TRICHOGRAMMA AUGMENTATION AS A COMPONENT OF THE MANAGEMENT OF CODLING MOTH IN WALNUTS


ABSTRACT

Field trials were carried out using different release rates of Trichogramma parasitoids in replicate 0.5 acre plots in two walnut orchards, one at Grimes in the Sacramento Valley and the other at Denair in the San Joaquin Valley. These field trials were designed to demonstrate whether Trichogramma has a significant impact on codling moth in walnut orchards, reducing the level of nut infestation through the season, and to assess what number of Trichogramma are needed per acre to have the desired impact. Four releases of Trichogramma were used during each of the three generations of the codling moth through the season and levels of damage were monitored by counts of infested nuts in the canopy of the trees at the end of each generation. Significant impacts of the Trichogramma releases were seen in both orchards. A release rate of 100,000 Trichogramma per acre per release provided effective control in Grimes, an orchard with low pressure from codling moth, but release rates of up to 400,000 Trichogramma per acre per release continued to give added benefit in Denair, an orchard with high pressure from codling moth. An incorrect timing of the release of the Trichogramma at Denair during the first codling moth generation led to unacceptable levels of damage in this high pressure orchard. Lab tests of various supplemental foods to support the longevity of Trichogramma indicated that honey, sucrose and fructose all support substantial improvements in the length of adult life, whereas aphid honeydew is not as good, and yeast extract and molasses are poor food sources. In addition, honey can be diluted as much as 1 part in 10 of water before it effectiveness as a food source is lost. Use of such supplemental foods could improve the effectiveness of the Trichogramma releases, allowing either a reduction in rate or frequency of release.

OBJECTIVES

1. To evaluate the impact of T. platneri release rate on codling moth in walnuts

2. Too determine the influence of walnut leaves on the longevity of T. platneri

3. To determine the importance of selected adult food sources in promoting the longevity of T. platneri

PROCEDURE

Trichogramma release rate trials. Release rate trials were carried out in two commercial orchards, one at Grimes in the Sacramento Valley and the other at Denair in the San Joaquin
Valley, both with a history of codling moth pressure. The Grimes site consisted of Ashleys interplanted with Hartleys and with occasional Franquettes as pollinators on a 30 x 30ft planting. The Denair site consisted of Serrs with Chico pollinators on a 24 x 24ft planting. A 10 acre end section of each orchard was used for the *Trichogramma* release rate study. The experimental orchard sections were arranged to provide 4 replicate blocks of 5 half acre treatment plots; the 5 release rate treatments being 0, 50,000, 100,000, 200,000 and 400,000 *Trichogramma* per acre. The blocks were completely randomized at Denair, but in Grimes the control plots for each block were confined to the accessible edges of the orchard to comply with the concerns of the grower and PCA. Four parasitoids releases were made at 10 day intervals during the oviposition period of the 1st generation of the codling moth and at weekly intervals through that of the 2nd and 3rd generations, as determined from pheromone trap catches and day degree models. The quality of the *Trichogramma* used for release was monitored by examination of an individual square from the cards used each week to determine the percent parasitism of the host eggs and the sex ratio of the emerging parasitoids.

The activity of the released *Trichogramma* was monitored using sentinel codling moth eggs on one occasion during each of the 3 codling moth generations in each orchard. Individual insectary-reared codling moth eggs were glued, with water-soluble glue, to small (2 x 1.5 inch) cards. A set of 3 cards were attached at each of 5 height intervals (7, 10, 13, 16 and 19ft) along a 1 inch diameter PVC pole. A single 20ft pole was placed in the canopy of a central tree, in each of the 20 half acre plots in each orchard, exposing the sentinel eggs to parasitism for a period of 4 days on each of the 3 sample dates (May 12, June 17 and August 12). The recovered sentinel eggs were returned to the laboratory to await the characteristic blackening of *Trichogramma* parasitised eggs.

Codling moth damage in the experimental plots was monitored through the season using 3 approaches; dropped nuts for the 1st generation, canopy sampling for each of the 3 generations, and ground nuts at harvest. The number of infested dropped nuts was monitored beneath 4 central trees (Ashley in Grimes or Serr in Denair) in each plot at weekly intervals from mid May to mid June. Canopy sampling, examination of 50 nuts from the entire lower canopy and 50 nuts from each of the two sides of 5 central trees (Ashley in Grimes or Serr in Denair) in each plot, took place at the end of each of the 3 codling moth generations (only the first two generations at Denair). A set of 40 nuts were collected from each of 5 central Ashley trees in each plot at harvest in Grimes and returned to the lab where they were stored at 2°C until they could be cracked open to determine levels of damage by codling moth.

