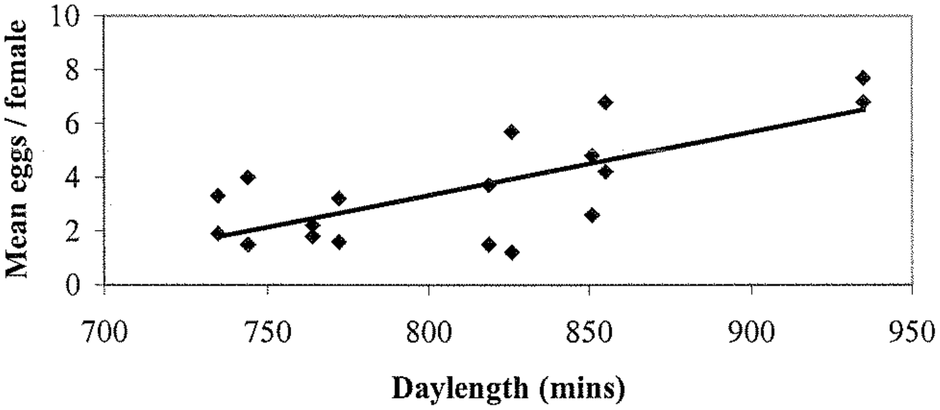
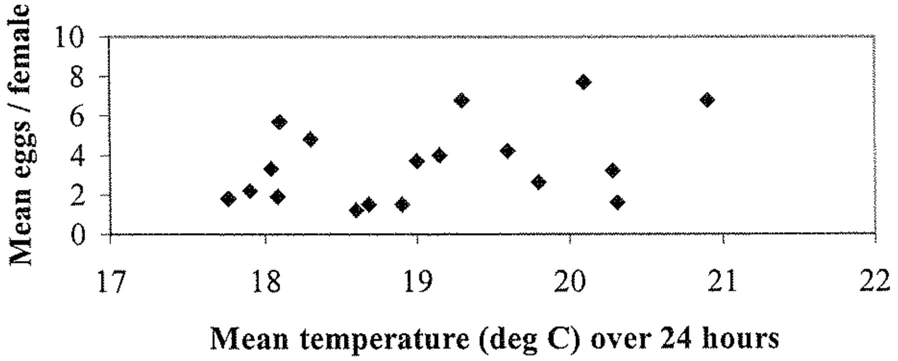


Figure 3.5. Mean numbers of eggs laid per female per day at different temperatures



PART 4: BIOLOGY OF *DIGLYPHUS ISAEA*

Although *D. isaea* is known to be an effective parasitoid of *L. bryoniae*, control failures have been common in commercial tomato crops during the early season and between April and June. These failures were difficult to explain because so little was known about the biology and behaviour of the parasitoid. Furthermore, early versions of the simulation model were based on published biological data (Nedstam, 1985; Minkenberg 1990) and did not accurately predict the performance of *D. isaea* in tomato crops. Analysis of the output of trial runs of the model indicated which aspects of the biology and behaviour of *D. isaea* were likely to have greatest impact on the control system and these subjects were further investigated. The data was subsequently fed back into the model. *Dacnusa sibirica* was also included in some of the early studies and these data are provided in the report.

4.1. DEVELOPMENT OF *DIGLYPHUS ISAEA* AND *DACNUSA SIBIRICA* AT CONSTANT AND FLUCTUATING TEMPERATURES

Objective

To measure development rates of *D. sibirica* and *D. isaea* under a fluctuating temperature regime.

Introduction

Nedstam (1985) and Minkenberg (1990) studied the development of *D. sibirica* and *D. isaea* under various constant and one fluctuating temperature regimes. However, the temperature fluctuations used in their experiments were smaller than those commonly observed in U.K. tomato crops. The present experiments were designed to examine the development of the parasitoids at fluctuating temperatures that resemble more accurately the conditions at the critical time of year (April/May).

Materials and method

In February 1997, single tomato plants (cv. Spectra) were placed in 12 cages housed in three incubators. Four cages were arranged randomly in each incubator and maintained at 18 ± 1 °C for 15h (1800-0900h) and 25 ± 1 °C for 9h (0900-1800h). The light regime was 16L:8D, with the photophase occurring between 0400-2000h. Ten 3-4 days old adult female *L. bryoniae*s were placed in each cage and allowed to lay eggs for 24 hours. The females were then removed. When the developing *L. bryoniae* larvae reached the second instar stage, five mated adult female *D. isaea* or *D. sibirica* were placed in each of eight cages for 24 hours. The development times from egg to adult emergence for both the *L. bryoniae* and parasitoid progeny were recorded. The experiment was replicated three times.

Results and discussion

The 18-25°C fluctuating temperature regime provided an overall mean of 21°C. The results in Tables 4.1 and 4.2 show that development rates of the parasitoids are very similar to those given by Nedstam (1985) and Minkenberg (1990) (see also Part 3 of this report for *L. bryoniae* results). There was no evidence to suggest that *D. isaea* or *D. sibirica* development times were disproportionately affected by fluctuating temperature.

The results also demonstrated that the strains of parasitoids used in these experiments were not atypical of their species and provided confidence to use the data of Nedstram and Minkenberg in the simulation model.

Table 4.1. The development period of *D. isaea* at five temperature regimes

	Temperature (°C)				
	15*	20*	25*	20.3* (18-22)	21** (18-25)
Mean	26.6	16.6	10.5	15.7	14.9
Sd	0.3	0.1	0.1	0.2	1.8

* Experiments by Minkenberg (1990)
** Experiments by HRI (1997)

Table 4.2. The development period of *D. sibirica* at four temperature regimes

	Temperature (°C)			
	15*	21*	24*	21** (18-25)
Mean	32.1	18.8	15.4	18.9
Sd	-	-	-	2.1

* Experiments by Nedstam (1985)
** Experiments by HRI (1997)

4. 2. THE EFFECT OF *LIRIOMYZA BRYONIAE* DENSITY ON *DIGLYPHUS ISAEA* EGG LAYING AND ADULT LONGEVITY

Introduction

Diglyphus isaea was thought to perform poorly at low *L. bryoniae* densities. The reasons could be either environmental or biological. Biologically, it was possible that the parasitoid required a regular feed in order to maintain successful reproduction. The following experiments examined the effect of low *L. bryoniae* density on the fecundity and adult longevity of the parasitoid.

4.2.(i): The effect of two *L. bryoniae* densities on *D. isaea*

Objective

To measure the fecundity and adult longevity of *D. isaea* at two extreme (*i.e.* high and low) *L. bryoniae* densities.

Materials and method

The experiment was completed by February 1997. Individual female *D. isaea* were placed in Petri dishes containing pieces of tomato leaflets either with or without *L. bryoniae* larvae. Two densities of *L. bryoniae* were provided:

1. High - between 1 and 10 larvae per day.
2. Low - 1 larva every third day.

The dishes were kept in a constant environment of 20 ± 2°C, 16L:8D. Each treatment was replicated 20 times. After 24 hours the *L. bryoniae* larvae were removed and checked for *D. isaea* egg laying or feeding activity. Fresh *L. bryoniae* larvae and tomato leaves were given each day until the parasitoid died. The number of eggs per female, the number of feeds on leaf miner larvae and the longevity of adult females were recorded. The data were analysed using L.S.D. on comparison of the two treatment means.

Results and discussion

The results in Table 4.3 show that at the high *L. bryoniae* density *D. isaea* fecundity was 335 times that of the female given a low *L. bryoniae* density. The longevity of females provided with high *L. bryoniae* densities was four times longer than that of females provided with low *L. bryoniae* densities.

Table 4.3. The effect of low (1 *L. bryoniae* larva every third day) and high (1-10 *L. bryoniae* larvae per day) host densities on the mean longevity (days), fecundity and feeding activity of *D. isaea*.

L. bryoniae density	Mean adult longevity (days)	Mean number of eggs per female	Mean number of larvae fed on per female
1-10 per day	45.5	201.1	39.2
1 every third day	10.6	0.6	2.1
L.S.D. (38df)	9.5	44.9	10.0

4.2.(ii): The effect of four low *L. bryoniae* densities on *D. isaea*

Objective

To measure the fecundity and adult longevity of *D. isaea* at an additional four "low" *L. bryoniae* densities.

Materials and method

The experiments were completed by August 1999. The method used was similar to that described in Experiment 4.2.(i) and included the following treatments:

1. One *L. bryoniae* larva per day
2. One *L. bryoniae* larva on each of days 1, 7 and 14.
3. One *L. bryoniae* larva on each of days 5 and 13.
4. One *L. bryoniae* larva on day 10.

The dishes were kept at 27-31°C and 16L:8D. Analysis was done using analysis of variance (ANOVA) and treatment means compared using least significant difference (L.S.D.).

Results and discussion

Table 4.4 shows that below one *L. bryoniae* larva per day *D. isaea* did not produce eggs and the longevity of the adults was significantly reduced.

Table 4.4. The effect of four low *L. bryoniae* densities on the mean longevity (days) and fecundity of adult *D. isaea* during a two week period.

L. bryoniae density	Adult Longevity	Mean number of eggs per female
1 per day	9.7	3.2
1 on days 1, 7 and 14	7.0	0
1 on days 5 and 13	6.6	0
1 on day 10	6.8	0
L.S.D., (36 df)	2.6	*

The combined results of experiments 4.2.(i) and (ii) show that the fecundity and longevity of *D. isaea* are significantly reduced at low *L. bryoniae* densities. *Diglyphus isaea* is unable to reproduce when provided with less than one *L. bryoniae* larva every third day. The reduced longevity of the wasp at the low *L. bryoniae* densities will also limit the time that she has to find a host.

In summary, the results show that the poor performance of *D. isaea* at low *L. bryoniae* densities is partly due to the fact that the parasitoid needs a regular food source to reproduce and maintain the population. Within a tomato crop her survival at low host densities will therefore depend on her ability to detect rapidly the low numbers of *L. bryoniae* larvae.

4.2.(iii): Longevity of *D. isaea* without *L. bryoniae* hosts

Objective

To examine the longevity of *D. isaea* in the absence of *L. bryoniae*

Introduction

The longevity of female *D. isaea* in the absence of *L. bryoniae* larvae and the time taken to lay eggs in the presence of *L. bryoniae* larvae were originally measured to establish the "fitness" of parasitoids used in experiments. However, the results also provided information about the potential survival of *D. isaea* within a crop with very low numbers of *L. bryoniae.*

Materials and methods

The experiment was completed by August 1999. Ten female *D. isaea* were placed individually in Petri-dishes containing tomato leaves (cv Espero) without *L. bryoniae* larvae. The dishes were checked daily and the number of dead and live *D. isaea* recorded. A further ten female *D. isaea* were placed individually in dishes containing tomato leaves (cv Espero) infested with five or more *L. bryoniae* larvae. The larvae were replaced daily and each one was examined for the presence of *D. isaea* eggs. The treatments were replicated 40 times.

Results and discussion

The average number of days that female *D. isaea* survived in the absence of prey and the time taken to lay eggs in the presence of prey are summarised in Table 4.5. The majority of the parasitoids died within four to five days if they were unable to feed on *L. bryoniae* larvae. This helps to explain why *D. isaea* establishment is poor early in the season when there are few mines per plant and when there may be several weeks between *L. bryoniae* generations with little or no food available to the parasitoids.

Eighty percent the female *D. isaea* produced eggs, and the majority started to lay within three to four days from release.

Table 4.5. Survival of female *D. isaea* in the absence of prey and time taken to lay eggs in the presence of prey.

Days	1	2	3	4	5	6	7	8	9	10
% survival without prey	95	93	90	73	30	20	8	8	8	0
% productive with prey	3	35	53	75	78	78	78	80	-	-

4.3. *DIGLYPHUS ISAEA* SEARCHING

Introduction

One possible explanation for the poor performance of *D. isaea* at low *L. bryoniae* densities is that they feed too infrequently to maintain egg production and their longevity is reduced (see section 4.2.). However, this may not be a problem if they can search efficiently at low host densities

There is little available literature specifically on the searching behaviour of *D. isaea* and it is difficult to extrapolate information from other species because the searching behaviour of hymenopteran parasitoids is very varied and generally complicated. Valerie *et al.* (1996) showed that *D. isaea* was more attracted to damaged plants than non-damaged plants. Cheah and Coaker (1992) showed that host finding in *D. isaea* involved initial plant attraction, that the parasitoid would search both mined and unmined parts of leaves and visual stimulation was not required at close contact. Coaker and Cheah (1993) also showed that host plant conditioning affected the behaviour of the parasitoid. The effect of host density on searching behaviour was not known.

4.3.(i): Small scale experiments

Objective

To determine the searching behaviour and times of *D. isaea* at different *L. bryoniae* densities.

Introduction

The following small-scale experiments set out to examine the searching ability of the *D. isaea* at various *L. bryoniae* densities and to provide data to improve the model. Prior to this, the model had assumed that the parasitoid could search one plant per day. This was based on estimates by Boot *et al.* (1990) but had never been verified experimentally.

Materials and method

The experiments were completed by August 1999. Observations of *D. isaea* searching behaviour were made on a single whole tomato plant with 4-5 fully expanded leaves in a Perspex cage (2 x 0.6 x 0.6m) under glasshouse conditions.

The treatments comprised three *L. bryoniae* densities; low (2-12 mines per plant), medium (22-100 mines per plant) and high (150-400 mines per plant). Each treatment was run with a single female parasitoid (5-6 day old, tomato and *L. bryoniae* conditioned) for one hour and all three were run on the same day. Each series of runs was replicated six times with the order randomised.

Females were released on to the side of the cage. The following observations were recorded for each of the females:

1. Time taken for the female to first search the plant
2. Searching and resting times on leaflets.
3. Time spent on the cage
4. Handling *L. bryoniae* times (*i.e.* time taken for parasitoid to begin and finish investigating a mine).
5. Host feeding or oviposition, and oviposition times.

Analysis was done using correlation coefficients on log transformations for whole plant analysis and using L.S.D. for comparison of treatment means on leaflets.

Results and discussion

Figure 4.1 shows that with increasing density of *L. bryoniae*, the time to start searching on the tomato plant decreased. The correlation of time to start searching whole plants against plant density was significant (-0.47, 16df). The results suggest that at low *L. bryoniae* densities *D. isaea* are less attracted to the tomato plant and are therefore less likely to find the host. The experiment could not determine whether the attraction at the higher densities was chemical or visual.

Figure 4.1. Time (Log) for *D. isaea* to first search tomato plant at different leaf miner densities

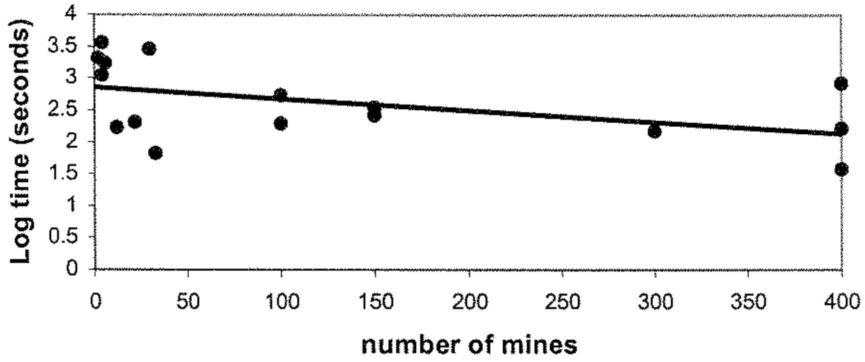
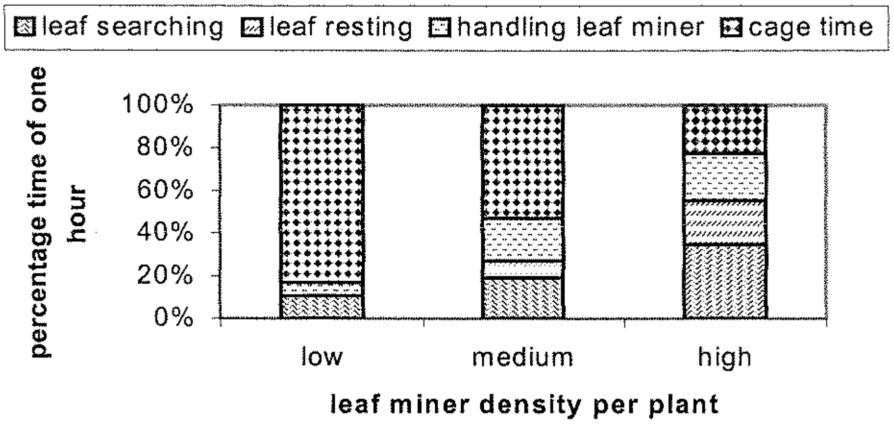


Figure 4.2. shows that the percentage of time spent by *D. isaea* on the cage decreased with increasing density of *L. bryoniae*. The greater time on the cage at low host densities included the

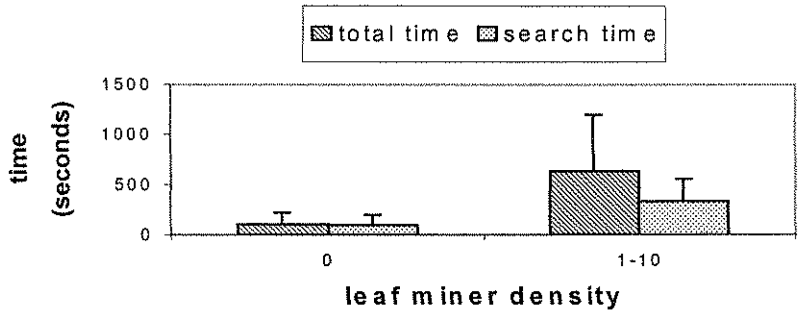
initial time before the parasitoid moved onto the plant and return visits during the period of observation. Figure 4.2. also shows that the time *D. isaea* spent searching on leaflets increased with increasing density of *L. bryoniae*. Resting on the leaf surface was only observed at the medium and high *L. bryoniae* density and reflects the tendency of the parasitoid to stay on the plant rather than return to the cage. Handling time appeared to decrease with increasing *L. bryoniae* density but the low density value was obtained from a single observation and therefore more replication is required to confirm this response. The fact that only one *L. bryoniae* larva was found by one female *D. isaea* at low host densities further suggests that *D. isaea* have difficulty locating prey at low densities. Also, this record was made at the upper limit of the low density category (*i.e.* 12 mines per plant) and therefore this observation may infact be more applicable to the medium density. Most of the time spent handling *L. bryoniae* was the result of feeding activity. Females spent on average 642.2 seconds (± 339.8s) handling larvae they were feeding upon, and this involved frequent stings of up to 60 seconds duration. Oviposition was not established. On high host density plants, stings of 25-30 seconds were observed but with no subsequent feeding activity. However, it was not possible to examine these mines to see if this behaviour was an indication of oviposition.

