Project Title	HNS: further evaluation of *Trichogramma brassicae* parasitoids on a commercial scale for long term biological control of carnation tortrix.
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Project leader:	John Buxton, ADAS
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Key words:	Light brown apple moth, Carnation tortrix moth, biological control, *Trichogramma* parasites, hardy nursery stock.

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Grower Summary

Headline

Weekly introductions of *Trichogramma brassicae* egg parasitoids to mixed HNS crops in a polythene tunnel gave good control of a natural infestation of the light brown apple moth, *Epiphyas postvittana*, on *Chaenomoles.* Two supplementary sprays of the IPM compatible insecticide *Bacillus thuringiensis* (Dipel DF) were needed to gain complete control.

Background and expected deliverables

- Carnation tortrix moth (*Cacoecimorpha pronubana*) and light brown apple moth (*Epiphyas postvittana*) are widespread pests of nursery stock, and cause damage to many species including *Photinia, Chaenomoles, Daphne* , *Euonymus*, and *Choisya*. Carnation tortrix is endemic to Mediterranean regions, while light brown apple moth is endemic to Australia, and so both are favoured by warm environments such as nursery stock grown under protection. Caterpillars of both species are similar in morphology and habit; they feed on the leaves and growing points, reducing crop quality. Control with insecticides is difficult because the caterpillars hide inside rolled up leaves and are protected from the spray deposits.

- The eggs of both species are laid in an egg mass, composed of 15-50 or more eggs, laid on the upper leaf surface. Egg masses are pale green in colour, and are often missed by growers during crop monitoring as they are very inconspicuous.

- Growers may have to spray up to 7 or 8 times during the summer, using IPM compatible insecticides such as Conserve or Dipel DF, in order to protect their plants and reduce the foliar damage.

- No biological control organisms have been recommended for these pests, but the project HNS 170, carried out in 2008, showed that the egg parasitoid wasp *Trichogramma brassicae* could successfully parasitise egg masses of carnation tortrix in cage tests.

- The aim of this project was to evaluate *Trichogramma* parasitoids on a commercial scale, by introducing them at weekly intervals to a polythene tunnel with a natural infestation of carnation tortrix, and to evaluate the economics of this approach.

Summary of the project and main conclusions

- Liners of *Chaenomoles* which were naturally infested with light brown apple moth were used in this work, which was based in a polythene tunnel of 500 m^2 area at Wyevale nurseries, Hereford. Carnation tortrix moth had been present in previous years, but light brown apple moth was the dominant species in this project.

- *Trichogramma* parasitoids supplied as black eggs on cards (as the product Tricholine from Syngenta Bioline) were introduced weekly at the rate of $20/m^2$ from mid June until early September 2009, as parasitized moth eggs on cards. A total of 10 introductions were made.

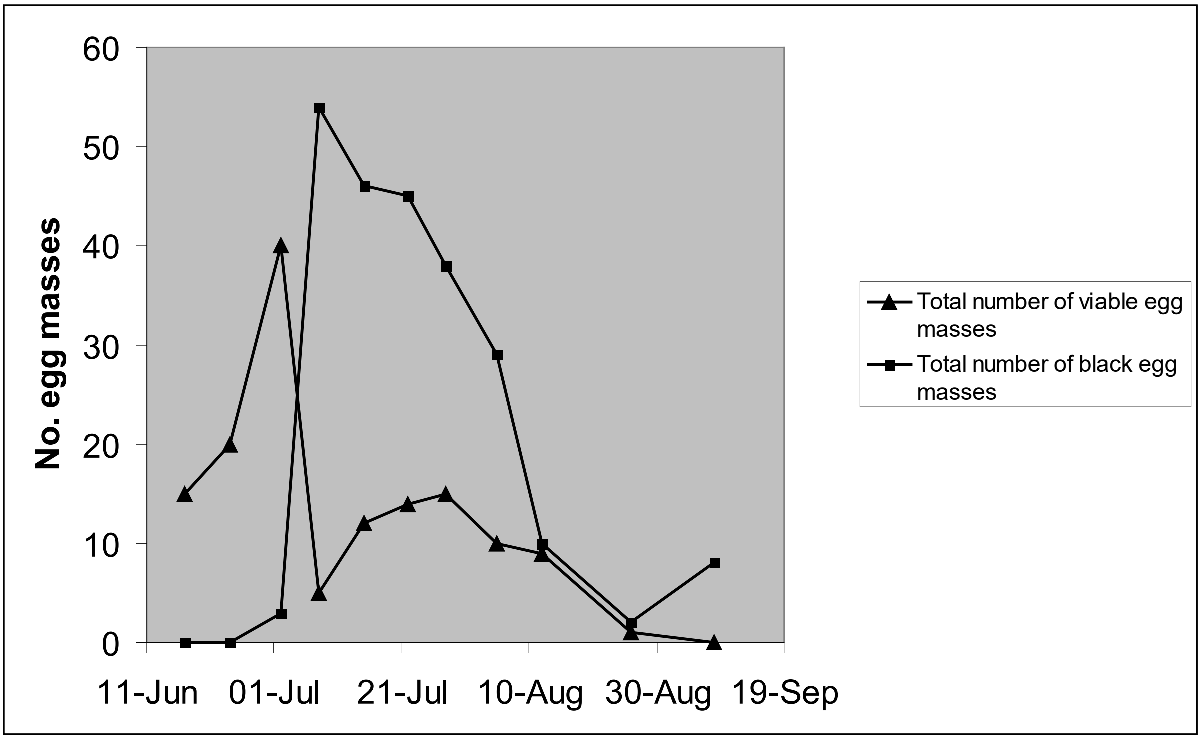
- The parasitisation of egg masses was low at first, but after four weekly introductions it reached over 90%. For the next 4 weeks it varied, but always exceeded 70%. The numbers of viable and parasitized egg masses over the period of the experiment are shown in Figure 1. In order to provide complete control, two supplementary sprays of Dipel DF were needed.

