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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the 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 FOR HNS 170

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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TABLE OF CONTENTS

GROWER SUMMARY **1**

HEADLINE 1

BACKGROUND AND EXPECTED DELIVERABLES 1

SUMMARY OF THE PROJECT AND MAIN CONCLUSIONS 2

FINANCIAL BENEFITS 3

ACTION POINTS FOR GROWERS 3

SCIENCE SECTION **5**

INTRODUCTION 5

MATERIALS AND METHODS 5

Incubation time of Trichogramma parasitoids on commercially-produced cards *6*

Cage tests on parasitism rate of carnation tortrix eggs *6*

RESULTS AND DISCUSSION 9

Incubation time of Trichogramma parasitoids on commercially-produced cards *9*

Cage tests on parasitism rate of carnation tortrix eggs *10*

CONCLUSIONS 15

TECHNOLOGY TRANSFER 15

REFERENCES 15

ACKNOWLEDGEMENTS 16

GROWER SUMMARY

Headline

- This project has clearly shown (in cage tests) that the egg-parasitic wasp *Trichogramma brassicae* can attack the egg masses of carnation tortrix moth (*Cacoecimorpha pronubana*), and thus has potential for use in a biological control or IPM programme for this pest.

Background and expected deliverables

The carnation tortrix moth (*Cacoecimorpha pronubana*) is a widespread pest of nursery stock and causes damage to many species including Photinia, Chaenomeles, Daphne, Euonymus and Choisya. It is endemic to Mediterranean regions and so is favoured by warm environments such as those provided by nursery stock grown under protection. Caterpillars feed on the leaves and growing points, reducing crop quality. Control with insecticides is difficult because the caterpillars hide inside rolled-up leaves where they are protected from spray deposits. The eggs are laid in a mass, composed of 15-50 or more eggs, on the upper leaf surface (see Figure 1). Egg masses are pale green in colour, and are easily missed during crop monitoring as they are very inconspicuous.

Figure 1. Newly laid egg mass of carnation tortrix.

Growers may have to spray many times during the summer in order to protect their plants and reduce foliar damage. No biological control organisms have been recommended for this pest, but the egg-parasitic wasp *Trichogramma brassicae* is commercially available, and has been used successfully against a wide range of moth species in Europe and elsewhere.

- The main aim of the project was to determine whether *Trichogramma* parasitoids would parasitise egg-masses of the carnation tortrix moth in small-scale cage tests.

Summary of the project and main conclusions

The work used liners of Chaenomeles, known to be heavily infested with carnation tortrix in one of the single polythene tunnels at A commercial nursery in the West Midlands. The crop was inspected closely for egg masses at intervals, and plants with an egg-mass were removed and placed singly in a fine-mesh insect cage together with moth eggs which had been previously parasitized by Trichogramma. (The commercial product is a small card, with parasitized eggs stuck onto a circle in the middle of the card).The percentage hatch of carnation tortrix eggs was recorded within the cages.

Assessments showed that a high level of parasitism could be achieved providing the egg-mass was pale green when Trichogramma parasitoids were active. Eggs that were parasitized turned black and so were easily recognizable (see Figure 2).

Figure 2. Egg mass parasitised by Trichogramma where all the eggs have turned black

If the egg mass was more mature (i.e. a darker green or yellower in colour) at the time of exposure to the Trichogramma parasitoids, then parasitism did not occur.

The age of the eggs therefore appears to be critical in determining the success of the parasitoid.

These results were obtained with parasitoids and eggs confined in small cages. It will be important to replicate this success with parasitoids released on a larger scale, such as in a commercial polythene tunnel.