The influence of walnut leaves on longevity of *T. platneri*. The longevity of the *Trichogramma* in the presence and absence of walnut leaf exudates, as a possible food source for the *Trichogramma* adults, was monitored in the laboratory at 25°C and a 16hr photoperiod. 10-20 freshly emerged *Trichogramma* adults were confined in each of a series of small ventilated glass vials. A small fresh walnut leaf, held in a moist cotton plug, was introduced into one half of the series of vials, while the other half of the series remained empty. The vials were monitored daily to determine longevity.
The importance of selected adult food sources for *T. platneri*. The influence of adult food sources in promoting longevity of the *Trichogramma* was determined in the laboratory at 25°C and a 16hr photoperiod. The carbohydrate food sources tested included honeydew (from the rosey apple aphid *Dysaphis plantaginea*), honey, molasses, and saturated solutions of sucrose and fructose, the two latter with and without the addition of yeast extract as a protein source. In a second series of tests, the ability of *Trichogramma* to use various dilutions of honey were tested under the same conditions. A series of 10 replicates glass vials containing from 30-50 freshly emerged *Trichogramma* adults were used for each food source and the food was replaced daily. The vials were monitored daily to note the point in time when all adult parasitoids had died.

RESULTS

*Trichogramma* release rate trials

Sentinel egg parasitism was used to estimated the activity of the released *Trichogramma* in each orchard plot and to determine the presence of any background parasitism in the control plots from naturally occurring *Trichogramma*. Only a small proportion of the sentinel eggs were missing after field exposure at Grimes (0-4%), although a more substantial loss was noted at Denair (15-22%). At both sites a significant proportion were damaged due to desiccation, abrasion or predation (25-73% at Grimes, 13-22% at Denair). As a result, percent parasitism was calculated as the proportion of recovered viable eggs that were parasitized. A single control plot during both the 1st and 3rd generations at Grimes and two control plots during the 1st generation at Denair received some parasitism, but otherwise there was no parasitism in the control plots indicating that there was not a resident *Trichogramma* population in the orchard. Where parasitism was detected in control plots they were downwind from a 400,000 release plot, and so it is likely that this parasitism resulted from movement of released rather than naturally-occurring *Trichogramma*. The loss or damage of sentinel eggs resulted in very variable levels of parasitism, both between sample dates and between plots, and there was only a very weak relationship between parasitism and parasitoid release rate in both orchards (Fig. 1a). The observed trend, however, did suggest that levels of parasitism increased rapidly over the lower release rates but increased only marginally above a release rate of 100,000 *Trichogramma* per acre. Similarly, there was considerable variability in parasitism within height sections of the canopy. Pooling all sets of sentinel egg data for each orchard there is an indication that levels of parasitism are greatest at the mid height section of the canopy, with reduced parasitism both in the lowest and highest sections of the canopy (Fig. 1b).

The quality of the *Trichogramma* used for the field trials was monitored through the season (Fig. 2). The percentage of the host eggs that were actually parasitized showed a rather variable but steady decline through the season from about 80% at the start of the season to less than 60% by the end of August. However, the sex ratio of the parasitoids that emerged showed no trend through the season, remaining relatively constant at 66% female the typical sex ratio for *Trichogramma*. This suggests that the quality of the *Trichogramma* rearing was consistent throughout the season but that the attack rate of host eggs on the *Trichogramma* cards declined through the season resulting in a decline of the release rates through the season.
The canopy sampling of infested nuts at Grimes shows levels of nut damage by codling moth in each of the 3 successive generations through the season (Fig. 3). There is significant variation in the levels of nut infestation between Trichogramma release rates for each of the 3 sample dates. The pattern of nut infestation during the 1st generation shows only a weak correlation with Trichogramma release rate, the level of damage in the 172,000 treatment being greater than expected. This anomalous pattern is lost in the two subsequent generations, where there is a much better negative correlation between nut infestation and Trichogramma release rate. Bars of the histograms with different letters indicate the treatments which differ significantly in levels of nut infestation. In general, during the 2nd and 3rd generations, levels of nut infestation are reduced up to a release rate of 100,000 Trichogramma per acre but no further reductions are detectable from greater release rates.