- Successfully parasitized egg masses turned black and so were easy to recognize. In all cases, even though the number of eggs within an egg mass varied, 100% of the eggs within an egg mass were successfully parasitized.

- The previous related project (HNS 170) showed that *Trichogramma* parasitoids could attack egg masses of carnation tortrix in cage tests. This project has shown that they are also effective against egg masses of the light brown apple moth in a commercial situation.

- Emergence of the parasitoids from the cards is delayed because they are delivered in a state of diapause and this needs to be considered when planning IPM programmes. Emergence can take between 9 and 13 days (mean 11 days) depending on temperature, and if the cards are put out immediately upon receipt, they deteriorate with overhead watering. This could adversely affect their performance. Best results would be gained by retaining the cards until hatching occurs, and then placing them in the crop immediately.

Figure 1. Number of viable and parasitized egg masses of light brown apple moth on Chaenomoles, following introduction of *Trichogramma* weekly from 17 June



Financial benefits

The *Trichogramma* parasitoid programme of 10 introductions cost a total of 31 p per m^2 over the season, (this cost includes the cost of the two sprays of Dipel DF which were needed), compared to a conventional insecticide programme using IPM compatible insecticides (six applications of Conserve, active ingredient spinosad, and two applications of Dipel DF, active ingredient *Bacillus thuringiensis*), which would cost approximately 27 p per m^2.

In addition, there would be less selection pressure for resistance in the tortrix population, which should allow existing chemicals to remain effective for longer, with financial benefits for growers in the long term. This is especially important given the fact that EC legislation is reducing the number of products available to growers.

Action points for growers

- *Trichogramma* parasitoids are freely available from at least two UK suppliers, as parasitized moth eggs on cards which can be hung on the crop.

- They can attack egg masses of both carnation tortrix moth, and light brown apple moth, and so have great potential for use in IPM programmes for protected nursery stock.

- Eggs which have been parasitized turn black and so are easily seen.

- Tests carried out in project HNS 170 in 2008 showed, however, that it takes between 9 and 13 days for the *Trichogramma* parasitoids to emerge and so if the cards were put out immediately they were received, it is likely that they would get damp and lose quality before this time, especially if overhead watering was in use.

- The delay in emergence of the parasitoids need to be considered when planning an IPM programme to ensure that emergence is synchronised with the presence of tortrix egg masses.

- Best effects would be gained by regular monitoring of a few cards in the office, and only putting them out on the crop once adult parasitoids had started to emerge.

- A weekly programme of introductions would be needed to maintain control of the tortrix, and supplementary sprays may be required, depending on pest pressure. The product Dipel DF is ideal for use in conjunction with *Trichogramma*, because it is completely safe to the parasitoids.