Financial benefits

- *Trichogramma* parasitoids are inexpensive; (2008 price was £8.00 for 10,000 parasitoids). Recommended rates are 1 card (containing 200 parasitoids) per 5-20 m² of crop. This is equivalent to 10-40 parasitoids per m² of crop, at a cost of 0.8-3.2p/m². However, it is likely that repeated releases during the summer months would be required for control of carnation tortrix, so the cost would increase *pro rata* depending on the number of releases. Assuming a total of 10 releases during the period May-September inclusive, this would cost a total of 8-32p/m² of crop per season. If control was effective after a few weeks, then the number of introductions could be reduced and thus the cost would also decrease. These costings are based on the full retail price as supplied by the manufacturer and discounts would be possible for a season-long programme from the supplier.
- Since *Trichogramma* prevents the caterpillars from hatching at all, crop damage is likely to be reduced and the cost of labour to apply regular insecticide sprays would also be either reduced or avoided completely.
- The likely costs, savings and viability of this approach cannot be determined accurately until field-scale trials have been completed.

Action points for growers

- This pilot study does not indicate whether or not *Trichogramma* will work on a commercial scale. However, *Trichogramma* parasitoids are freely available from at least two UK suppliers as parasitized moth eggs on cards which can be hung on the crop. Growers could therefore try the parasitoids to determine their possible impact on carnation tortrix control on their own nurseries.

- Tests showed that it takes between 9 and 13 days for the *Trichogramma* parasitoids to emerge from commercially-available cards. If these were put out immediately they were received, it is likely that they would get damp and lose quality before parasitoid emergence had occurred, especially if overhead watering was in use.
- Best results are likely to be gained by regular monitoring of a few cards after receipt, only putting the whole batch of cards out on the crop once adult parasitoids have started to emerge.
- To be effective over the season, it is likely that repeated introductions of parasitoids would be needed. Further research work is needed to determine the frequency of introductions necessary and the economics of this approach.

SCIENCE SECTION

Introduction

Carnation tortrix moth (*Cacoecimorpha pronubana*) is widespread in the HNS industry and damages a very wide range of shrubs, both outdoors and under protection. Female moths lay egg masses, which vary in size from 15-50 plus 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

A newly potted crop of Chaenomeles liners at Wyevale Nurseries, Hereford, was selected for this work because it was already naturally infested with carnation tortrix, and because IPM methods for other pests were being practised by the nursery. In total there were ca. 1,500 liners in a polytunnel. At intervals between June and September 2008, these plants were carefully examined for egg masses of carnation tortrix. Searching for egg masses was very time-consuming, as they are pale green in colour when first laid, and very hard to spot amongst the closely-spaced liner plants.

Incubation time of Trichogramma parasitoids on commercially-produced cards

Trichogramma brassicae parasitoids were supplied by Syngenta Bioline as the product 'Tricholine', with 200 parasitised eggs of the Mediterranean flour moth (*Ephestia kuehniella)* on each card. Initially, cards were used as soon as they were delivered, but in later experiments, the cards were incubated in the laboratory until the first wasps emerged and were then immediately used in experiments. The time taken for each batch of 'Tricholine' to emerge was recorded. Spare cards were retained and observed in the laboratory to estimate how long the wasps remained active.

Cage tests on parasitism rate of carnation tortrix eggs

A total of five experiments were done. For each experiment, individual plants with an egg mass present were placed in a fine mesh cage inside the poly tunnel at Wyevale Nurseries, and one *Trichogramma* card was hung on the plant. The number of eggs in each egg mass was counted *in situ* using a hand lens, and the stage of development (in terms of colour) was noted. The age of individual egg masses varied as they were selected from naturally-infested liners for each experiment. Cages were replicated (the number of replicates in each experiment depended on how many infested plants were found), and monitored at intervals until egg hatch had occurred. The number of eggs that had hatched, and the number that had turned black, were recorded for each replicate. In addition, leaves containing black eggs were removed and incubated in petri dishes in the laboratory. The time taken for a new generation of *Trichogramma* parasitoids to emerge was recorded.

The cages were shaded by covering with green mesh. Temperatures in the tunnel were recorded by placing a TinyTag recording device inside one of the cages; this recorded maximum, minimum and mean temperatures during the experimental period.