Figure 4.2. The effect of leaf miner density on *D. isaea* searching



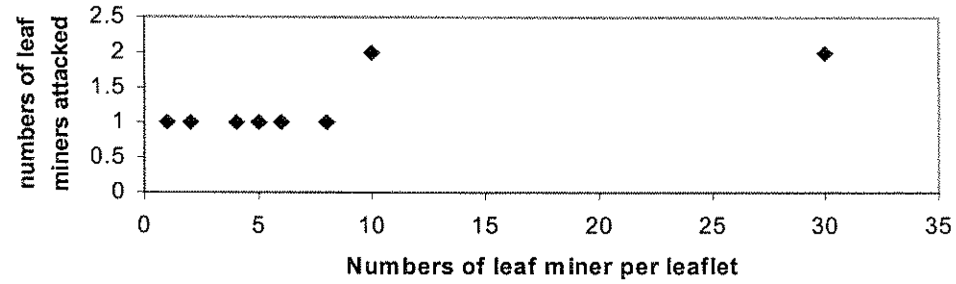
In Figure 4.3. it can be seen that time spent on tomato leaflets (total and searching time) was significantly greater (L.S.D. 88.3, 55df) on leaflets with one or more mines than leaflets without mines.

Figure 4.3. The effect of *L. bryoniae* density on total time on leaves and searching time of *D. isaea*



Figures 4.2. and 4.3. strongly suggest that there is a host density dependence on the searching behaviour of the parasitoid. However, as Figure 4.4. shows the actual numbers of larvae attacked per leaflet before the parasitoids moved onto another leaflet appears to be independent of the *L. bryoniae* density.

Figure 4.4. The effect of leaf miner density (per leaflet) on numbers attacked by *D. isaea*



The leaflet searching times determined from these experiments have been used to estimate how long it would take a female *D. isaea* to search the equivalent of one mature tomato plant in a commercial crop. The approximations are shown in Table 4.6. One approximation is based on the assumption that the parasitoid searches an entire plant with twenty fully expanded leaves and seven leaflets per leaf; although observations suggest that it would not systematically search in this way. However, based on this assumption the times for searching a plant at medium and high host densities are about one day. This is very close to the unverified estimates of Boot *et al.* (1990) that had previously been incorporated in the simulation model. The parasitoids on low host density plants however, completed the search twice as fast as parasitoids on high host density plants because they spent less time on each leaflet.

In the second approximation, the proportion of time that *D. isaea* appears to be uninterested in the plant (*i.e.* time spent on the cage, see Figure 4.2.) has been taken into account. In this scenario, the parasitoid does not move directly from one leaflet to the next but instead rests between leaflets. This increases the searching time of a whole plant with low host density from 0.4 day to 2.4 day.

In reality, the proportion of time spent on the cage in the experiments was probably greater than the proportion of time the parasitoid would spend off plants in a tomato crop. The real searching time per plant within the commercial tomato crop is therefore probably between the two approximations.

Table 4.6. The effect of *L. bryoniae* density on the mean time *D. isaea* spends on leaflets and the calculated equivalent time to search a whole tomato plant.

L. bryoniae density per plant	Mean number of mines per leaflet searched (sd)	Mean total time spent on leaflet in hrs. (sd)	Calculated time to search a whole plant, without leaving that plant	Calculated time to search a whole plant allowing for off-plant resting time.
High (150-400)	2.71 (2.69)	0.11 (0.11)	15.8h (1 day)	0.6 day
Medium (22-100)	1.78 (2.65)	0.12 (0.22)	16.8h (1 day)	0.9 day
Low (2-12)	0.12 (0.33)	0.05(0.09)	7.0h (0.4 day)	2.4 day

4.3 (ii) Glasshouse experiments

Objective

To determine searching ability of *D. isaea* within a glasshouse

Materials and method

Trap plants were set up in tomato crops in one or two glasshouse blocks on five dates between 29 March and 19 July 1999. On the first day of each experiment, 50 to 100 female *D. isaea* were released in one location. The trap plants were either replaced or examined at one to three day intervals and the *L. bryoniae* larvae examined for parasitism or host feeding. When the experiments were set up on 30 June and 19 July there was a low background level of *D. isaea* that had not been released. The experiment was run on 19 July without releasing *D. isaea* as a control. Although there was no true replication, the results indicated some important trends, which help to explain field observations. The methods and results are summarised in Table 4.7.

Results and discussion

Diglyphus isaea failed to find and parasitise *L. bryoniae* larvae on trap plants in overcast conditions during March (daily average of 4.3 hours sunshine, 96 J/cm^2, 19°C). Only one egg was laid over a six day period during slightly brighter conditions in April (daily average of 177 J/cm^2, 19.2°C). On 12 May, there were short periods of sunny weather (daily average of 144 J/cm^2, 19.0°C) and 11 eggs were laid on one of three trap plants. Under improved conditions in June and July (daily average of 205 J/cm^2, 20°C) *D. isaea* located and parasitised mines on all trap plants.

Table 4.7. Summary of trap plant methods and results.

Date set up	No of days run	Mean mines /plant	Age of *D. isaea*	Distance of trap plant(s) from release point	Results No. of plants + parasitised larvae	 % parasitism
29.03	3	15	2	0m, 4m, 8m, 11m (rS)	0	0%
29.03	3	23	2	11m (rS)	0	0%
28.04	6	87	2	0m, 4m, 8m, 11m (rS)	1	<1%
28.04	6	50	2	11m (rS)	0	0%
12.05	5	113	2	0m, 2.5m (rN), 5m (rS)	2	1%
12.05	5	120	5	0m, 2.5m (rN), 5m (rS)	0	0%
30.06	5	54	2	0m, 2.5m (NSEWrb), 5m (NSEWrb)	All (9)	20%
19.07	4	73	None released	0m, 2.5m (NSEWrb), 5m (NSEWrb)	All (9)	7%
19.07	4	85	2	0m, 2.5m (NSEWrb), 5m (NSEWrb)	All (9)	23%

(r) = plants distributed within a row of tomatoes
(b)= plants distributed between the rows of tomatoes
S= Trap plants to the South of the release point,
N= Trap plants to the North of the release point etc

The ability of *D. isaea* to find the trap plants correlated better with host density on those trap plants than with the distance from the release point. For example, in the experiment starting on 12 May, *D. isaea* was found on the plant next to the release point on day 4 and on the plant furthest from the release point on day 6 but on no other plants throughout the experiment. On both occasions *D. isaea* were found on the plant with the greatest number of mines on that day.

In June and July all trap plants were parasitised but there was a significant correlation between the numbers of *D. isaea* per plant and the numbers of *L. bryoniae* larvae per plant (0.63, $p<0.01$, 14df). This indicates that the parasitoids are most attracted to plants with a higher mine density. Figure 4.4. shows the number of parasitised larvae on plants with different larval densities set up on 19 July in two adjacent glasshouses. *D. isaea* was released in zone 4 but not in zone 3. Where *D. isaea* was released, only 8% parasitism was observed on the plant at the release point, which was infested with 39 *L. bryoniae* but 34% parasitism was observed on a plant 2.5m from the release point which was infested with 119 active miners. These results correlate well with the small-scale host searching results where it was shown that *D. isaea* spent significantly less time on plants with fewer mines. Where it was possible to watch *D. isaea* emerging from the release tube, at least half immediately flew away from the plant on which they were released, regardless of whether it was infested with *L. bryoniae*.

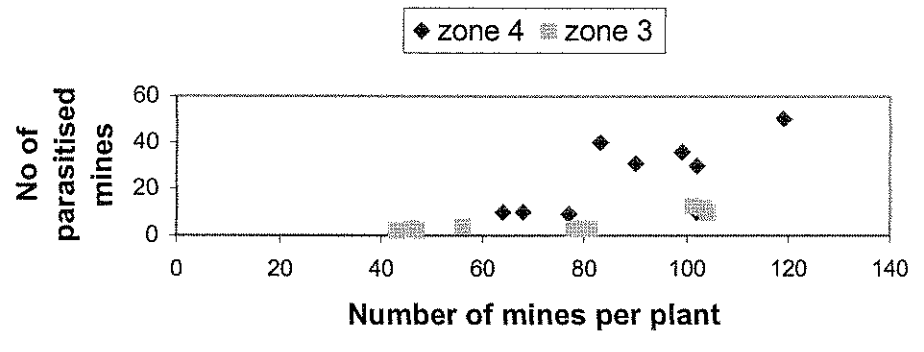


Figure 4.4. The number of mines parasitised by *D. isaea* on plants with different mine densities

4.4. OVERALL CONCLUSIONS

The poor performance of *D. isaea* at low *L. bryoniae* densities was found to be a result of both the biology and searching behaviour of the parasitoid.

The parasitoid requires a regular feed (*i.e.* at least one *L. bryoniae* larva every third day) to reproduce and therefore maintain a population within the glasshouse. The reproductive potential of the parasitoid increases at higher *L. bryoniae* densities.

The longevity of adult *D. isaea* is also reduced at low *L. bryoniae* densities and this limits the time she has to find the hosts.

Small-scale studies of the searching behaviour of *D. isaea* at low *L. bryoniae* densities indicate that the parasitoids are unable to locate *L. bryoniae* easily. It was found that the females are not attracted to tomato plants at low *L. bryoniae* densities. Also, time spent searching tomato plants decreased with low *L. bryoniae* density. The results obtained in the glasshouse experiments also suggest that the parasitoid is better at locating *L. bryoniae* on tomatoes when the *L. bryoniae* density is high.

The results have demonstrated a density dependence on the biology and searching behaviour of *D. isaea* that had not been previously proven and provided new data to the model.

PART 5: THE IMPACT OF *LIRIOMYZA BRYONIAE* DAMAGE ON MARKETABLE YIELD OF TOMATO PLANTS.

It is inevitable that some leaf miner damage must be tolerated when using biological control strategies based on parasitoids. However, at the start of this project it was impossible to estimate the point at which damage would become unacceptable and additional control measures required because the impact of leaf miners on marketable yield of tomatoes was so poorly understood. Reports in the scientific literature of effects of leaf damage on tomato yield are extremely varied. At one extreme, there was no difference in yield between plots with 1.2% and 75% loss of leaf area (Linquist, 1974). In contrast, Ledieu and Helyer (1985) reported large losses at relatively low pest densities; 30 mines per leaf (probably 5-8% loss of leaf area) were claimed to cause 10% loss of yield. Another study demonstrated 0%, 16% and 40% loss of yield at 25%, 50% and 75% leaf reduction respectively (Stacey, 1983). The experiments were conducted very differently; some used determinate tomato cultivars, some used simulated herbivory and some were based on small plots with inadequate replication. None of the studies reported in the literature were particularly relevant to modern tomato cultivars or growing techniques currently employed in the UK.

A direct correlation between leaf miner damage and yield loss is difficult due to problems associated with the manipulation of leaf miner populations on whole plants and crops. The first experiment in this study avoided such difficulties by using simulated herbivory. This was followed by a second experiment based on real herbivory.

5.1. IMPACT OF LEAF REDUCTION ON TOMATO YIELD

Objectives

1. To correlate numbers of *L. bryoniae* mines to loss of photosynthetic leaf area.
2. To reduce photosynthetic leaf area by simulated herbivory and correlate this to the yield of specific tomato trusses and whole tomato plants.
3. To estimate the effect of *L. bryoniae* damage on yield of tomato plants.

Introduction

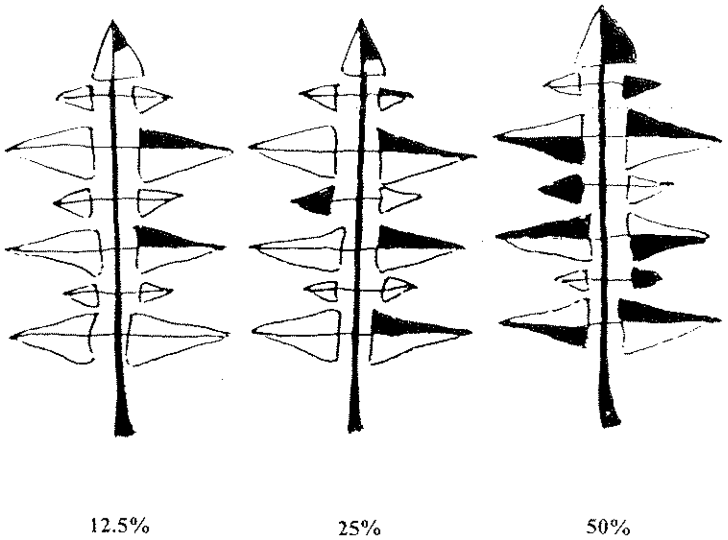
This experiment was done in 1996 in two parts; the first part correlated numbers of mines to loss of leaf area and the second part correlated loss of leaf area to yield. The latter focussed on the truss with most recently set fruit and the closest six leaves because the truss attracts assimilates predominantly from this area (Ho & Hewitt, 1996). Two cultivars, Liberto and Solairo, with different types of leaf canopy were included to determine whether the "leafier" Solairo plants were more tolerant to damage.

Materials and method

Tomato leaves (cv Ferrari) infested with *L. bryoniae* were obtained from a commercial crop on four dates in May and June. On each occasion, the individual areas of approximately 40 fully developed mines were measured by image analysis.

The correlation between loss of photosynthetic leaf area and tomato yield was done over four months in tomato crops in four sections of MFU Block 1 at Stockbridge House. The experimental design was a plaid Latin Square comprising two cultivars and four degrees of damage, with four replicates per treatment. The crop was planted in the first week of January 1996. There were 15 plants per plot and additional side shoots were taken in March to increase the number of stems to 20 per plot. The plants were grown hydroponically on rockwool slabs with excess feed running to waste and trained according to the Cordon "V" system. Trusses with fruit just beginning to swell were marked in early May. In the four damage treatments, three leaves above and three leaves below the marked trusses were trimmed to reduce their area by approximately 0%, 12.5%, 25% and 50%. The method of trimming is shown in Figure 5.1. Ten marked trusses on single stem plants were taken to harvest and the yield recorded. The actual areas of the trimmed leaves were measured by image analysis when removed by routine de-leafing in mid-June. Whole plot yields were recorded from two weeks before treatment to October.

Figure 5.1. Diagram to show method of leaf trimming - black areas were removed.



Results

The mean size of L. bryoniae mines, recorded on four occasions in May and June 1996, are shown in Table 5.1. The mean area of the mines was similar on all occasions and the overall mean was 162mm^2.

The mean area of untrimmed leaves of Liberto and Solairo when removed by routine deleafing in mid-June was 733cm^2 and 845cm^2 respectively. The actual reductions in leaf area in each treatment compared to untrimmed controls are shown in Table 5.2.