Science Section

Introduction

Carnation tortrix moth (*Cacoecimorpha pronubana*) and light brown apple moth (*Epiphyas postvittana*) are both widespread in the HNS industry and damage a very wide range of shrubs, both outdoors and under protection. Over the past 5 years, apple moth has extended its range northwards and, where it occurs, tends to displace carnation tortrix moth. Female moths of both species lay egg masses, which vary in size from 15-50 or more eggs, like overlapping scales on the dorsal leaf surface. Egg masses are translucent, pale green when first laid, turning a darker green or pale yellow just before hatching. Newly hatched larvae disperse rapidly by crawling or 'ballooning' on silken threads before starting to feed on the leaf lamina. As the larvae grow they eat shoot tips and leaves, and as they mature, leaves may be bound together with silk so that the caterpillars can feed in the protected growing points. This causes a loss of quality and a reduction in the percentage of saleable plants. For liners, this damage is not too serious as plants can be trimmed back before sale, but on finished plants the damage can significantly affect marketability and quality. During the summer months, there can be up to three generations of the pest under protection, so numbers can rapidly build up to become a major pest problem.

Although there are several insecticides available which can be used in a chemical control programme, the IPM compatible products, such as Dipel DF (*Bacillus thuringiensis*), are only effective against young larvae and also need frequent (sometimes weekly) application, which takes time and is expensive in terms of labour costs.

If a biological control method could be found for carnation tortrix, it would offer another dimension to the IPM programme, and would also allow sustainable control of this pest. The aim of this project was to evaluate the egg-parasitic wasp *Trichogramma brassicae*, a potential biological control agent, to determine if it could successfully attack the egg masses of carnation tortrix.

Materials and Methods

The host nursery had a low population of tortrix moths present in April 2009, and this was identified as light brown apple moth, rather than carnation tortrix moth. As the two species are extremely similar (both belong to the lepidopteran family *Torticidae*) and the site at Wyevale nurseries was ideal for the project, it was decided to work with this species.

The tunnel of ca. 500 m^2 in area contained HNS liners of mixed species, including 11 liner trays of *Chaenomoles*, each containing 18 plants, which were distributed regularly within the tunnel.

All assessments were made on these plants, because from previous work it was known to be very susceptible to tortrix, and also the leaf morphology made recognition and identification of egg masses relatively easy.

Parasitoid introduction

Trichogramma brassicae parasitoids were introduced as the product “Tricholine” supplied by the company Syngenta Bioline. The wasps arrived as pupae within the eggs of the flour moth*, Ephestia kuehniella*, on small cards, 200 per card and 50 cards per box, making a total of 10,000 parasitoids per box.

Cards were clipped to plastic labels, which were spaced evenly within the tunnel, with an approximate density of 1 card per 10 m^2 of crop. The cards were clipped to the reverse of the labels, so that they were shaded from bright sunlight. A previous project (HNS 170) had shown that there was always an interval of about 11 days between arrival of the cards and emergence of parasitoids, so all introductions were kept in the laboratory at 15-20ºC until the first wasps were seen, and then the cards distributed at the trial site immediately.

The first introduction to the trial site was made on 17 June, and they were then introduced weekly, with the final one on 26 August (10 introductions in all)

Supplementary sprays of Dipel DF

Examination of the growing points and shoots of the *Chaenomoles* liners in mid July showed that, despite a high % of parasitized egg masses, live tortrix larvae were present and so a decision was made to apply a corrective spray of Dipel DF, to maintain control to commercial standards. This product, which is based on the bacterium *Bacillus thuringiensis*, acts as a stomach poison when larvae consume treated foliage. It is specific to caterpillars and has no adverse effect on parasitoids such as *Trichogramma*.

Dipel was applied on 18 July and again on 25 July. These were the only pesticide applications made in the tunnel over the trial period.

Assessments

At weekly intervals from mid June to early September 2009, every *Chaenomoles* liner (a total of 198 plants) was examined carefully, and leaves checked for the presence and number of egg masses of apple moth. Egg masses, which were always on the upper leaf surface, were divided into the following categories:

Emerged: these were silver in colour and the tiny hole where the 1st instar caterpillar had emerged could be seen with a hand lens.

Viable: these were pale green, darker green or yellow in colour, turgid and varied in number from 15-50+.

Parasitized: these had turned black.

It was noted that emerged egg masses remained on the leaf for many weeks, but eventually they disintegrated and were not visible.

In addition to egg mass assessments, counts were made of the number of shoots with tortrix damage on each plant. This was maintained for only the first three assessments, because over time it became impossible to distinguish between old and new larval damage.