Experiment 1

This was set up on 26 June 2008. A total of 12 plants with an egg-mass were found within the liner crop and placed individually in fine mesh cages. One 'Tricholine' card was placed in each cage. Cards had arrived on 25 June 2008. The experiment was assessed on 2 July. Subsequent experiments used a different approach in that new 'Tricholine' cards were ordered, incubated in the laboratory and only introduced into the experimental cages once the first wasps emerged.

Experiment 2

This was set up on 15 July 2008. A total of five plants with a carnation tortrix egg mass were found within the liner crop and these were used in the experiment. *Trichogramma* parasitoids were just emerging at this time. Assessment was done on 25 July 2008.

Experiment 3

This was set up on 28 July 2008, using parasitoids which had first emerged on 27 July 2008. A total of six plants with an egg mass were found within the liner crop, and each was placed individually in a fine mesh cage as before. Assessments were carried out on 4 August 2008.

Experiment 4

This was set up on 11 August 2008, using parasitoids which had first emerged on 10 August 2008. A total of four plants with an egg mass were found within the liner crop and placed in mesh cages as before. Assessments were done on 22 August 2008, which was when the first black egg masses were seen (11 days after parasitoid introduction).

Experiment 5

This was set up on 26 August 2008. A total of five plants with an egg mass were found within the liner crop and placed in the mesh cages. Parasitoids first emerged on 28 August 2008 and were immediately introduced into the cages. Assessments were done on 6 September 2008 when black eggs were first seen (nine days after parasitoid introduction).

Results and discussion

Incubation time of Trichogramma parasitoids on commercially-produced cards

The results of these tests are shown in Table 1.

Table 1. Incubation time of *Trichogramma* in different commercial batches

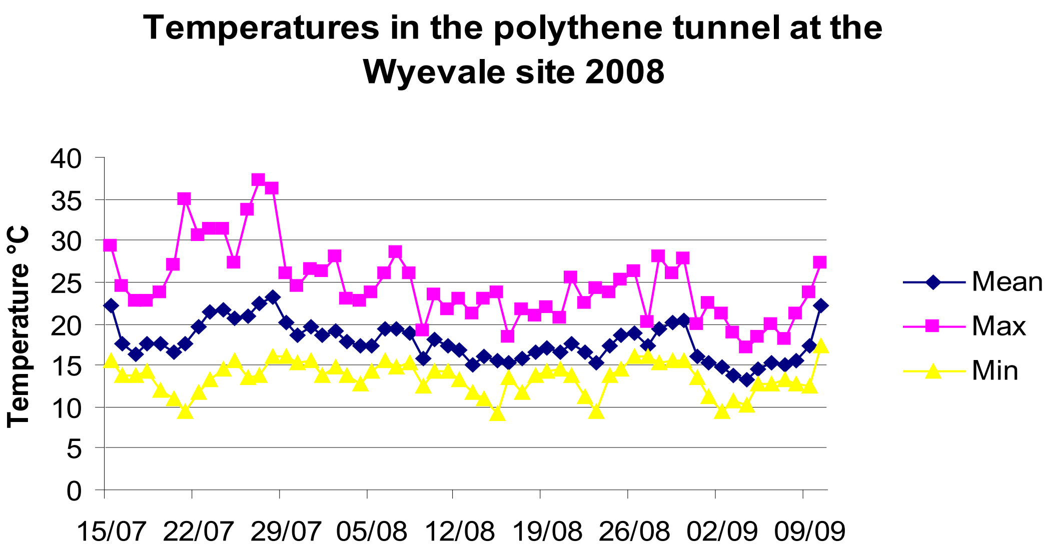
Parasitoid Batch number	Date arrived	Date hatch commenced	Time for emergence (days)
1	25 June	7 July	12
2	2 July	15 July	13
3	8 July	21 July	13
4	18 July	27 July	9
5	19 August	28 August	9
Mean			11

Overall mean time from receipt of the cards to parasitoid emergence was 11 days (Table 1), but was variable and in any case was much longer than stated in the literature supplied by the producer. This is important, because if growers placed the cards out immediately upon receipt they would be exposed to overhead irrigation for some days before hatch occurred, causing damage which would reduce the percentage emergence of the parasitoids. Ideally, cards should be kept safe and observed daily until hatching was seen to begin, and only then placed amongst the crop.