The mean yields of marked trusses in all treatments are shown in Table 5.3. The average yield per plot of all marked trusses was 6.0 kg. There were no significant differences between trimming treatments or cultivars. The mean plot yields, accumulated between May and October 1996, from all trimming treatments are shown in Table 5.4. The average plot yield for this period was 366kg. There was no evidence of differential effects although Class 1 yields were higher for Solairo than Liberto ($P<0.05$). There was no evidence of any effect on yield due to trimming.

Table 5.1. The mean size of *L. bryoniae* mines recorded on four occasions in May and June 1996.

Date	Mean mine size (sd)
24 May	150 mm^2 (70)
5 June	186 mm^2 (42)
12 June	159 mm^2 (67)
21 June	154mm^2 (43)
Overall mean	162mm^2

Table 5.2. Reduction in leaf area recorded in each treatment when leaves were removed by routine deleafing in mid-June.

Intended leaf reduction (%)	Achieved leaf reduction - cv Liberto (%)	Achieved leaf reduction - cv Solairo (%)
0	0	0
12.5	12.3	0
25	26.1	15.5
50	44.5	35.3

Table 5.3. Mean yields (kg) per plot of marked trusses in all treatments.

Cultivar	Trimming treatment	Class 1 fruit (kg)	Total fruit (kg)
Solairo	0	5.5	6.1
	12.5	5.4	6.3
	25	5.7	6.4
	50	5.3	6.0
Liberto	0	5.4	6.0
	12.5	5.1	5.7
	25	5.0	5.6
	50	5.4	6.2
sed (within cv)		0.37	0.33
Approx df		12	11

Table 5.4. Mean yields per plot, accumulated between May and October 1996, from all treatments.

Cultivar	Trimming treatment	Class 1 fruit (kg)	Total fruit (kg)
Liberto	0	240	369
	12.5	233	371
	25	229	360
	50	233	363
Solairo	0	270	362
	12.5	277	372
	25	274	366
	50	268	364
sed (within cv)		10.6	10.6
Approx df		5	4

Discussion

Untrimmed leaves of Liberto and Solairo were 733cm^2 and 845cm^2 respectively and the area of a fully developed *L. bryoniae* mine was 162mm^2. Based on these measurements, numbers of *L. bryoniae* mines have been correlated to percentage loss of photosynthetic leaf area (Table 5.5.).

Table 5.5. Correlation of numbers of mines to loss of photosynthetic leaf area (figures in bold correspond to leaf damage in the second part of the experiment - see Table 5.2.).

Number of mines per leaf	Equivalent loss of leaf area: cv Liberto (mean leaf area = 733cm^2)	Equivalent loss of leaf area: cv Solairo (mean leaf area = 845cm^2)
56	**12.3%**	10.7%
81	17.9%	**15.5%**
118	**26.1%**	22.6%
184	40.1%	**35.3%**
201	**44.5%**	38.5%

There was no difference in yield from the marked trusses, demonstrating that either the untrimmed leaves were producing more assimilates than the trusses required, or the plants were in some way able to compensate for the reduction in leaf area.

When days are long and light is relatively good, tomato plants probably produce more assimilates than are required to supply all of the fruits present (Cockshull, pers. com.). Thus some reduction in leaf area could be accommodated between May and August without loss of yield. However, over production of assimilates seems unlikely to explain the results obtained in this experiment when leaf reduction was greater than 25%; some changes (e.g. physiological or physical) must also occur to enable the plant to compensate for such large localised losses of photosynthetic area.

In normal circumstances the marked trusses would be expected to source assimilates from the six leaves that were trimmed in this experiment. An obvious means of compensation for leaf reduction would be additional growth of these leaves. In fact, compensatory growth, equivalent to approximately 10-15% of the area of the whole leaf, was observed in cv Solairo, and this would have contributed to the pool of assimilates locally available to the marked trusses. However, no such growth was recorded in cv Liberto. The yields of marked trusses were equally consistent across leaf reduction treatments on that cultivar, so other factors must be involved.

Another possible explanation is that after leaf trimming the marked trusses attracted assimilates from beyond their normal range. Although a truss normally sources assimilates locally, this is not absolute (Ho, 1996). In some circumstances it may be supplied by twelve leaves immediately above and below it (Ho & Hewitt, 1996). However, if that were true in this experiment, the sourced areas would be expected to be deprived of assimilates and, as a consequence, growth of fruit retarded in those areas. There was no evidence to support this because whole plant yields were similar in all treatments during each month of the experiment.

It follows that there must also be a compensatory change in the sourced area. It is known that trimming upper leaves allows more light to pass to lower leaves thus increasing their photosynthetic production. Marked trusses may therefore have attracted extra assimilates from lower parts of the plant without depriving fruit in that area.

Although, the experimental advantages of mechanical simulation of insect feeding compared to real herbivory are generally indisputable, the experience of this project suggests that artificial techniques are not entirely suitable for the study of leaf miner damage to tomatoes. It had been assumed that the most important consequence of leaf miner feeding was the massive loss of leaf photosynthetic tissue and this would override more subtle effects on the plant's physiology; e.g. changes in vegetative growth, transpiration, photosynthetic rate and production of secondary metabolites. As the plant seems capable of compensating for very large localised reductions in leaf photosynthetic area between May and August, the less obvious physiological effects could be of greater importance. Furthermore, it has been hypothesised that leaf trimming allows lower parts of the plant to produce more assimilates, and thereby compensate for the damage. If this is true, trimming is clearly an inappropriate method for this type of investigation. A second experiment, using real herbivory, was therefore done.

5.2. IMPACT OF LIRIOMYZA BRYONIAE DAMAGE ON TOMATO YIELD

Objective

To measure the impact of *L. bryoniae* feeding damage on marketable yield of tomato plants.

Introduction

Following the simulated herbivory experiment (section 5.1.), it was hypothesised that leaf trimming could allow lower parts of the plant to produce additional assimilates and thereby compensate for the damage. A second experiment was designed that used real *L. bryoniae* herbivory. Preliminary studies demonstrated that insecticides could be used to stop the development of *L. bryoniae* populations without affecting tomato yield. In addition, a template was developed to aid the measurement of leaf area lost due to leaf miner damage.

Materials and Methods

The experiment was done in four identical glasshouses, each of 190 m^2, containing tomato plants (cv Solution). They were planted in early February 1998 at a density of 2.3/m^2 and grown hydroponically in rockwool slabs with excess feed running to waste. Liriomyza bryoniae were released into one corner of three of the glasshouses from early March and the populations manipulated to provide damage ranging from 0 to 60% loss of photosynthetic leaf area. Liriomyza bryoniae development was stopped by applying three applications of heptenophos (Hostaquick) at five-day intervals beginning on 22 June.

For each glasshouse, total yields were recorded for the seven complete trusses that were present on the plant on 22 June. Yields for Class I and total fruit were cumulated on a weekly basis and a percentage Class I figure was also determined. Yields were also cumulated by weeks (over a ten week harvesting period) to ascertain any differential harvest effects over that time. Analysis of variance was performed on the following in each week:

i) total yield in that week
ii) cumulative total yield
iii) Class I yield in that week
iv) cumulative Class I yield

More detailed assessments were made on 100 individual plants. Twenty-five plants were selected from each of the four glasshouses to provide an overall range of damage from zero to approximately 60% loss of photosynthetic area on the leaves positioned 13 to 22 from the top of the plant. Eight trusses were labelled on each of the selected plants; truss 1 being the first truss with open flowers at the top of the plant. Tomatoes were picked three times per week as they ripened. As each truss matured, the leaf positioned immediately above the truss was removed and the number of mines, total leaf area and loss of photosynthetic leaf area due to leaf miner activity (expressed as a percentage of the whole leaf) were assessed. The following records were made of each fruit as it ripened:

i) Truss reference number
ii) Weight
iii) Size
iv) Class
v) Colour (Tomato colour chart 301.1 codes 1-9)
vi) Gold spot (0<5%, 1=5-20%, 2=21-50%, 3=51-75%, 4>75%)
vii) Marbling (0 to 4 = none to 40% cover of surface area)
viii). Uneven ripening (0 to 4 where 4 = more than 50% of fruit surface at least two colour stages different from the rest).
ix). Cracking (0=none, 1=0-25%, 2=25-50%, 3=51-75%, 4>75%)

In addition, firmness was measured on one fruit from each truss. The different parameters were analysed by regression analysis.

Results and discussion

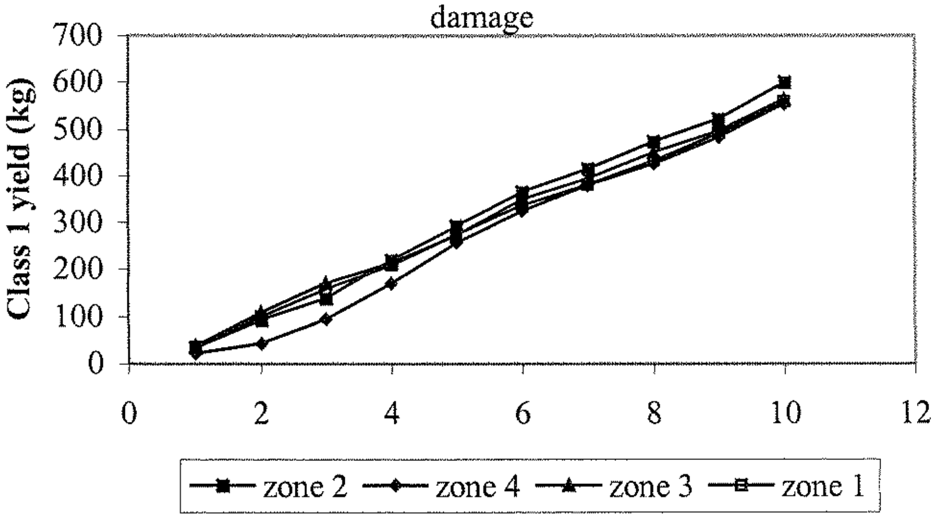
Table 5.6 shows the average amount of leaf damage and their ranges in the different glasshouses infested with *L. bryoniae* at different leaf positions on the plant on 22 June 1998.

Table 5.6. Leaf miner damage on leaves at eight positions on tomato plants in four glasshouses.

Leaf position (numbered from first fully expanded leaf)	Mean (range) percentage loss of photosynthetic leaf area in four different glasshouses:			
	Zone 1	Zone 3	Zone 4	Zone 2
1	1 (1-2)	1 (0-1)	1 (0-1)	1 (0-1)
4	3 (1-5)	1 (1-3)	1 (0-1)	1 (0-5)
7	9 (1-15)	6 (2-9)	3 (1-10)	2 (1-10)
10	15 (6-22)	5 (3-10)	3 (1-6)	1 (1-4)
13	31 (15-46)	15 (6-30)	8 (2-17)	2 (1-7)
16	38 (20-50)	18 (5-25)	9 (3-15)	4 (1-20)
19	37 (20-60)	26 (12-35)	8 (2-21)	5 (2-10)
22	47 (40-60)	18 (10-33)	10 (2-22)	5 (1-21)

The overall behaviour of Class I and total yield was virtually identical in the four glasshouses (Figure 5.2). Although there were significant differences between the four glasshouses on a week by week basis, there was no obviously consistent pattern to it. At the end of the measurement period glasshouse 2, which had the least leaf miner damage, had a Class I yield 7.5% in excess of the other three glasshouses, the total yield showing a similar excess. However, given the variability these differences were not significant.

Figure 5.2. Cumulative Class 1 yields in four glasshouses (zones 1, 2, 3 and 4) with different amounts of leaf miner damage



There was a close association between number of mines and percentage of photosynthetic leaf area lost due to leaf miner activity; 100 mines per leaf causing approximately 50% leaf area damage. Leaf size was slightly reduced at the highest leaf miner damage levels (Correlation coefficient: -0.261, $P<0.05$) (Figure 5.3).

When 100 individual plants with different amounts of damage were marked and assessed, the weight and class of fruit on individual trusses were correlated with the various leaf damage assessments on the adjacent leaf. Despite the reduced leaf size, there was no significant effect on either truss yield or class I truss yield with up to 50% loss of photosynthetic area and over 100 mines/leaf on the adjacent leaves; e.g. results for truss 5 are shown in Figure 5.4 (Correlation co-efficient: -0.108). In addition, no significant effect of up to 50% leaf miner damage was observed on fruit size, fruit colour, gold spot, cracking, softness, ripening or marbling.

5.3. OVERALL CONCLUSION

The results of both the simulated and real herbivory experiments indicate that the standard round tomato cultivars, Liberto, Solairo and Solution, can tolerate substantial damage by *L. bryoniae* without suffering loss of yield or fruit quality. As a general guide, it should be not be necessary to resort to remedial action against leaf miners until there is over 35% loss of photosynthetic leaf area in a stratum of approximately six leaves.

It would be more convenient for growers if this "threshold" could be expressed in terms of numbers of mines rather than percentage of leaf area lost due to leaf miner activity. However, this is unreliable because both leaf and mine sizes are variable depending on cultivar, season and nutrional status of the plant.

Figure 5.3. Size of leaves compared to numbers of *L. bryoniae* mines per leaf.

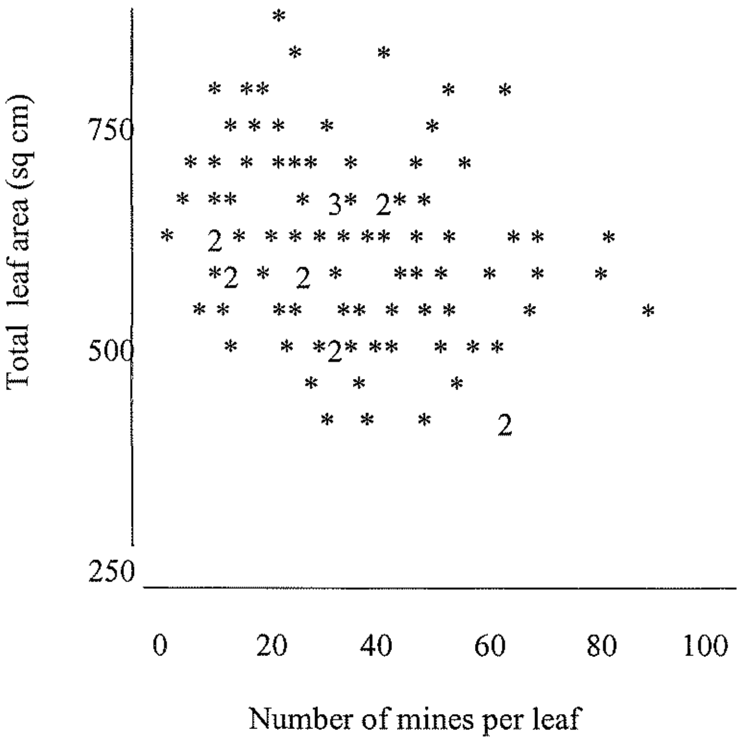
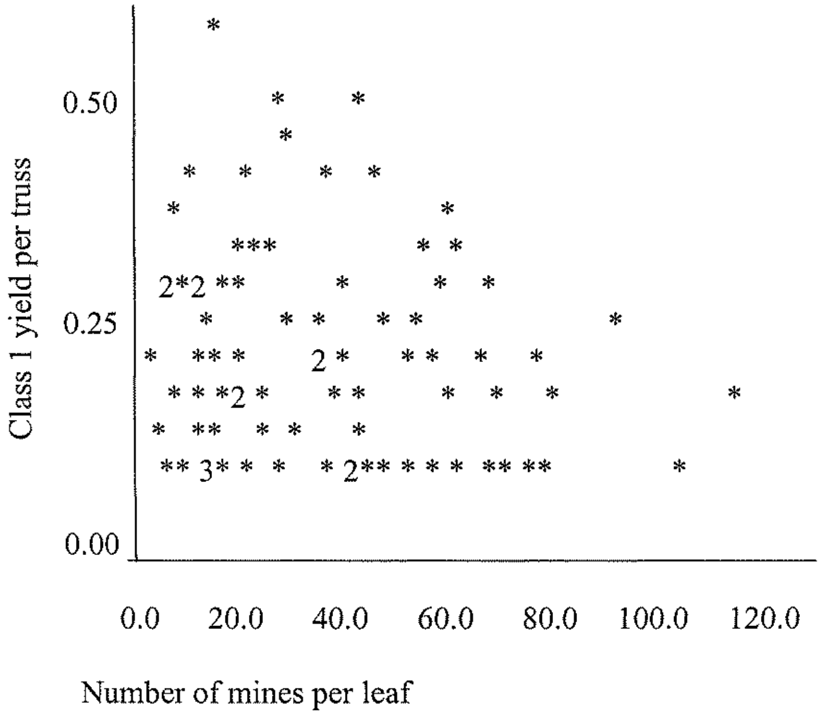


Figure 5.4. The total class 1 yield per truss compared to the number of mines per leaf on adjacent leaves.