Results and Discussion

The results of the assessments for larvae and egg masses are shown in Table 1.

Table 1: Assessments of light brown apple moth parasitism by *Trichogramma* on Chaenomoles

Date*	Total number of damaged shoots**	Total number of emerged egg masses ***	Total number of viable egg masses	Total number of black egg masses	% parasitisation of unhatched egg masses
17 June	198	26	15	0	0.0
24 June	230	22	20	0	0.0
2 July	429	49	40	3	7.0
8 July	N/A	37	5	54	91.5
15 July	N/A	33	12	46	79.3
22 July	N/A	39	14	45	76.3
28 July	N/A	56	15	38	71.7
5 August	N/A	48	10	29	74.4
12 August	N/A	74	9	10	52.6
26 August	N/A	106	1	2	67.0
8 September	N/A	78	0	8	100.0

Notes:
* Tricholine cards put out weekly from 17 June until 26 August inclusive (10 introductions in all)
** After the assessment on 2 July, counts of the number of damaged shoots were discontinued, as it was impossible to distinguish between old and new damage on the plants.
*** Emerged egg masses remained visible on plants for several weeks after larvae had emerged, but eventually they disintegrated and fell off the leaves.

Successfully parasitized egg masses turned black and so were easy to recognize. In all cases, even though the number of eggs within an egg mass varied, 100% of the eggs within an egg mass were successfully parasitized.

The assessments showed that light brown apple moth egg masses were successfully parasitized by *Trichogramma* parasitoids when introduced weekly at the rate of 20/m^2. There was a lag time of about 2 weeks after introduction before parasitized black egg masses were seen, (Table 1) but from then on the % parasitism of egg masses increased rapidly. On the 8 July, after only 4 introductions, the % parasitism exceeded 90% and afterwards, although it decreased, was still greater than 70% for a further 4 weeks. Considering that the *Chaenomoles* liner plants were spaced evenly within the tunnel and were surrounded by liners of other HNS species, the searching ability of the parasitoids is clearly excellent. The cards were distributed at an even density of approximately 1 card/10 m^2 in this trial. Within the tunnel, the air was relatively still and this would benefit the parasitoids flying ability. Knutson (1997) summarized the biology of *Trichogramma*, and its use in classical biological control programmes. The parasitoid locates egg masses by “homing in” on the pheromones and chemical signals left behind when the tortrix moth oviposits. Tiny wing scales are often left in the vicinity of egg masses and these act as chemical and visual cues for the parasitoids, (Nordlund and Beevers, 1981). The wasps in the family *Trichogrammatidae* are even smaller than *Encarsia* parasitoids, and range in size from 0.2 to 1.1 mm in length (Knutson, 1997). Despite their small size, they can be effective outdoors, and have been used in biological control programmes for pests such as codling moth on fruit trees (Hassan, 2006), so there may be further potential for their use in IPM programmes for nursery stock outdoors in the future.

Despite the high % parasitism of egg masses in this trial, it is clear that complete control of the apple moth caterpillars was not obtained, as two supplementary sprays were needed. Only one rate of release was used in this trial, and it is possible that higher release rates would be more effective. However, at the rate used, (20/m^2 per week) the costs were roughly equivalent to a chemical programme, and higher rates would incur a cost penalty. The costs of a season long programme (including two supplementary sprays of Dipel DF) at 31 p/m^2 are similar to the costs of a full chemical programme. Additionally, as emerged egg masses were observed on the date of the first introduction (17 June) there is potentially a benefit from introducing parasitoids at an earlier date.

There are many species and strains of *Trichogramma* available worldwide, and some species are more effective against certain pests than others (Hassan *et al*, 1988). Within Europe, only *T. brassicae* and *T. evanescens* are commercially available at present. (Greatrex, pers. comm).

It is not known whether the latter species would be as effective as *T. brassicae*, but the results of this project, and the cage tests carried out in project HNS 170 have shown that *T. brassicae* can parasitise both carnation tortrix and light brown apple moth, which is useful, as sometimes both species can occur concurrently on a nursery.

Conclusions

Trichogramma brassicae parasitoids can provide good, but not complete control of light brown apple moth when introduced on a weekly basis during the summer months.

The cost of this programme, including two supplementary sprays, is similar to the costs of a complete chemical programme.

Technology transfer

The results of this project will be detailed in an article for HDC News in spring 2010, and at relevant seminars as appropriate.

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Appendix

Picture of Tricholine cards attached to host plant, with wasps emerging.