Observations on several batches of 'Tricholine' showed that adult parasitoids were only active for a maximum of 2 or 3 days when confined in petri dishes. It is possible that in a field situation, where the adult wasps might have access to nectar or honeydew, the life-span might be longer, but this was not tested in this project. Ruberson & Kring (1993) found that the life span of *Trichogramma pretiosum* provided with honey was 3 days, whereas if they were allowed to host feed in addition to access to honey, average lifespan increased to 11 days.

Figure 1 shows the maximum, minimum and mean temperatures during the experimental period. Mean temperatures remained between 15°C and 20°C for the majority of this time, and there were few periods when the maximum exceeded 25°C.

Figure 1. Temperatures in the experimental polytunnel at Wyevale Nurseries.



Cage tests on parasitism rate of carnation tortrix eggs

Examination of the plants with a x10 hand lens showed that the eggs of carnation tortrix were pale green and translucent when first laid (Plate 1) then turned a darker green and finally a yellow brown colour , at which time the head capsule was visible as a dark spot in the centre of the egg. Once hatching had taken place, eggs turned a silvery clear colour and the emergence hole could clearly be seen.

Experiment 1

The results are given in Table 2. No parasitism occurred and all carnation tortrix eggs hatched normally (Table 2). 'Tricholine' cards from this batch were retained. Observation of these showed that none of the *Trichogramma* and had emerged at the time the trial was assessed on 2 July, so there was never any chance that parasitism would occur. In fact, the parasitoids first emerged on 7 July (see Table 1 above).

Plate 1. Newly laid egg mass of carnation tortrix.

Table 2. Experiment 1 - percentage hatch of carnation tortrix eggs and parasitism rate (%) by *Trichogramma brassicae*

Plant/cage number	**Total eggs in egg mass**	**Stage at start of experiment**	**No. black eggs**	**No. hatched eggs**	**% parasitism**	**% hatch**
1	15	Pale green	0	15	0	100
2	17	Pale green	0	17	0	100
3	21	Darker green	0	21	0	100
4	16	Pale green	0	16	0	100
5	18	Pale green	0	18	0	100
6	29	Dark green	0	29	0	100
7	26	Dark green	0	26	0	100
8	62	Pale green	0	62	0	100
9	30	Pale green	0	30	0	100
10	43	Pale green	0	43	0	100
11	59	Dark green	0	59	0	100
12	13	Dark green	0	13	0	100

Experiment 2

The results of Experiment 2 are given in Table 3. Significant parasitism (52-72%) occurred on three of the five caged plants. This result was the first evidence in this project that *Trichogramma* parasitoids were able to attack and parasitise egg masses of the carnation tortrix moth. Examination with a x10 hand lens showed clearly the black colouration of eggs which had been parasitised (Plate 2)). The time between parasitoid introduction and the first sign of black eggs was 10 days.

Table 3. Experiment 2 - percentage hatch of carnation tortrix eggs and parasitism rate (%) by *Trichogramma brassicae*

Plant/cage number	Total eggs in egg mass	Stage at start of experiment	No. black eggs	No. hatched eggs	% parasitism	% hatch
1	36	Pale green	26	10	72	23
2	54	Pale green	28	26	52	48
3	45	Darker green	0	45	0	100
4	21	Pale green	12	9	57	43
5	66	Darker green	0	66	0	100

Plate 2. Egg mass parasitised by Trichogramma where all the eggs have turned black

The variability in the parasitism rates may indicate that eggs close to hatching may be unsuitable for parasitoid oviposition. *Trichogramma* wasps take between 8 and

17 days to complete their development inside the host egg and emerge, but this is dependent on temperature, with an average quoted as 9 days (Knutson, 1997).