PART 6: IPM COMPATIBLE REMEDIAL TREATMENTS

No matter how carefully biological control systems are managed, there will always be occasions when they fail and it is therefore important to have another control measure in reserve. For over a decade, tomato growers have used the organophosphorus insecticide, heptenophos (Hostaquick), as a second line of defence against *L. bryoniae* but only as a last resort because it is non-specific and disrupts IPM of other pests and biological pollination. An IPM compatible remedial treatment to support parasitoids is urgently required.

The parasitic nematodes, *Steinernema feltiae*, are produced as a commercial product (Nemasys, MicroBio Limited, Cambridge, U.K.) for the control of glasshouse sciarid flies in soil and other growing media. The nematodes are supplied as infective third stage juveniles in an inert carrier that is mixed with water to form a suspension and then applied to the target as a drench or spray. The infective juveniles move in free water, locate insect prey (*eg.* by detecting insect excretory products and CO_2 gradients), and penetrate the host through natural body openings or directly through the cuticle. Once inside, the nematodes release symbiotic bacteria, which in combination with the nematodes kill the host. Reproduction occurs within the dead insect when resources are depleted and infective juveniles are released into the growing media.

In 1986, Kaya described the use of *S. feltiae* against foliage feeding insects but noted that results in the field were generally erratic due to inactivation by desiccation. Williams and Macdonald (1995) subsequently reported some success with foliar sprays containing large numbers of *S. feltiae* against three species of leaf miners; *L. bryoniae*, *Liriomyza huidobrensis* and *Chromatomyia syngenesiae*. The nematodes were further evaluated against *L. bryoniae* on tomato plants within this project.

Steinernema feltiae must be compatible with *Diglyphus isaea* if it is to be used as a second line of defence. In a similar control system, Sher and Parella (1996) reported both positive and negative interactions when *Steinernema carpocapsae* and *Diglyphus begini* were used together to control *Liriomyza trifolii* on chrysanthemum. The compatibility of *S. feltiae* and *D. isaea* was investigated in this project.

6.1. PRELIMINARY STUDIES

In a series of laboratory-scale experiments in 1995 and 1996, *S. feltiae* (Nemasys) were applied to excised tomato leaves infested with *L. bryoniae* larvae and observed for 5 days at 21°C, 16:8 L:D. The nematodes entered the mines and located, penetrated and reproduced within the leaf miner larvae. The miners died approximately 48 hours after application of treatments and the progeny of the nematode invaders emerged 48 hours later. The nematodes appeared to enter the leaves primarily through feeding/egg laying punctures on the upper surface of the leaves. Second and third instar leaf miner larvae were most vulnerable to attack. Leaf miner pupae were not directly attacked but nematodes sometimes penetrated third instar larvae just before pupation and then produced progeny within the dead pupae.

During 1997, the preliminary studies progressed to small-scale experiments on plants in glasshouses. In each of a series of experiments, synchronised leaf miner infestations were established by confining adult females on individual leaves within transparent sleeves. The females were allowed to feed and lay eggs for two days and then removed from the glasshouse. The various treatments were applied when the majority of leaf miner larvae were second and third instars. In the first such experiment, suspensions of up to 10,000 nematodes per ml were sprayed on to leaves that were infested with an average of 23 leaf miners. The results, which were similar in all treatments, were disappointing with only 16-18% of the larvae killed. The spray droplets had dried rapidly, restricting the mobility of the nematodes so that they couldn't gain entry to the leaves. Several experiments were then designed to explore methods of improving spray cover and delaying drying time; these included the use of different sprayers, the use of adjuvants and various methods of maintaining artificially high humidity. There was no difference between sprayers because all those tested provided good cover on the upper surface of the leaves. Water alone gave greater recovery of live nematodes from leaves than spray mixtures containing Savona (fatty acid soap), Codacide (rapeseed oil and emulsifier), Headland Guard (sticker and latex) or Agral (non-ionic surfactant). Attempts to raise relative humidity by dampening the floor and/or periodic misting with water after application of Nemasys had little impact on spray drying time. However, throughout the experiments the greatest percentage kill was always achieved in replicates with the largest number of leaf miner larvae and this appeared to override all the other factors that were investigated. The leaves with greater leaf miner densities had more surface damage and it was hypothesised that the nematodes had more opportunities to gain entry before the water evaporated.

Throughout the above experiments the standard Nemasys formulation, vermiculite, left unacceptable deposits on the tomato leaves and fruit. MicroBio Ltd subsequently produced a revised formulation based on clay; the clay particles being separated from the nematodes by their differential settling rates during preparation of the spray mixture. Other formulations were tested in the latter stages of the project but none performed better than the clay based product.

The knowledge gained from the preliminary studies was used to design larger-scale studies in both experimental and commercial tomato crops.

6.2. CONTROL OF *LIRIOMYZA BRYONIAE* IN TOMATO PLANTS WITH TWO APPLICATION RATES *OF STEINERNEMA FELTIAE*

Objective

To identify an effective and economic application rate for the use of *S. feltiae* (Nemasys) for the control of *L. bryoniae*.

Introduction

Preliminary experiments had shown that *L. bryoniae* were susceptible to the parasitic nematode *S. feltiae* (Nemasys). The following experiment was designed to evaluate a specially prepared clay formulation of *S. feltiae* as a remedial treatment against high population densities of *L. bryoniae* in a glasshouse tomato crop.

Materials and method

This experiment was completed during April 1997 in a tomato crop (cv. Liberto) grown in rockwool following practices commonly used by the U.K. tomato industry. A single fully expanded leaf was enclosed in a plastic sleeve on each of 40 plants. Fifteen three to four day old adult female *L. bryoniae* were released in each sleeve, allowed to lay eggs for ten days and then removed. Twenty four leaves with high leaf miner densities were selected for use in the experiment when the larvae were predominantly second and third instars. The following three treatments were each applied to eight of the selected leaves:

1. High rate (1 pack of Nemasys in 5l of water; *i.e.* 10,000 nematodes ml^{-1}).
2. Low rate (0.1 pack of Nemasys in 5l of water; *i.e.* 1000 nematodes ml^{-1}).
3. Control (water only)

The nematode products were applied high volume to maximum leaf retention using a Hozelock sprayer on the finest setting. Sprays were applied at the end of the day and the vents closed over night to maintain humidity (70-89% for first 24h). Samples were taken from the spray mixtures and the numbers of nematodes counted using a haemocytometer. After four days, the leaves were removed and the *L. bryoniae* larvae examined by dissection to determine the proportion that contained nematodes.

Results and discussion

The high and low rate spray mixtures contained an average of 9500 ml^{-1} and 950 ml^{-1} live infective juveniles respectively. The results in Table 6.1. show that both rates of Nemasys reduced *L. bryoniae* numbers significantly compared to untreated controls ($P<0.001$). The higher rate resulted in 89% control of *L. bryoniae* larvae. However, at £16 (plus VAT) per pack (50 million nematodes), the treatment would cost £77 per 1000 plants and this was considered to be too expensive. Although the lower rate provided only 62% control, this could be adequate to support an existing population of *D. isaea* and the cost of £7.30 per 1000 plants would be more acceptable.

Table 6.1. The effects of two rates of Nemasys on the control of *L. bryoniae* in infested tomato plants.

Treatment	Mean (± sd) number of leaf miner per leaf	Mean (± sd) number of leaf miners with nematode	Mean number (± sd) of each leaf miner instar killed			Mean (± sd) proportion of leaf miner killed
			1st	2nd	3rd	
1pkt / 5 l water	61.8 (26.6)	55.5 (27.1)	7.07 (2.9)	42.6 (23.6)	7.5 (7.0)	0.89 (0.08)
0.1pkt/ 5 l water	32.1 (7.4)	19.4 (7.2)	3.2 (1.6)	13.7 (6.8)	3.3 (2.4)	0.62 (0.23)
Water	45.7 (10.4)	0	0	0	0	0.0

6.3. THE EFFECT OF *STEINERNEMA FELTIAE* ON THE LEAF MINER PARASITOID, *DIGLYPHUS ISAEA*

Objective

To determine whether the use of *S. feltiae* (Nemasys) against *L. bryoniae* disrupts the establishment of *D. isaea*.

Introduction

To achieve effective and economic control of *L. bryoniae*, a remedial treatment is required that is compatible with the primary biological control agent, *D. isaea*. The previous studies have shown that *S. feltiae* has the potential to fulfill this role but the nematodes only provide a single knock down treatment. As an established population of *D. isaea* can provide season long control of *L. bryoniae*, it is essential that the nematodes do not significantly effect the growth of parasitoid population.

Preliminary studies

In December 1997, laboratory studies were carried out to determine whether *S. feltiae* could parasitise *D. isaea* under ideal conditions. Sowthistle leaves infested with the chrysanthemum leaf miner, *Chromatomyia syngenesiae*, and *D. isaea* were dipped in a high concentration of nematodes (50,000 ml^{-1}). The leaves were then placed in petri-dishes on damp filter paper in order to maintain a high humidity. After three days, the *C. syngenesiae* and *D. isaea* larvae were dissected separately, and the presence or absence of nematodes inside recorded. After three days, 86% of the *C. syngenesiae* larvae contained breeding populations of nematodes. Thirty percent of the *D. isaea* larvae contained nematodes but only one or occasionally two infective juvenile nematodes were found in each larva indicating that *S. feltiae* could penetrate but were unable to reproduce in *D. isaea*. The infected *D. isaea* were still alive.

Materials and method

This experiment was completed during August 1998 in a mature tomato crop (cv. Espero) grown in rockwool following practices commonly used by the U.K. tomato industry. The crop was infested evenly with *L. bryoniae* and its parasitoid *D. isaea*. At the time of treatment, 70% of the live leaf miner were parasitised by *D. isaea* at leaf position 12 and 36% were parasitised at leaf position 8. Alternate rows of tomatoes were treated with *S. feltiae* using 0.1 pack of Nemasys per 5 litres of water sprayed to maximum leaf retention with a Hozelock PP 5 litre sprayer. Immediately after spraying, 15 treated and 15 untreated plants were selected at random. On each of these plants, leaves at positions 8 and 12 were enclosed individually in perforated plastic bags after the spray dried. Twelve days after spraying, the numbers of adult *L. bryoniae* and *D. isaea* that had emerged into the bags were counted. The data were analysed separately for each leaf position using ANOVA. Four days after treatment, 20 leaves were collected from leaf position 11 on treated plants and *L. bryoniae* larvae (dead and live) and *D. isaea* (larvae and pupae) were removed from the leaves, dissected and examined for the presence of *S. feltiae*.

Results and discussion

The effect of Nemasys treatment on the numbers of *L. bryoniae* and *D. isaea* emerging from tomato leaves is shown in Table 6.2. At leaf position 12, where there were up to 75 mines per leaf and 25% leaf damage, the nematode treatment significantly reduced (*P*=0.015) the number of *L. bryoniae* adults by 51% compared to untreated controls. However, there was no significant reduction in numbers of *L. bryoniae* adults emerging at leaf position 8, where there were less than 35 mines per leaf and 15% leaf damage. These results are consistent with previous data showing that control is improved where there is more leaf damage and more entry points for the nematodes. There was no significant reduction in the number of *D. isaea* adults emerging from treated plots at either leaf position. Furthermore, parasitism by *D. isaea* improved from 70% to 82% in the treated leaves with greater damage.

Table 6.3. shows the effect of Nemasys treatment on the *D. isaea* larvae, *D. isaea* pupae and *L. bryoniae* larvae that were examined by dissection four days after application of sprays. Very small numbers of nematodes were found in 7% and 6% of the *D. isaea* larvae and pupae respectively. This was consistent with data from the preliminary studies and further suggests that the nematodes are unable to reproduce in *D. isaea*. There were few live *L. bryoniae* larvae remaining at the end of the experiment but 40% contained nematodes. The mortality of *L. bryoniae* larvae was clearly the result of the activities of both *S. feltiae* and *D. isaea*. However, it was difficult to separate the effects of the two natural enemies; for example 10% of the dead *L. bryoniae* larvae contained nematodes but it was impossible to say whether they entered before or after the larvae died.

Although more detailed studies would be required to determine whether the presence of either natural enemy influences host selection and performance of the other, overall, it may be concluded that *S. feltiae* can be used to control *L. bryoniae* with minimal disruption to *D. isaea* establishment.

Table 6.2. The effect of Nemasys treatment on the numbers of *L. bryoniae* and *D. isaea* emerging from tomato leaves twelve days after application of sprays.

Treatment	Number of emerging *L. bryoniae*		Number of emerging *D. isaea*	
	Mean number per leaf	Square-root transformed data	Mean number per leaf	Square-root transformed data
Nemasys – leaf 12	2.9	1.5	13.0	3.5
Untreated– leaf 12	5.9	2.3	13.8	3.5
Sed		0.3		0.5
Nemasys – leaf 8	7.7	2.6	5.6	2.19
Untreated– leaf 8	8.7	2.7	5.3	2.05
Sed		0.4		0.37

Table 6.3. The effect of Nemasys treatment on *L. bryoniae* larvae and *D. isaea* larvae and pupae examined by dissection four days after application of sprays.

	Number parasitised	Number unparasitised	% parasitised	Mean mines per leaf	Mean % damage
D. isaea larvae	2	28	7	86 (active or not)	22%
D. isaea pupae	7	102	6		
Live *L. bryoniae*	2	3	40		
Dead *L. bryoniae*	7	69	10		

6.4. CROP SCALE EVALUATION OF *STEINERNEMA. FELTIAE* FOR THE CONTROL OF *LIRIOMYZA BRYONIAE* IN PROTECTED TOMATOES

Objective

To determine whether *S. feltiae* can be used effectively and economically as a remedial treatment for *L. bryoniae* control in tomato crops.

6.4.(i). A crop at HRI Stockbridge House

Materials and method

This experiment was completed in 1999 in a mature tomato crop (cv. Espero) grown in rockwool following practices commonly used by the U.K. tomato industry. The crop was artificially infested with *L. bryoniae*. It was originally intended to apply treatments when the leaf miner population exceeded 80 mines per leaf but an invasion of *D. isaea* restricted the increase in pest numbers and the experiment began with fewer *L. bryoniae* larvae.

The glasshouse was divided into 16 plots, each containing a double row of 24 tomato plants. Immediately before treatment, one leaf was taken from leaf position 10 on every second plant (excluding guards) in each plot (*i.e.* n=10). Each leaf was sealed in a plastic bag and the number of emerged *L. bryoniae* and *D. isaea* adults were counted after 14 days. On 9 June, half the plots (randomised block design) were treated with the clay formulation of Nemasys. The product was applied to maximum spray retention with a Hozelock PP 5 litre sprayer at the rate of 0.1 pack per 5 litres of water. Ten 1ml samples were extracted from the spray tank and the numbers of live and dead nematodes counted. Sprays were applied during the evening and the vents were kept closed overnight, maintaining humidity above 85%. Twenty-four hours after treatment muslin bags were placed over the leaves remaining at leaf position 10 (n=10). Six days later these leaves were removed and placed in plastic bags for a further week when the number of emerged *L. bryoniae* and *D. isaea* adults were counted. The data was square-root transformed, then analysed by ANOVAR. Treatment means were then compared using a T test.

Results and discussion

Table 6.4. shows the average number of *L. bryoniae* and *D. isaea* adults emerging from leaves collected from treated and untreated plots before and after treatment. Although there was no significant difference between the numbers of *L. bryoniae* or *D. isaea* in treated and untreated plots at each assessment date, there were significant changes with time within treatments ($P<0.05$). The numbers of *L. bryoniae* decreased in the treated plots by 23% but increased in the untreated plots by 9%.

Two factors were thought to have contributed to the poor control of *L. bryoniae* in this experiment. It is possible that there was insufficient leaf damage to give sufficient entry points for the nematodes. In addition, the actual number of live *S. feltiae* recorded from the spray mixture in this experiment was 398 per ml, which was much lower than the target of 1000 per ml. A crop with more leaf miners per leaf was sought to repeat the experiment (see 6.4.(ii)).

Table 6.4. The average numbers of *L. bryoniae* and *D. isaea* adults emerging from leaves collected from treated and untreated plots before and after treatment.

	Number of *L. bryoniae* / leaf				Number of *D. isaea* / leaf			
	Treated		Control		Treated		Control	
	Mean No	Square root No	Mean No	Square root No	Mean No	Square root No	Mean No	Square root No
Pre-treatment	53	5.7	45	4.7	37	5.7	26	4.7
Post-treatment	41	4.8	49	5.6	26	4.8	32	5.6
Sed		0.37		0.37		0.44		0.44

6.4 (ii). A crop at a commercial tomato nursery

Materials and methods

This work was completed in June and July 1999 in a mature commercial crop of cherry tomatoes (cv Favorita) with a large but uneven infestation of *L. bryoniae*, 29% parasitism with *Dacnusa sibirica* and small numbers of *D. isaea*. On 30 June, three plots, each containing three double rows of plants, were treated with *S. feltiae* using 0.1 pack of Nemasys (clay formulation) per 5 litres of water sprayed to maximum spray retention with a hydraulic sprayer. Three double rows adjacent to the treated areas but with fewer leaf miners were used as an untreated control plot. Ten samples were extracted from the spray mixture and the numbers of live and dead nematodes counted in each. Treatments were applied in the evening and the vents were kept closed overnight, maintaining humidity above 85%. Twenty-four hours before treatment and 48 hours after treatment, ten leaves were collected at random from leaf position 6 in the centre row of each plot. Each leaf was sealed in a plastic bag for nine days, when the number of emerged *L. bryoniae* and *D. isaea* were counted.

Results and discussion

The number of live *S. feltiae* recorded from the spray mixture was 1277 per ml. Table 6.5. shows the average number of *L. bryoniae* and *D. isaea* adults emerging from leaves in treated and untreated plots before and after the treatment. The data could not be analysed statistically due to the variability between plots. The results from the most heavily infested plot ("hot-spot") have been separated because there was a difference in treatment response. At the start of the experiment, there were 164 *L. bryoniae* larvae per leaf in the hot-spot and this was reduced by 51% following application of nematodes. There was no apparant control of *L. bryoniae* larvae in the other treated plots, which had an average of 38 larvae per leaf before treatment. The numbers of *D. isaea* per leaf increased in all plots by between eight and eleven times between the pre and post-treatment assessments. The increase was greatest in plots with the most leaf miners regardless of application of nematodes

Table 6.5. The average numbers of *L. bryoniae* and *D. isaea* adults emerging from leaves in treated and untreated plots before and after the application of nematodes.

	Mean number of *L. bryoniae* / leaf			Mean number of *D. isaea* / leaf		
	Treated hot-spot	Treated	Control	Treated hot-spot	Treated	Control
Pre-treatment	164	38	22	0.7	0.4	0.4
Post-treatment	81	90	82	7.6	3.9	3.3

Moisture retention is considered a key factor in *L. bryoniae* control using *S. feltiae* as the nematodes need to move across the leaf surface in order to find an entry point. In this experiment the nematodes were sprayed at 2100h, the vents were closed and the leaves were still wet in the morning giving the best opportunity for survival under grower conditions.

The majority of the nematode infested leaf miner larvae were found close to the leaf tip, which probably reflects the distribution of spray on the leaf and the subsequent drying time.

After the experiment had finished, there was a sudden increase in *D. isaea* numbers, which together with the activity of *D. sibirica*, rapidly brought the leaf miner population under control. The model could be used to compare the relative merits of a nematode treatment that provides a "one-off" 50% control with extra releases of *D. isaea* which provide continuous control.

6.5. OVERALL CONCLUSION

Under suitable conditions, the parasitic nematode, *S. feltiae* (Nemasys), can provide a significant reduction in numbers of *L. bryoniae* larvae in tomato leaves without disrupting an existing population of the parasitoid, *D. isaea*.

A clay-based formulation of the nematodes was applied high volume to maximum leaf retention at the rate of 1000 infective juveniles per ml of water. Two factors were shown to be particularly important in achieving a reasonable level of control:

1. After spraying, the leaves must remain moist long enough to allow the nematodes to locate and enter mines.
2. The time that the leaves must remain moist depends on the leaf miner population density. It is shorter at higher densities because there are more entry points.

Figure 6.1. shows the percentage control achieved at different mine densities under carefully controlled conditions in an experimental crop (section 6.2) compared to that achieved by a grower in a commercial crop (section 6.4(ii)). It is clearly more difficult to achieve good results on a large scale but at least 50% of the leaf miner larvae were killed where there were sufficient entry points on the leaves. The latter appears to occur when there are over 80 mines per medium sized leaf, which equates to 20-30% loss of leaf area. Such damage is not expected to affect tomato yield in standard round cultivars (see Part 5).

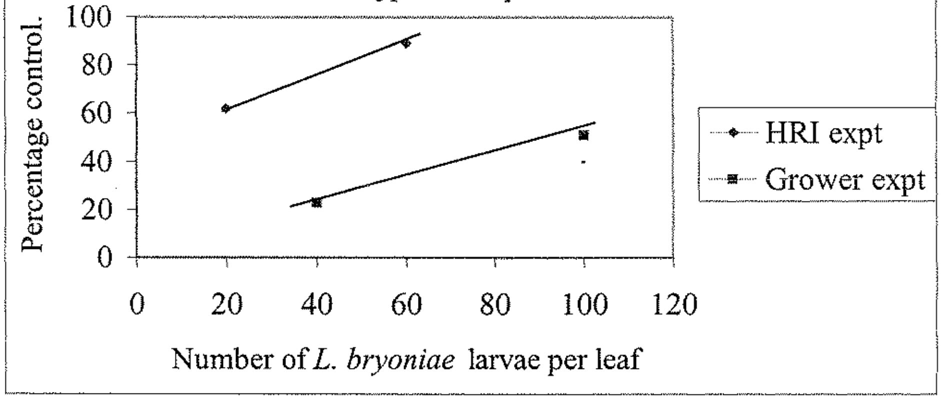


Figure 6.1. The effect of *Steinernema feltiae* on *Liriomyza bryoniae* control at different mine densities in two types of experiment.

A 50% reduction of numbers of *L. bryoniae* larvae should be sufficient to slow down the growth of the population and allow *D. isaea* to gain control.

There may be scope to improve the level of control by changing the spraying technique or using adjuvants to decrease leaf drying time. Preliminary studies in collaboration with Simon Piggott (Imperial College) have shown that the inclusion of a polyacrylamide gel in the spray mixture delayed leaf drying time and increased survival of nematodes by 29%. However, such treatments are not currently approved and further work would be required to fully evaluate this technique.

The control method may benefit from further studies to improve our understanding of the interspecific interference and intraguild "predation" between *D. isaea* and *S. feltiae.* Similar studies involving closely related species have been reported by Sher and Parella (1999).

A clay-based formulation of *S. feltiae* (Nemasys®T) has now been developed specifically for foliar application to tomato plants.

PART 7: *LIRIOMYZA BRYONIAE* INVASION AND POPULATION DEVELOPMENT IN COMMERCIAL TOMATO CROPS

Crop invasion by *L. bryoniae* was monitored at commercial nurseries over three seasons to determine whether the timing of adult emergence was consistent with the results obtained in experiments reported in Part 2 of this report. *Liriomyza bryoniae* populations were monitored throughout the season at selected nurseries from different parts of the country to verify the *L. bryoniae* population development and the control by parasitoids predicted by the simulation model. Where discrepancies between predicted and real responses were highlighted, further experimental work was organised and the model was modified. Data collected in 1997 and 1998 suggested that *L. bryoniae* populations could spread very rapidly from "hot-spots" of pest activity and this could lead to control failure. More specific studies were done in 1999 to investigate this and further improve parasitoid release strategies.

Objectives

1. To verify that adult *L. bryoniae* emerge in response to raised temperatures in glasshouses.
2. To verify the predictions of the simulation model by monitoring the development of *L. bryoniae* and *D. isaea* populations through the season.
3. To compare the impact of different *D. isaea* release strategies on control of *L. bryoniae*.

Materials and methods

Liriomyza bryoniae invasion: The first occurrence of *L. bryoniae* and the subsequent population development were monitored in selected crops before parasitoid release began at four nurseries in the north and south of England (*i.e.* Hazlewood VHB, Runcton and Zuider Zee; EVS, Camblesforth; AVN, Isle of Wight). Two nurseries were included in 1997, three in 1998 and two in 1999. Daily average temperatures were also recorded. The timing of the first occurrence of *L. bryoniae* and subsequent development at different glasshouse temperatures were compared to that predicted from existing data (Minkenberg and Helderman, 1990; Part 3 of this report).

Liriomyza bryoniae population development: The crops described above were monitored weekly from planting until *L. bryoniae* was under control. At low *L. bryoniae* densities, in 1997 and 1998, 100 plants were monitored in ten rows per glasshouse, with half the rows located in areas where *L. bryoniae* had been most prevalent in the previous season and the other half spread evenly around the rest of the crop. As the *L. bryoniae* density increased to one mine per five plants, 60 individual plants, in ten lots of six, were marked per glasshouse. The numbers of plants monitored was further reduced to a minimum of ten plants as *L. bryoniae* numbers continued to increase. New mines were marked, counted and recorded each week. Daily temperature and light levels were also recorded where available. In 1999 the monitoring system was refined further as outlined in Part 8 of this report.

Spread of *L. bryoniae* from "hot-spots" of activity: In 1999, a hot-spot of *L. bryoniae* activity, located in the center of a glasshouse (EVS, Block 52, cv *Elegance*), was monitored for eight weeks until mines were found on all monitored plants. Seventeen plants were marked, with four at 3.5m intervals to the north, south, east and west of a central plant. The numbers of new mines on each of the marked plants were counted weekly. In a second glasshouse (Block 55, cv Aranca), the density of mines was recorded weekly recording zones of 6.4 m^2 throughout the glasshouse until *L. bryoniae* were widespread.

Parasitism assessments: Fifty mines containing active *L. bryoniae* larvae were collected weekly from each of the monitored glasshouses and sent to HRI Stockbridge House for *L. bryoniae* parasitism assessments. A glasshouse (EVS, Block 51, cv Aranca) was selected with greater numbers of *L. bryoniae* in the eastern than western ends and both areas were monitored to evaluate *D. isaea* establishment at different mine densities. After *D. isaea* release, weekly parasitism assessments were made in the two areas until mine numbers stabilised.

Control strategies: In 1996 and 1997, experiments organised by the States of Jersey and BCP Ltd. compared the efficacy of releasing *D. isaea* weekly from planting to delaying release until the *L. bryoniae* density reached a threshold of approximately one active mine per plant. Further experiments compared the efficacy of two *D. isaea* release strategies, both of which began when the *L. bryoniae* population reached the threshold of one active mine per plant. The first strategy involved a single release of 14,820 *D. isaea* per ha followed by two further releases of 2470 per ha at weekly intervals. The second strategy comprised eight separate *D. isaea* releases of 2470 per ha at weekly intervals.

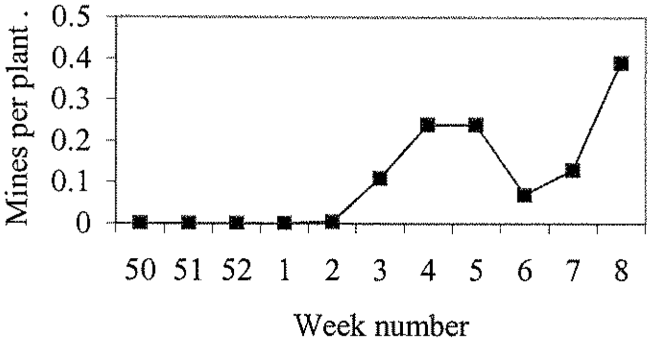
Results and discussion

Liriomyza bryoniae invasion into crops

Data from 83% of monitored crops (*i.e.* two in 1997, five of seven in 1998 and 12 of 14 in 1999) supported the results from previous small scale studies (see Parts 2 and 3). For example, in block 13 at EVS in 1997/8, the crop was planted in week 50 and with an average temperature of 15°C, the first mines were observed after 28 days. The experimental data predicted 20 days to adult emergence, 2 days pre-oviposition period and 7 days to egg hatch; *i.e.* a total of 29 days. The distinct peaks in the first *L. bryoniae* generation indicated that all adults emerged during a short period (Figure 7.1). After the first generation peak, the average temperature increased to 20°C and the second generation appeared after approximately four weeks; again as predicted. It follows that temperature data can be used to predict the appearance of the first mines in crops with an over-wintering *L. bryoniae* population.

In several monitored crops, the first record of *L. bryoniae* was later in the season but in each case the infestation could be attributed to the pest migrating in from outside or from other crops in the locality.

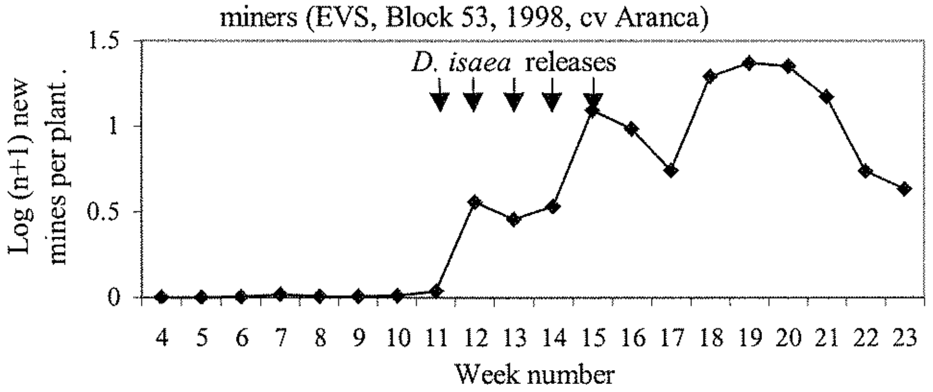
Figure 7.1. Early season development of leaf miner populations in block 13 at EVS 1998



Liriomyza bryoniae population development

Liriomyza bryoniae populations showed a typical development pattern in most round tomato crops. The first generation emerged in small numbers over three to four weeks after the glasshouse temperature was raised. The emergence was synchronised and the first two generations clearly defined. By the third generation, the *L. bryoniae* generations had started to overlap and usually become widespread, which improved the chance of *D. isaea* becoming established. Growers usually released *D. isaea* only during the third and fourth generations of leaf miners. They then became established, prevented the next flush of leaf miners and provided control for the rest of the season (e.g. Figure 7.2).

Figure 7.2. Typical pattern of leaf miner population development in round tomatoes with an over-wintering population of leaf miners (EVS, Block 53, 1998, cv Aranca)



A number of factors influenced the timing and size of the *L. bryoniae* population peaks. These included planting date, season, numbers and timing of parasitoid releases and tomato cultivar. At EVS in 1997/8, *L. bryoniae* numbers peaked in the different glasshouses between mid April and early May; peaks occurring earlier in crops planted in week 50 than in crops planted in week 1. In 1999, the cooler spring slowed down *L. bryoniae* development and they peaked later, between early May and early June.

Cultivars were observed to influence the size of the population peaks. For example at EVS in 1998, *L. bryoniae* numbers peaked in cv Aranca at 17 mines per plant (n=4, range=10-22) while in adjacent crops of cv *Elegance* numbers peaked at 68 mines per plant (n=3, range=57-78). In the same season at AVN, numbers in two crops of cv Espero peaked at 9 and 11 mines per plant but reached over 400 mines per plant in the more susceptible cv Campari. The influence of cultivar on *L. bryoniae* population development was further investigated (see Part 3).

Whilst monitoring *L. bryoniae* populations, flushes of new mines were observed after periods of warm sunny weather and this factor was further investigated (see Part 3).

EVS improved their control of leaf miners each season throughout the project. In 1998, this was attributed to the implementation of the monitoring methods, which improved decision making and timing of *D. isaea* releases (*i.e.* the first *D. isaea* was released seven weeks earlier than in 1997). In 1999, further improvements were made and the overall cost of leaf miner control was reduced from £2,663 per ha in 1998 to £1,473 per ha in 1999.

Spread of *L. bryoniae* from "hot-spots" of activity:

The crop was planted in week 1 and the first mines were observed in week 6. The numbers of new mines per plant and the timing of their spread from the "hot-spot" area correlated with the emergence of each new *L. bryoniae* generation. The second generation peaked in week 9 and then there was a decline in numbers until the third generation emerged four to five weeks later. When there were few mines, they occurred randomly within the monitored area, but they became more evenly spread when the third generation emerged.

Growers should expect the *L. bryoniae* population to become asynchronised and to spread throughout the glasshouse at the start of the third generation. This is a good time to start to release *D. isaea* because they have a continuous supply of prey. The simulation model should predict the timing of the third generation from glasshouse temperature and monitoring data, and recommend the optimum time to start the control programme.