Experiment 3

The results of Experiment 3 are given in Table 4. As in Experiment 2, the results were variable, with parasitism occurring on some egg masses but not on others. Leaves from cages 3, 4 and 6 (containing black eggs) were removed after this assessment (13 August 2008) and kept in petri dishes, on moist filter paper, until emergence of new parasitoids was observed. In this experiment, the time from black eggs being observed until hatching was nine days.

Table 4. Experiment 3 - percentage hatch of carnation tortrix eggs and parasitism rate (%) by *Trichogramma brassicae*

Plant/cage number	Total eggs in egg mass	Stage at start of experiment	No. black eggs	No. hatched eggs	% parasitism	% hatch
1	36	Pale green	0	36	0	100
2	41	Pale yellow	0	38	0	93
3	26	Pale green	24	2	92	8
4	20	Pale green	16	4	80	20
5	21	Pale green	0	21	0	100
6	22	Pale green	9	13	41	59

Experiment 4

The results of Experiment 4 are given in Table 5. In this experiment, most of the egg masses found were yellow or dark yellow in colour, indicating that they were unsuitable for *Trichogramma* oviposition. This was confirmed by the hatch of caterpillars at the time of assessment. Where the egg mass was pale green in colour, and therefore likely to have been more recently laid, 56% of the eggs were seen to be parasitised 11 days after active parasitoids were first introduced. The actual age of each egg mass was unknown.

Table 5. Experiment 4 - percentage hatch of carnation tortrix eggs and parasitism rate (%) by *Trichogramma brassicae*

Plant/cage number	Total eggs in egg mass	Stage at start of experiment	No. black eggs	No. hatched eggs	% parasitism	% hatch
1	31	Dark yellow	0	31	0	100
2	16	Pale green	9	7	56	44
3	12	Pale yellow	0	12	0	100
4	19	Dark yellow	0	19	0	100

The leaf with black eggs (Plant 3) was removed on 22 August 2008 and kept in a sealed petri dish on damp filter paper in the laboratory. New parasitoids emerged on 1 September 2008 (10 days after eggs turned black).

Experiment 5

The results of Experiment 5 are given in Table 6.

Table 6. Experiment 5 - percentage hatch of carnation tortrix eggs and parasitism rate (%) by *Trichogramma brassicae*

Plant/cage number	Total eggs in egg mass	Stage at start of experiment	No. black eggs	No. hatched eggs	% parasitism	% hatch
1	15	Pale green	14	1	93	
2	20	Pale green	20	0	100	
3	56	Darker green	48	8	85	
4	25	Pale green	25	0	100	
5	30	Pale green	27	3	90	

Observations made using a x10 hand lens indicated that a few eggs had not hatched or turned black, but were very dark yellow and appeared less turgid. It is possible that host feeding by the parasitoids had occurred on these eggs. In this final trial, the percentage parasitism was the highest recorded and also the least variable in that all the replicates showed evidence of parasitism. This may be due to younger egg masses having been selected, and also perhaps to temperatures being higher in early September when this final experiment was taking place (Figure 1), which may have favoured parasitoid activity.

Conclusions

- *Trichogramma brassicae* parasitoids can successfully parasitise egg masses of carnation tortrix moth in cage tests.
- The parasitoids are available commercially from at least two biological control companies and so could be utilized by growers in their IPM programmes.
- The age of the egg mass appears to influence the success of parasitism.
- Repeated introductions would probably be needed to provide effective control over a season.

- Further experience is needed on a larger scale and in a more realistic environment such as a polythene tunnel.

Technology transfer

- The results of this project were discussed at HDC sponsored ICM workshops for HNS growers in October 2008.
- An article for HDC Project News will be produced in spring 2009.

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