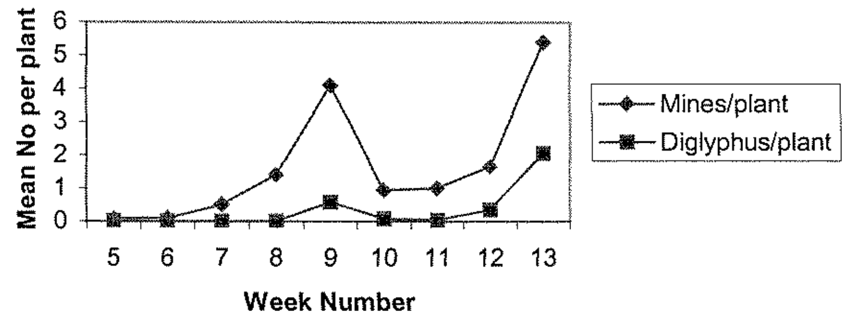
Although the first mines were found in the center of the glasshouse, as the population developed the numbers increased most rapidly around the paths and periphery of the glasshouse. The spread of *L. bryoniae* was also more rapid along rows than between rows. This may have been because these plants were more exposed to light, which increases *L. bryoniae* activity and egg laying (see Part 3). It is also probable that *L. bryoniae* use paths and rows as flight routes. This pattern was consistent with the population development observed in other glasshouses and at other nurseries. It follows that growers should monitor around paths and hot-spots in order to decide when to release the first *D. isaea*.

Diglyphus isaea establishment

To control *L. bryoniae* populations during the early season in round tomatoes, release of *D. isaea* began at thresholds ranging from 0.01 to 1.39 new mines per plant per week. The parasitoid was then released weekly until either *D. isaea* parasitism or host feeding had killed at least 50% of the leaf miner population. An average of 12,000 (range 500-35,250) *D. isaea* per ha was used at EVS with between one and eleven releases per glasshouse depending on the timing of outbreaks and numbers of *L. bryoniae*. However, no *D. isaea* parasitism was observed until mine numbers averaged 1.5 active mines per plant. In some glasshouses, *D. isaea* had been released for four weeks before there were sufficient mines for them to become established.

In the glasshouse with variable mine densities (EVS, Block 51), *D. isaea* was released weekly but failed to establish at the western end where mine numbers remained below one new mine per plant throughout February and March. In the eastern area, where there were more mines, numbers of parasitised mines closely followed numbers of new mines (Figure 7.3). However, parasitism levels declined when mine numbers fell below one new mine per plant between generations

Figure 7.3. The average number of new mines and mines parasitised by *Diglyphus isaea* per plant per week at EVS (Block 51)



Diglyphus isaea established at any time throughout the late winter and early spring if there were sufficient mines.

These observations are consistent with results of the host density experiments (see Part 4) and the large scale *D. isaea* release experiments (see "Control Strategies" below). They support the view that *D. isaea* releases are wasted at the start of the season and between synchronised generations when numbers of new mines are below one per plant per week. The failure of *D. isaea* to establish is due to their poor searching ability at low host densities and short longevity when they fail to find prey (see Part 4).

The host density threshold appears to be lower later in the season; for example at AVN in mid-April 1998, *D. isaea* established at 0.02 and 0.05 active mines per plant. This is probably due to improved searching ability at warmer temperatures and longer days. It is clear that a different action threshold is required for late season *L. bryoniae* infestations but this is beyond the scope of this project.

Control strategies

The impact of several parasitoid release strategies was monitored on known *L. bryoniae* populations in tomato crops at different nurseries and during different seasons. These data were used to verify and develop the simulation model (Part 1).

In the experiments carried out by the States of Jersey and BCP Ltd., an action threshold for *D. isaea* release was compared to weekly releases from planting. *Diglyphus isaea* did not establish until mine densities averaged 0.8 mines per plant. *Liriomyza bryoniae* populations peaked at a similar time and at similar numbers regardless of the release strategy. This indicated that pre-threshold releases of *D. isaea* were wasted. In 1997, when the same number of *D. isaea* were concentrated into three releases at weekly intervals rather than spread over eight weeks, the more concentrated releases reduced *L. bryoniae* numbers at the peak by approximately half (Sampson and Walker, 1998). This strategy relies on an accurate knowledge of mine numbers and should only be used by growers who are prepared to carefully monitor *L. bryoniae* populations.

Trials by BCP Ltd in other commercial crops of round tomatoes support the use of an action threshold of one mine per plant before starting to release *D. isaea* in the early season. However, there have been situations where endoparasitoids such as *Dacnusa sibirica* and *Opius pallipes* have made an important contribution to the overall control. These parasitoids can reduce the rate of increase of *L. bryoniae* numbers and help *D. isaea* to exert control before unacceptable crop damage occurs. Time of year and cultivar differences need to be considered in this respect.

OVERALL CONCLUSIONS

The following conclusions apply to round tomato crops that become infested by *L. bryoniae* that have survived from the previous crop.

Timing of Release

1. It has been shown that *D. isaea* will establish in tomato crops at any time of the year providing there is a sufficient and continuous supply of *L. bryoniae* larvae.

2. The approximate density of *L. bryoniae* larvae required for survival and reproduction of *D. isaea* is one per plant. *Diglyphus isaea* releases made at lower mine densities are wasted early in the season.

3. Early season *D. isaea* releases made between generations are wasted as adults need a continuous supply of *L. bryoniae* larvae to survive and reproduce (at least one larva is required per female every three days; see Part 4).

Release Strategy

Once there are sufficient mines for *D. isaea* establishment, *L. bryoniae* control is improved by concentrating releases of the parasitoid into three weeks rather than spreading them over six or more weeks.

Decision Making

1. The use of a release threshold depends on accurate monitoring of *L. bryoniae* populations and a good understanding of *L. bryoniae* population development. The growers who monitored *L. bryoniae* populations during the course of this project gradually improved their *L. bryoniae* control.

2. *Diglyphus isaea* will establish in hot-spots of *L. bryoniae* activity where the mine density exceeds one active mine per plant. Improved control can be achieved by releasing parasitoids into specific areas exceeding the establishment threshold even when the average number of mines throughout the whole glasshouse may be less than one per plant.

PART 8. DESIGN AND EVALUATION OF SAMPLING PROCEDURES

Objective

To design and evaluate a system for sampling *L. bryoniae* and *D. isaea* populations that may be used by non-specialist nursery staff.

Introduction

The success of the IPM programme and ultimately the use of the simulation model in commercial tomato crops will depend upon the accuracy of information collected by crop monitors. The monitoring system must provide a precise estimate of both *L. bryoniae* and *D. isaea* populations and be practical for use by non-specialist nursery staff. A suitable monitoring system was developed and evaluated within this project.

Materials and methods

In 1998, nine commercial tomato crops were monitored weekly. One hundred plants were monitored in each of ten rows per glasshouse, with half the rows located in areas where *L. bryoniae* had been most prevalent in the previous season and the other half spread evenly around the rest of the crop. As the *L. bryoniae* density increased to one mine per five plants, 60 individual plants, in ten lots of six, were marked per glasshouse. The numbers of plants monitored was further reduced to a minimum of ten plants as *L. bryoniae* numbers continued to increase. New mines were marked, counted and recorded each week.

At the end of the 1998 season, the data was collated, the sampling system was evaluated and the procedures were revised (see results section). The same glasshouses were monitored in 1999 using the revised system detailed below. To ensure that the system provided an accurate estimate of the insect populations, mine numbers were also counted.

The 1999 Monitoring Procedure

1. Within each glasshouse, 10 sampling areas, each of 100 plants, were chosen with half located in areas where *L. bryoniae* had been most prevalent in the previous season and the other half spread around the rest of the crop. As numbers of *L. bryoniae* can increase most rapidly at the ends of rows and next to glasshouse walls, such areas were included in the sampling procedure.

2. The presence or absence of new mines per area per week was recorded.

3. When eight of the sample areas were infested with *L. bryoniae*, the sample size was reduced from 100 plants to a group of 10 plants in each area.

4. The sampling process continued, recording the presence or absence of new mines per area per week.

5. When eight of the sample areas were infested with *L. bryoniae*, the sample size was reduced to two separate plants in each area (*i.e.* 20 individual plants in all).
6. The numbers of new mines per plant per week were recorded on the 20 individual plants.
7. When the number of new mines per week reached 20 per plant, the sample size was reduced to one plant in every other sampling area (*i.e.* 5 plants in total).
8. The numbers of new mines per plant per week were recorded on the 5 individual plants until numbers stopped increasing.

The *D. isaea* population was monitored in parallel to the leaf miners. This began one week after the first release of parasitoids. Each week, 50 mines were collected at random from the glasshouse. Only one mine was taken per plant and they were selected from around leaf position 12 to 14. The proportion of larvae killed by host feeding and parasitism were recorded.

Results and discussion

Results in 1998: The monitoring system provided a good estimate of *L. bryoniae* numbers and helped growers to decide when to release parasitoids but it was too time consuming. A revised system was designed.

Refinement of procedures for *L. bryoniae*: Table 8.1. shows how by changing the sample size the average number of new mines can be determined without counting anything but the number of clean plants. The argument is based on the Poisson distribution, and provides an effective estimate until the threshold of 1 new mine per plant is reached. As the population increases further, however, *L. bryoniae* spread throughout the glasshouses and the number of clean plants quickly diminishes. The last column shows that with a sample of 100 plants, while the average number of mines is around 1 per 100 plants, then a set of 10 samples should yield about four completely clean ones. One can then switch to samples of ten plants and simply by counting the number of clean samples out of ten we have a good estimate of the average number of mines while it is below 1 per 10 plants. Switching now to single plants, we have a good estimate of average number down to the threshold, since at the threshold level we would still expect approximately four 'randomly sampled plants' to be clean.

Results in 1999: EVS achieved control of *L. bryoniae* in all glasshouses without crop damage whilst reducing control costs by 44%. The improved control was attributed directly to improved monitoring which in turn aided decisions on when to release parasitoids. The monitoring was considered effective and practical; taking between 20 and 90 minutes per week per ha depending on plant size and *L. bryoniae* numbers. The leaf miner population reached the *D. isaea* release threshold before fruit picking began, so existing staff were able to do the monitoring and no extra labour costs were incurred. Further reductions in monitoring time may be expected as staff become more practised.

Table 8.1. Expected number of 'clean' samples (out of 10) for different damage levels using different sample sizes.

Sample size:		1 plant		10 plants		100 plants	
Mean new Mines per Plant	prob-ability	Prob(0)	Exp no of clean samples	prob(0)	Exp no Of clean Samples	prob(0)	Exp no of clean samples
1 per 100	0.01	0.990	10	0.904	9	0.366	4
1 per 50	0.02	0.980	10	0.817	8	0.133	1
1 per 20	0.05	0.951	10	0.599	6	0.006	0
1 per 10	0.10	0.905	9	0.349	3	0.000	0
1 in 5	0.20	0.819	8	0.107	1	0.000	0
1 in 2.5	0.40	0.670	7	0.006	0	0.000	0
1 in 2	0.50	0.607	6	0.001	0	0.000	0
1 in 1.5	0.67	0.512	5	0.000	0	0.000	0
1 in 1	1.00	0.368	4	0.000	0	0.000	0

OVERALL CONCLUSION

The monitoring system evaluated in 1999 (detailed above) proved to be effective and manageable by non-specialist nursery staff.

A SUMMARY OF THE KEY DEVELOPMENTS FROM THE PROJECT

The overall aim was to develop a control strategy for tomato leaf miner (*Liriomyza bryoniae*) that was compatible with the rest of the tomato IPM programme, reliable throughout the season and manageable by non-specialist staff. The studies focussed on the use of the parasitic wasp, *Diglyphus isaea*, and the parasitic nematode, *Steinernema feltiae*, for the control of leaf miners that survive in glasshouses and infest classic round tomato crops soon after planting.

Liriomyza bryoniae-Diglyphus isaea simulation model.

- A computerised mathematical model was developed to simulate the interactions between *L. bryoniae* and *D. isaea* under variable environmental conditions.

- The model manager incorporates pest monitoring information (*i.e.* numbers of mines and the proportion parasitised) and the model then takes into account pest and parasitoid performance under the current environmental conditions and provides a prediction of the population development of both species. The output can be viewed graphically and the user can introduce variables, such as additional releases of parasitoids, to explore the best and worst scenarios.

- Comparisons with data collected by crop monitoring demonstrated that the output of the model was reasonably realistic.

- The model has been a very important tool in understanding the dynamics of the *L. bryoniae-D. isaea* system. It highlighted key areas within the system where further experimentation was necessary; *e.g.* searching behaviour and activity patterns of *D. isaea*, activity patterns of *L. bryoniae* and effect of environmental factors other than temperature on both species.

Liriomyza bryoniae survival between crops and crop invasion

- No diapause was detected in individuals from populations that had been reared for two generations under short days in tomato growing conditions.

- Pupal development and adult emergence time was temperature dependent. *Liriomyza bryoniae* survived in cold glasshouses between tomato crops as pupae in a quiescent state. Their development resumed upon the return of normal tomato production temperatures and all adults emerged within three to four weeks.

- The experimental data were confirmed by monitoring the appearance of *L.bryoniae* in commercial crops.

- Short day length triggered the production of a greater proportion of dark coloured pupae but there was no evidence to suggest that pupal colour was associated with a delayed development time under the test conditions. However, it was speculated that dark pupae have a thicker cuticle, which may be an adaptation to aid survival by preventing desiccation during prolonged periods of adverse conditions.

Biology of *Liriomyza bryoniae*

- The development time for *L. bryoniae* used in these experiments was consistent with data published by other scientists, which provided confidence to collate the data for incorporation in to the simulation model.

- There was no evidence to suggest that leaf miner development time was disproportionately affected by fluctuating temperature as may be experienced in tomato crops during April and May. Mean daily temperatures were therefore used to predict development in the simulation model.

- Leaf temperature was slightly lower than air temperature when the plants had an unrestricted water supply and there was no evidence to suggest that temperature regulation was impaired in leaves that were damaged by leaf miners. It was therefore concluded that measurements based on air temperature were acceptable for use in the simulation model. The situation was different when the water supply to the plants was switched off temporarily; the leaves then appeared to be unable to regulate their temperature and became increasingly warmer than the air.

- *Liriomyza bryoniae* females fed more actively and laid more eggs under conditions of greater light intensity or duration; *e.g.* three times more eggs per day under bright conditions in May compared to dull conditions in March. This information helped to explain why the control system became unstable between April and June and indicated that a correction factor should be incorporated into the simulation model.

- Preliminary experiments suggested that some tomato types and cultivars were more susceptible to damage than others (*e.g. L. bryoniae* laid more eggs in cherry tomato leaves than round tomato leaves) but this could not be further investigated in this project. A correction factor would probably be required if the simulation model was extended to include tomato types other than the classic round cultivars.

- There were indications that plant condition affected *L. bryoniae* fecundity but this could not be further investigated with the resources available in this project.

Biology of *Diglyphus isaea*

- The results demonstrated a host density dependence on the performance of *D. isaea* that had not been previously proven.

- The poor performance of *D. isaea* at low *L. bryoniae* densities was found to be the result of both the biology and searching behaviour of the parasitoid.

- The parasitoid required a regular feed (*i.e.* at least one *L. bryoniae* larva every third day) to produce eggs and therefore maintain a population in a tomato crop. The reproductive potential of *D. isaea* increased at higher *L. bryoniae* densities.

- The longevity of adult *D. isaea* was reduced at low *L. bryoniae* densities, which limited the time that was available to find a host.

- *Diglyphus isaea* were unable to locate *L. bryoniae* larvae when the latter were present at low densities in the early part of the season.

- Female *D. isaea* were not specifically attracted to tomato plants with low leaf miner densities. Furthermore, the time the parasitoids spent on the plants and the time they spent searching individual leaflets decreased with low *L. bryoniae* density. The time required to search the equivalent of a plant with low density of leaf miners in a mature tomato crop was estimated to be between 0.4 and 2.4 days. The new data were incorporated in the simulation model.

Impact of *Liriomyza bryoniae* damage on tomato yield

- The results of both simulated and real herbivory experiments indicated that classic round tomato cultivars could tolerate substantial damage by *L. bryoniae* without suffering loss of yield or fruit quality. As a general guide, it should not be necessary to resort to remedial action against leaf miners until there is over 35% loss of photosynthetic leaf area in a stratum of approximately six leaves.

IPM compatible remedial treatment

- Under suitable conditions, *S. feltiae* (Nemasys), provided a significant reduction in numbers of *L. bryoniae* larvae in tomato leaves without disrupting an existing population of *D. isaea*.

- A clay-based formulation of *S. feltiae* was developed specifically for foliar application to tomato plants. This is now marketed as Nemasys®T.

- After spraying, it was important that leaves remained moist long enough for nematodes to locate and enter mines. The actual time that the leaves had to remain moist depended on the leaf miner population density. It was shorter at higher densities because there were more entry points.

- It was more difficult to achieve good results in commercial crops than experimental crops but at least 50% of leaf miner larvae were killed where there were sufficient entry points on the leaves. The latter appeared to occur when there was over 20-30% loss of leaf area due to *L. bryoniae* activity. This is consistent with their use as a second line of defence.

- A 50% reduction of numbers of *L. bryoniae* larvae was sufficient to slow down the growth of the population and allow *D. isaea* to gain control.

Sampling procedures

- A monitoring system was developed for commercial crops that proved to be effective and manageable by non-specialist nursery staff.

Evaluation of control strategies

- *Diglyphus isaea* established in tomato crops at any time of the year providing there was a sufficient and continuous supply of *L. bryoniae* larvae.

- In the early season, the approximate host density required for *D. isaea* establishment was one *L. bryoniae* larva per plant. This was broadly consistent with the results of the small scale experiments summarised above. *Diglyphus isaea* released at mine densities below this "threshold" in the early season probably died before laying eggs and therefore were wasted.

- Early season *D. isaea* releases between generations were wasted as adults need a continuous supply of *L. bryoniae* larvae to survive and reproduce.

- *Diglyphus isaea* established in hot-spots of *L. bryoniae* activity where the mine density exceeded one active mine per plant even when the overall average in the crop was below the threshold.

- Once there were sufficient mines for *D. isaea* establishment, *L. bryoniae* control was improved by concentrating releases of the parasitoid into three weeks rather than spreading them over six or more weeks.

- The use of the release threshold depended on accurate monitoring of *L. bryoniae* populations and a good understanding of *L. bryoniae* population development.

- Growers who adopted the new monitoring procedures and control strategies increased the effectiveness and reduced the cost of control programmes based on *D. isaea*.

RECOMMENDATIONS FOR FURTHER RESEARCH

Specific tomato/leaf miner research

- Modification of the control strategy for other "types" of tomato, particularly those which are considered to be more susceptible to leaf miners (eg cherry tomatoes).
- Modification of the control strategy for situations where there is only a very small carry-over of leaf miners from the previous crop.
- Modification of the control strategy to combat sudden large mid-season invasions of leaf miners.

These topics were considered suitable for Industry/HDC funding and will be brought to the attention of the TGA and HDC Protected Crops Panel.

Broader issues

The consortium agreed that many of the questions raised by the studies in this project were common to the improved understanding of tritrophic interactions between other annual crops, their pests and natural enemies, and therefore should be addressed in a broader context. The influencing factors were considered to be wide ranging, encompassing aspects of plant architecture, plant toxins, plant nutrition, environmental conditions and microclimate of the plant surface, and therefore should be studied by multidisciplinary teams. The following subjects were given highest priority:

- The measurement and definition of the microclimates of boundary layers on plant and insect surfaces.
- The relationship between the nutritional status of the plant and the performance of herbivorous invertebrates.
- Physiological responses of plants to invertebrate herbivory and the subsequent impact on yield.

These topics have a strong strategic element that was considered suitable for MAFF funding and they will be brought to the attention of MAFF's Chief Scientist's Group.

TECHNOLOGY TRANSFER

Publications

- Jacobson, R. J. (1997). Integrated pest management of leaf miners in tomatoes. *HDC Project News*, 42, 1-3.
- Jacobson, R. J. (1998). Integrated pest management of leaf miners in tomatoes. *Project Information Leaflet (MAFF, LINK - Technologies for sustainable farming systems)*, PB1788. 2 pp.
- Jacobson, R. J. (1998). Improving leaf miner control in tomato crops. *Agriculture LINK (July 1998)*, 1(1), 4.
- Sampson, C. & Walker, P. (1998). Improved control of *Liriomyza bryoniaae* using an action threshold for the release of *Diglyphus isaea* in protected tomato crops. *Med. Fac. Landbouww. Univ. Gent,* 63(2b), 415-422.
- Jacobson, R. J. (2000). Early season control of tomato leaf miner. *HDC Fact Sheet*, 8/00, 4pp (In Press)

Presentations

- Jacobson, R. J. & Croft, P. (1996). Update on research related to pests in tomatoes. Tomato Growers Crop Study Day. HRI, Stockbridge House. 18 July 1996.
- Jacobson, R. J. (1996). Update on research related to pests in tomatoes. Tomato Growers Crop Study Day. HRI, Efford. 10 September 1996.
- Jacobson, R. J. (1996). Weak links in tomato IPM. Published in: *Abstracts, HDC/ADAS/HRI National Tomato Conference,* Wellesbourne, 1 October, 1996 (ed K. E. Cockshull), 7.
- Jacobson, R. J. (1996). HRI Research Programme on Pests of Tomatoes. Presentation to VHB Directors and Senior Managers. HRI, Wellesbourne. 12 December 1996.
- Jacobson, R. J. & Sampson, C. (1997). Improving Leaf Miner Control in Tomato crops. Published in: *Abstracts, HDC/HRA 1997 Tomato Conference,* Wellesbourne, 2 October, 1997 (ed K. E. Cockshull), 4.
- Jacobson, R. J., Sampson, C. & Croft, P. (1998). A summary of the HRI leaf miner research programme. Presentation to Northern Tomato Growers Group. HRI, Stockbridge House. 16 July 1998.
- Jacobson, R. J. & Sampson, C. (1999). Controlling leaf miners without chemicals. *In Abstracts, Tomato Conference*, Coventry, England, 7 October 1999 (Ed. F. Sheppard), 26-28.

Project Management Meetings

14 May 1996
22 October 1996
14 April 1997

7 October 1997
11 March 1998
13 October 1998

29 January 1999 – Interim meeting
14 April 1999 – Interim meeting
16 September 1999

REFERENCES

Boot, B J; Minkenberg, O P J M; Rabbinge, R; De Moed, G H. 1992, Biological control of the leafminer, *Liriomyza bryoniae* by seasonal inoculative releases of *Diglyphus isaea*: simulation of a parasitoid-host system. Netherlands Journal of Plant Pathology. 98: 203-212.

Cheah, C.A. and Coaker, T.H. 1992. Host finding and discrimination *in Diglyphus isaea*, a parasitoid of the chrysanthemum leaf miner *Chromatomyia syngenesiae*. Biocontrol Science and technology. (2): 47-51.

Coaker, T.H. and Cheah, C.A. 1993. Conditioning as a factor in parasitoid host plant preference. Biocontrol Science and Technology (3): 277-283.

Helyer, L and Ledieu, M. 1990. The seasonal variability in tomato leaf miner (*Liriomyza bryoniae* (Kalenbach) life cycle. *IOBC BullETIN* 13(5): 83-86.

Heinz K. M., Nunney L. & Parrella M. P. 1993 Toward predictable biological control of *Liriomyza trifolii* (Diptera: Agromyzidae) infesting greenhouse cut chrysanthemums. *Environmental Entomology* 22(6): 1217-1233.

Ho, L. C. (1996). Tomato, pp. 709-717. *In* Zamaski, E. & Schaffer, A. A. (eds.) *Photoassimilate Distribution in Plants and Crops*, Marcel Dekker Inc., New York.

Ho, L. C. & Hewitt, J. D. (1996). Fruit development, pp. 201-239. *In* Atherton, J. G. & Rudich, J. (eds.) *The Tomato Crop*. Chapman and Hall, Bury St Edmunds, United Kingdom.

Kaya, H. K. (1986). *Steinernema feltiae*: use against foliage feeding insects and effect on non-target insects. In *Fundamental and Applied Aspects of Invertebrate Pathology. Proceedings of the fourth International Colloquium of Invertebrate Pathology* (Samson, R. A., *et al.*, Eds) Wageningen, The Netherlands. pp 268-270.

Le Dieu, M. S. and Helyer, N. L.,+ 1985. Observations on the economic importance of tomato leaf miner. Agriculture, Ecosystems and Environment, 13: 103-109.

Linquist, R.K., 1974. Effects of leaf miner larvae on yields of greenhouse tomatoes. Ohio Agricultural Research and Development Centre Research Summary, 73: 25-29.

Minkenberg O. P. J. M. 1989 Temperature effects on the life history of the eulophid wasp *Diglyphus isaea*, an ectoparasitoid of leafminers (*Liriomyza* spp.), on tomatoes. *Annals of Applied Biology* 115: 381-397.

Minkenberg, O.P.J.M. 1990. On seasonal inoculative biological control. PhD thesis. Landbouw Universiteit te Wageningen. 230 pages.

Minkenberg O. P. J. M. & van Lenteren J. C. 1986 The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. *Agricultural University Wageningen Papers* 86(2): 1-50.

Minkenberg, O. P. J. M. and Helderman, C. A. J. 1990. Effects of temperature on the life history of *Liriomyza bryoniae* (Diptera: Agromyzidae) on tomato. *Journal of Economic Entomology* 38: 117-125.

Minkenberg, O.P.J.M and Ottenheim, J.J.G.W. 1990. Effect of leaf nitrogen content of tomato plants on preference and performance of a leafmining fly. *Oecologia* 83: 291-298.

Nedstam, B. 1985. Development time of *Liriomyza bryoniae* kalt. (Diptera: Agromyzidae) and two of its natural enemies, *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) and *Cyrtogaster vulgaris* Walker (Hymenoptera: Pteromalidae) at different constant temperatures. Med. Fac. Landbouww. Univ. Gent. 50: 411-417.

Parella M. P. 1984 Effect of temperature on oviposition, feeding and longevity of *Liriomyza trifolii* (Diptera: Agromyzidae). *Canadian Entomologist* 116: 85-92.

Parella M. P. 1987 Biology of *Liriomyza*. *Annual Review of Entomology* 32: 201-224.

Patel K. J. & Schuster D. J. 1983 Influence of temperature on the rate of development of *Diglyphus intermedius* (Hymenoptera: Eulophidae) Girault, a parasite of *Liriomyza* spp. (Diptera: Agromyzidae).

Sampson, A C and Walker, P. 1998. Improved control of *Liriomyza bryoniae* using an action threshold for the release of *Diglyphus isaea* in protected tomato crops. Med. Fac. Landbouww. Univ. Gent. 63/2b: 415-422.

Stacey, D.L., 1983. The effect of artificial defoliation on the yield of tomato plants and its relevance to pest damage. Journal of Horticultural Science, 58: 117-120.

Sher, R. B. & Parella, M. P. (1996). Integrated biological control of leaf miners, *Liriomyza trifolii*, on greenhouse chrysanthemums. *IOBC Bulletin* 19(1): 147-150.

Sher, R. B. & Parella, M. P. (1999). Biological control of the leaf miner, *Liriomyza trifolii*, in chrysanthemums: Implications for intraguild predation between *Diglyphus begini* and *Steinernema carpocapsae*. *IOBC Bulletin* 22(1): 221-224.

Valerie, F.L., Bagneres, A.G. and Clement J.L. 1996. Role of plant volatiles in the search for a host by parasitoid *D. isaea* (Hymenoptera: Eulophidae). Journal of Chemical Ecology (22): 541-558.

Williams, E. C. and O. C. Macdonald. 1995. Critical factors required by the nematode *Steinernema feltiae* for the control of the leaf miners *Liriomyza huidobrensis*, *Liriomyza bryoniae* and *Chromatomyia syngenesiae*. *Annals of Applied Biology* 127: 329-341.

APPENDIX:
DESCRIPTION OF THE *LIRIOMYZA BRYONIAE-DIGLYPHUS ISAEA* SIMULATION MODEL

History of the model

The model initially began as an empirical model, consisting of a set of equations, which aimed to calculate the number of *Liriomyza bryoniae* (Leafminer) and *Diglyphus isaea* (parasite) in the next generation. It was intended as a simple aid to decision making that could be performed every week, after estimates of insect abundance, through monitoring, had been made. This model included many correction factors, and it was felt that its accuracy could be improved. The decision was therefore taken to move to a more biologically based simulation model.

The initial simulation model was a deterministic (no randomness included) model. The model simulated the basic biology of both the leafminer and the parasite, using data that had been collected as part of the project and from the literature (Heinz *et al.*, 1993; Minkenberg, 1989, 1990;Minkenberg & Helderman, 1990; Minkenberg & van Lenteren, 1986; Parella, 1984,1987; Patel & Schuster, 1983). The interaction between the leafminer and parasite, in terms of host feeding and parasitism, was modelled using data from the experiments done during the project, and the equation of Boot *et al.* (1992). The aim of this model was to give the model a firm biological basis and to also try to use the model to provide predictions that could be used to determine strategies for the introduction of *Diglyphus isaea*, which would provide control of *L. bryoniae*. The predictions from this model were compared with crop monitoring data, and there was a lack of fit. Although the model was able to simulate the peaks and troughs of the *L. bryoniae* population reasonably well, the timing of the peaks did not coincide with the observed data.

Several reasons were proposed for this lack of fit, and the model was amended appropriately in the light of new experimental data. However, the fit was still not acceptable for the accurate predictions needed for the development of robust control strategies. At this point it was decided to try and incorporate some of the variability of the natural populations into the model. This necessitated the development of a stochastic model, which also altered the emphasis of the model. By moving to an individual-based stochastic model, the model became a research tool aimed at understanding the dynamics of the leafminer-parasite system, rather than a decision-support model. There are two main reasons for this: stochastic models produce a range of predictions, rather than a single prediction. Therefore comparison with observed data is far more difficult, as one realisation of the model may provide a good fit, but another may not, due to the randomness included into the model. Secondly, the time required to run an individual-based model is much greater than that for a population–based model, as changes have to be made to each individual separately. Hence, the usefulness of the model for providing quick predictions based on monitoring data is severely reduced. However, the individual-based model has enabled key areas of the biology of the system where information is lacking, such as mid-season immigration of *L. bryoniae* and the searching behaviour of *D. isaea* to be identified.

Description of the inidividual-based stochastic simulation model

The model was written in FORTRAN and will run either on a PC or DEC Alpha Workstation. Information about the spatial dynamics of the system was not included in the model, which simulates a maximum of 500000 individuals, in each instar, of either *L. bryoniae* or *D. isaea*. A time step of one day was assumed in the model.

Initialisation of the model

The model is initialised using data that is either input from file or manually by the user of the model. The data required by the model are: weekly mean temperatures in the glasshouse, the number of plants per hectare in the glasshouse (*Ph*), the size of the glasshouse in hectares (*G*), the number of new *L. bryoniae* mines counted during monitoring (M_c), the sample size (number of plants counted) for the number of mines counted (S_m), the proportion of the counted mines that were parasitised (P_p) and the number of days to be simulated. Once these have been input into the model, the number of parasitised and unparasitised mines in the whole glasshouse are calculated using the following equations:

$$M_u = \left\{ \frac{M_c \times (1 - P_p)}{S_m} \right\} \times Ph \times G \qquad (1)$$

$$M_p = 1 - M_u \qquad (2)$$

where M_u is the number of mines not parasitised and M_p is the number of mines that are parasitised.

When the numbers of parasitised and unparasitised *L. bryoniae* has been calculated, they are assigned to age classes based on a stable-age distribution, which was simulated by running the model. The proportion of *L. bryoniae* in each age class are shown in the table below, the proportions reflect the fact that the number of *L. bryoniae* mines counted only includes the three active larval instars.

Stage	Egg	1st Instar	2nd Instar	3rd Instar	Pupae
Proportion	0.4	0.2	0.4	0.4	1.8

The number of adult *L. bryoniae* to be simulated at the start of the model is then estimated by dividing the number of unparasitised mines counted, by the number of eggs laid per day by an adult female *L. bryoniae*. The number of eggs laid is calculated using the following equation, derived from regression equations fitted to a combined dataset of experimental and published data (Minkenberg 1990).

$$E = [1.66 \times T] - 18.3 \qquad (3)$$

where *E* is the number of eggs laid by a single female per day and *T* is the temperature (°C).

If some of the mines counted were parasitised, the model then assigns the *D. isaea* to instars, based on the assumption that 50% were juveniles and 50% being pupae. The number of parasite adults is assumed to be 4% of the number of parasitised mines, based on a simulated stable-age distribution.

Development and survival

L. bryoniae

The development of *L. bryoniae* is simulated using a modified box-car train method (Goudriaan & DeWit 1978). During initialisation, the total longevity of all stages is determined for each leafminer, by sampling a normal distribution. The mean of the normal distribution is calculated as the inverse of an equation relating the temperature to the development rate of the stage. The standard deviations used when sampling the normal distribution were calculated from published data (Minkenberg, 1990).

The equation describing the relationship between temperature and the longevity of each stage is:

$$D = \frac{1}{a + (b \times T)} \qquad (4)$$

where D is the longevity, T is temperature (°C), a and b are constants.

For the pre-reproductive period and post-oviposition period of the adult, the following equation was used. The inverse is not required, since the equation was fitted to actual times as opposed to rates, as in equation (4)

$$D = a + (b \times T) \qquad (5)$$

where D, T, a and b are as previously defined.

The values of a and b plus the standard deviations used for each developmental stage are shown in the following table. The values were calculated using linear regression lines fitted to a combined dataset of experimental data from the project and published data (Minkenberg, 1990).

	Stage	a	b	Standard Deviation
	Egg	-0.094	0.017	0.24
	1st Instar	-0.58	0.050	0.48
	2nd Instar	-0.069	0.023	0.54
	3rd Instar	-0.36	0.038	0.61
	Pupa	-0.052	0.0064	0.64
	Total Longevity	-0.044	0.0078	4.5
Adult	Pre-reproductive	4.4	-0.14	1.0
	Post oviposition	1.0	0.0	1.8

The age of each individual within a stage is incremented by one day during each time step. If the age of the individual is greater than its longevity then it is assumed to move into the next instar (and a new longevity sampled), unless it is an adult, in which case it dies.

The survival of each stage is set as a constant, as shown in the table below, the values being estimated from a combined dataset of experimental data from the project and published data (Minkenberg 1990). For each individual a uniform (0,1) distribution is sampled, and this sampled value compared with the survival constant. If the sampled value is greater than the constant, the individual dies.

Stage	Egg	1st Instar	2nd Instar	3rd Instar	Pupa	Adult
Survival Probability	0.79	0.78	0.94	0.98	0.96	1.0

D. isaea

The development and survival of *D. isaea* is simulated using the same methodology as for *L. bryoniae*, the only differences being a lack of post-ovipositional longevity for adult *D. isaea,* and the need for 2 sets of parameters for equations 4 and 5 to describe the longevity and reproductive period, respectively, of the adults. The first set of parameters is applicable only at temperatures below 20°C, whilst the second set of parameters apply when the temperature is above 20°C.

The values of *a, b,* the standard deviation and the probability of survival for each stage, calculated from a combined dataset of experimental data from the project and published data (Minkenberg 1990) are shown in the table below.

Stage			*a*	*b*	Standard deviation	Probability of survival
Juveniles			-0.12	0.013	0.41	0.93
Pupae			-0.078	0.011	0.25	0.99
Adults	Longevity	Below 20°C	0.08	-0.0024	5.1	1.0
		Above 20°C	-0.24	0.014		
	Pre-reproductive Period	Below 20°C	-1.0	0.16	0.38	
		Above 20°C	6.6	-0.22		

Reproduction

L. bryoniae

Adult leafminer are assumed to reproduce if their age is greater than their pre-reproductive period and less than the beginning of the post-oviposition period. The number of eggs laid by each individual is sampled form a normal distribution with a temperature- and age-dependent mean and a standard deviation of 7.2. The equations for relating egg production per individual to temperature, based on the data of Minkenberg (1990) are given below.

$$r = 3T - 33.0 \qquad \{4.0 < A \leq 7.0\} \tag{6}$$

$$r = (T - 11.0) \times \left[\frac{19.0 - A}{4.0}\right] \qquad \{7.0 < A \leq 19.0\} \tag{7}$$

$$r = (T - 11.0) \times (A - 1.0) \qquad \{A < 4.0 \text{ or } 19.0 < A\} \tag{8}$$

where r is the number of eggs laid per day by an individual, T is temperature (°C) and A is the age of the adult *L. bryoniae*. These equations account for the decline in egg laying with age, as observed by Minkenberg (1990).

D. isaea

Since the reproduction of *D. isaea* is equivalent to parasitism of the leafminer, its reproduction is dealt with in the section on parasitism.

Host Feeding by *D. isaea*

The number of eggs laid per day by a single *D. isaea* female is calculated according to the temperature using equation 5 with values of $a = 3.4$ and $b = 0.62$. These values are derived from a linear regression of equation 5 to the data of Minkenberg (1990). If the number of mines is greater than a threshold of one mine per plant then host feeding occurs. Each individual *D. isaea* is assigned a rate of host feeding sampled from a normal distribution with a mean based on the number of eggs that can be laid and a standard deviation of 1.97. This rate is then halved due to experimental data suggesting that host feeding occurs at half the rate of parasitism.

The number of prey available to be fed upon is adjusted from the number oflarvae in instars one to three per plant to take account of the experimental data showing the effect of prey density on the number of plants searched, and also the daylength. At densities below 100 mines per plant, the parasites are assumed to search 36 plants, whilst at densities below 500 mines per plant, but greater than 100 mines they are assumed to search 12 plants. At higher densities, the parasites are assumed to be able to search 6 plants per day. Daylength is accounted for by multiplying the number of mines available by the number of hours of light divided by 24.

The number of prey that are killed by hostfeeding is then calculated using the following equation:

$$N_a = \left\{ \frac{P \times A}{P + A} \right\} \times 0.25 \qquad (9)$$

where N_a is the number of prey fed upon, P is the number of prey available and A is the rate of hostfeeding. The constant takes account of the sex ratio and probability of successful host feeding of *D. isaea*.

The number of mines fed upon in each *L. bryoniae* larval instar is calculated according to the ratio of the number of mines in each instar. The number of *L. bryoniae* larvae is updated appropriately.

Parasitism

Parasitism is assumed to only occur on 1st and 2nd instar *L. bryoniae* larvae. The number of larvae available for parasitism is adjusted from the number per plant as describe in the section on host feeding to account for the number of plants searched at a particular daylength. The attack rate of the *D. isaea* is sampled from a normal distribution with a mean based on the calculated maximum rate of egg laying, according to the temperature, and a standard deviation of 3.4. The number of leafminer larvae parasitised is then calculated using a modified version of equation 9, where the constant is omitted.

The total number of mines parasitised is then calculated by summation, and halved to account for the sex ratio of the *D. isaea*. The number of mines parasitised in each instar is calculated according to the ratio of the total numbers in the two instars. The number of *D. isaea* eggs and *L. bryoniae* larvae are updated appropriately, with longevities assigned to the *D. isaea* eggs as described earlier.

Releases of *D. isaea* during the simulated growing season

The number and timing of releases of *D. isaea* have to be coded into the program manually. The week number of the simulation is calculated from the day number (time step), and if a release is necessary, the appropriate number of *D. isaea* to be released are added into the simulation. The longevities and reproductive rates are determined as described earlier.

Output

Several types of output are possible from the model. The numbers of *L. bryoniae* and *D. isaea* in each instar are output on each time step (day). For comparison with historical data, the number of new mines per week is calculated using a variable that is set to zero at the beginning of a new week, and to which all the new mines produced during the next seven time steps are added. This variable is then output to file every seven time steps along with the week number and temperature for use with Microsoft Excel™.

Comparison of model output with historical monitoring data.

Due to the stochastic nature of the model, 30 runs of the model, with the same conditions, were required to assess the possible outcomes predicted by the model. Obviously, presenting 30 graphs showing the comparison with historical monitoring data would not be sensible. The graphs below show selected worst, best and average case scenarios predicted by the stochastic model. To see how the predictions of the stochastic model compare with those of the determinisitic model, the predicted output of the deterministic model is included on the graphs.

Two data sets were used for the comparison. The first was that obtained from Block 8 of Areton Valley Nurseries in 1998. Block 8 was 0.28 hectares with a crop density of 29000 plants per hectare. The variety of tomato was Espero, which is a standard round tomato. The number and timing of *D. isaea* introduced into block 8 are shown in the table below.

Week Number	20	21	22	23	24	26
Number of *D. isaea* introduced (per hectare)	750	2250	1500	1500	1500	10000

The second data set came from English Village Salads Block 52 in 1998. The block size was one hectare. The crop density was 18000 plant per hectare at planting, which was increased to 28000 per hectare in week nine. The variety of tomato was Aranca, which is intermediate between a standard round and a cherry variety. The number of *D. isaea* introduced into the block were 1250/ha in week 12, 2500/ha in each of weeks 13 to 19 and 1750/ha in week 20.

The comparison of the model output with the counted new mines per plant per week for EVS block 52 is shown in Figure 1, whilst that for AVN block 8 is shown in Figure 2. For EVS block 52 it is clear that the determinisitic model predicted the timing of the leafminer population peak well, but it overpredicted the numbers of new mines quite markedly, and also predicted an earlier peak which was not reflected in the counts of new mines per plant per week obtained through crop monitoring. The stochastic model provides a much better fit to the monitoring data in terms of numbers and timing up to week 15, at which point it predicted control of the population. As for which of these models provides the best fit, it is dependent on the criteria used to define a good fit. In terms of timing and numbers, the stochastic model is best up to week 15, and there are several possible reasons for the diversion from the monitoring data at this time. The first reason is that there could have been a mid-season invasion of the crop by adult *L. bryoniae*, which neither of the models are programmed to cope with. It is not known whether a mid-season invasion occurred as the monitoring data only counts the number of new mines, and not the number of adults in the crop. Data on the number of adult *L. bryoniae* caught on sticky traps, which could show whether an invasion of adult *L. bryoniae* occurred during this period, is not available. It is also possible that the monitoring data overestimated the number of new mines due to empty mines being counted. The other possibility is that the model simply over predicted the number or parasitisation rate of *D. isaea* which led to greater control than actually occurred, and further information about the searching behaviour of *D. isaea* should alleviate this problem.

The fit of both models to the AVN block 8 is more difficult to judge as neither model manages to predict the large increase in numbers after week 27. The determinisitic model provides a better prediction of the timing of the peak, although it is overpredicting the numbers of new mines up to week 27. The stochastic model provides a much better numerical fit to the observed data up to week 27, at which the number of new mines predicted remains low. This could be due to the reasons suggested above for EVS block 52.

The fact that the stochastic model provides a good numerical fit to two independent sets of data until later in the season suggests that something is happening in the mid season which is not accounted for in the model. It therefore indicates that further information about the mid-season dynamics of the *L. bryoniae* – *D. isaea* interaction is required to improve the model.

It should be remembered that the model is only as accurate as the data with which it is provided, and that the monitoring data (collected before the revised monitoring scheme was developed) may well underestimate the population of mines in the glasshouse early in the season. Monitoring may also overestimate the numbers later in the season, due to empty mines being included in the counts of new mines. The monitoring data were often considered to be an exact reflection of the number of *L. bryoniae* in the crop during the season, when in fact it would have been subject to error. Therefore a lack of fit of the model to the monitoring data does not necessarily mean that the model is not accurate, it may be due to errors made during the monitoring process.

The model is unable to take account of any immigration of *L. bryoniae* into the glasshouse during the growing season, and hence it is difficult for the model predictions to provide a good match to the observed mine numbers. However, it is felt that by building the model using established data and underlying biological principles, it does represent the biology of the system in the most realistic manner. The only caveat being that, as mentioned in the main report, further information is required to enable the model to represent fully the *L. bryoniae* and *D. isaea* system dynamics.

Dr D Skirvin
Author of Appendix

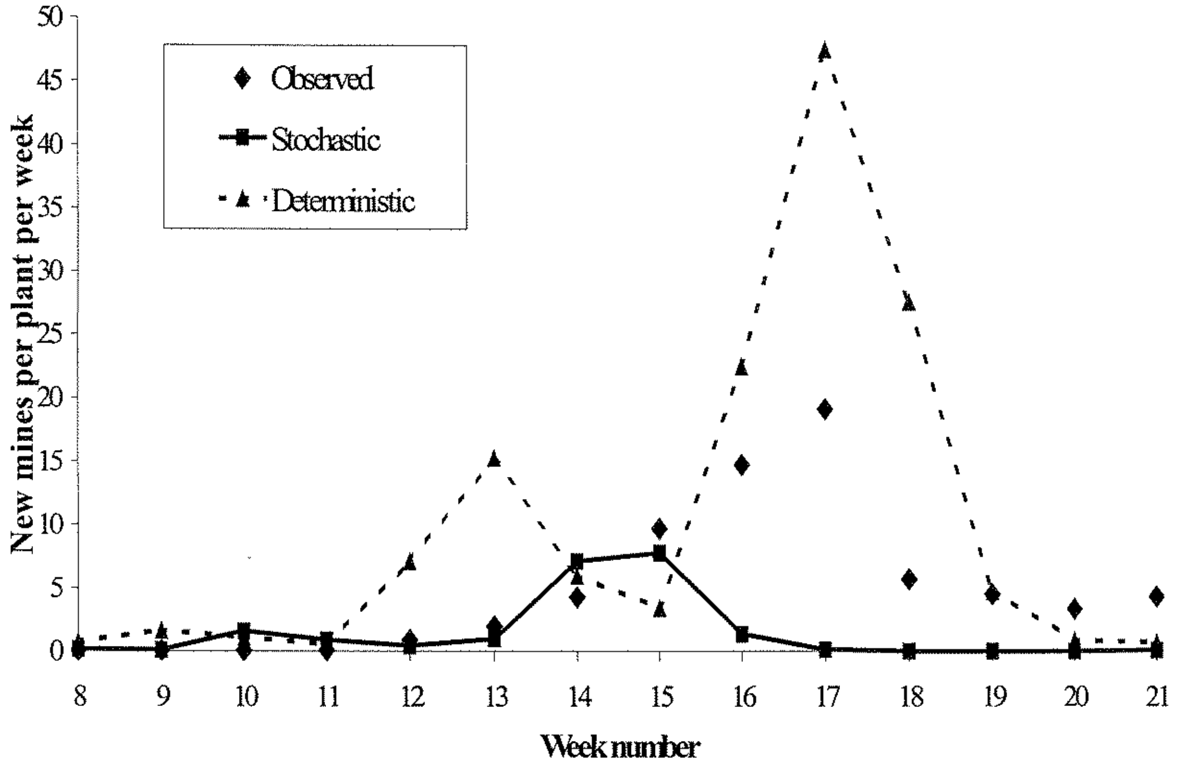


Figure 1: The number of new mines per plant per week observed and predicted for EVS52 in 1998.

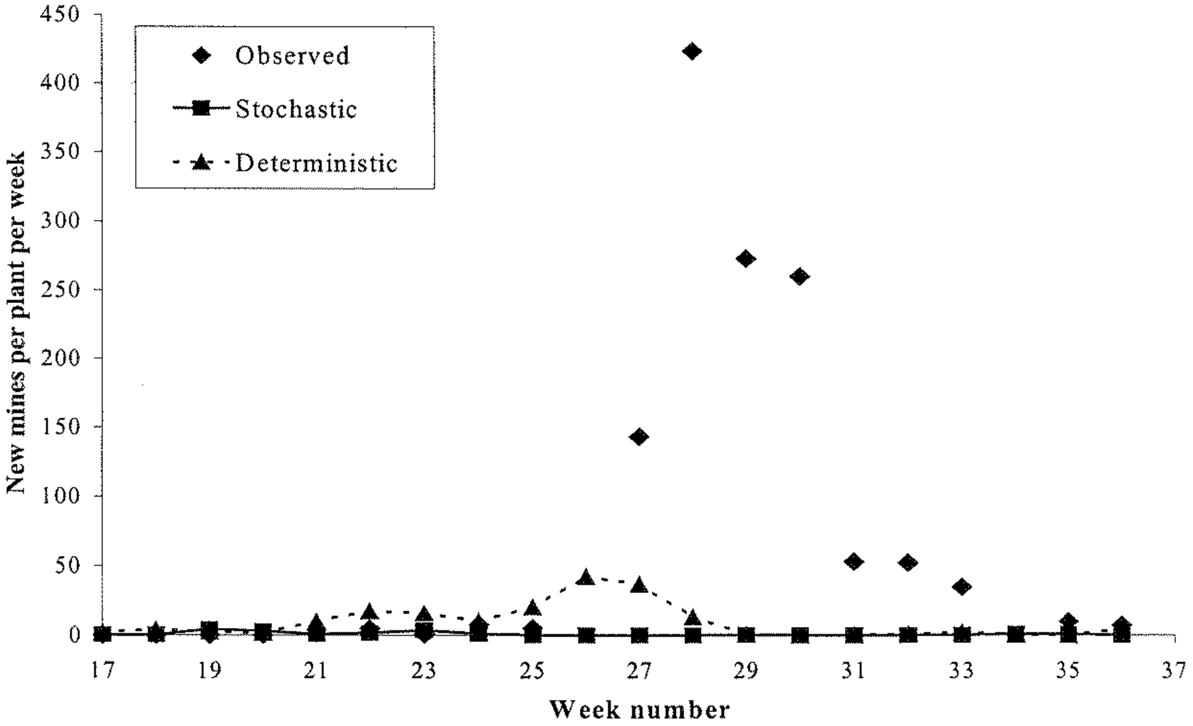


Figure 2: The number of new mines per plant per week observed and predicted for AVN8 in 1998.

of new mines per plant per week observed and predicted for AVN8 in 1998