Comparison of the levels of nut damage monitored by dropped nut counts and canopy nut samples during the first generation at Grimes revealed that both sampling methods provided similar patterns of nut infestation between treatments, although a lower level of damage was indicated in the control plots from the dropped nut sample than from the canopy sample. More importantly, however, there was no significant variation detectable between treatments from the smaller sample sizes of the dropped nut samples, in contrast to the significant variation between the larger sample size canopy samples. Similarly, the canopy nut samples at harvest, using visual cues for nut infestation, showed the same pattern of nut damage as observed from the cracked ground nuts, but only the larger sample size of the canopy nut sample provided significant variation in levels of damage between treatments. These latter results suggest that the more consistent sample sizes of the canopy nut sampling are likely to provide a more valuable measure of nut damage than can be achieved from dropped nuts sampling and harvest nut cracking.

At Denair sampling continued only through the first two generations of the codling moth due to the build up of unacceptably high levels of damage that required the intervention of insecticide treatment. At Denair there were two distinct peaks to the first generation flight of the codling moth and the Trichogramma releases were timed to cover only the first peak (generally the largest and most significant peak). However, the second peak of the first generation that occurred in late May was by far the greater peak of flight and oviposition in 1994 in the San Joaquin Valley. Canopy sampling indicates the tremendous increase in damage between early and late June (Fig. 4) that resulted from the second peak of 1st generation flight. Each of the 3 canopy samples from Denair show significant variation in nut infestation between treatments, with the Trichogramma releases providing the same level of control as the conventional section of the orchard where Lorsban was used. During the early part of the 1st generation the Trichogramma provided good control, but the absence of releases through the very strong 2nd peak of the 1st generation flight allowed a build up of codling moth in the orchard which the Trichogramma releases were then not able to adequately suppress, although release rate effects remained significant. This highlights the importance of correct timing for Trichogramma releases so that the parasitoids are active in the canopy of the trees when codling moth eggs are being laid. In contrast to the Grimes orchard, the impact of the release rates continued to increase over the range of treatments used under this high pressure situation. The dropped nut counts at Denair monitored damage only from the 1st peak of the 1st flight, nuts infested from the 2nd peak did not drop from the trees. Comparison of dropped nut counts and canopy nut samples for the 1st peak of the 1st generation of the codling
moth revealed similar patterns for both sampling methods, as at Grimes. In this case, however, the variation between treatment plots was significant for both methods, as the sample size of the dropped nuts was larger in this higher pressure orchard.

**The influence of walnut leaves on longevity of *T. platneri***

The possible influence of walnut leaf exudates on the longevity of *Trichogramma* proved very difficult to monitor in the lab. The introduction of a small fresh walnut leaf into the glass vials containing the *Trichogramma* invariably resulted in the formation of condensation on the sides of the vial. Such condensation is inevitably lethal for the *Trichogramma* as they are held by the relatively strong surface tension of the water droplets and subsequently drown. As a result the longevity of the *Trichogramma* in these trials was always severely reduced despite attempts to increase the level of ventilation or provide absorbent paper to soak up the condensation. We have eventually found an alternative container, plastic dialysis tubing, that appears not to develop condensation from the confinement of fresh foliage and does not generate electrostatic charges (as does most plastic) that can hold small insects, such as *Trichogramma*, captive on the sides of a vial. The dialysis tubing will be used in 1995 to assess the value of walnut foliage exudates as a carbohydrate food source for adult *Trichogramma*.

**The importance of selected adult food sources for *T. platneri***

A series of different supplemental foods were tested for their effect in prolonging the longevity of adult *Trichogramma*. This is a particularly important feature of *Trichogramma* augmentation as the searching adult females need to survive for as long as possible in the field to allow them the time to find the very low densities of codling moth eggs. Very significant differences were seen in the ability of the food sources to support *Trichogramma* longevity (Fig. 5a) with a span of from 2 days for water to 22 days for fructose. Molasses, sucrose plus yeast extract and fructose plus yeast extract were no better than water in promoting the longevity of the *Trichogramma*. The very small droplets of honeydew from the dusky veined aphid on walnuts dried up too quickly in the lab and so honeydew from the very large droplets produced by the rose apple aphid was used instead as a source of honeydew. The honeydew did prolong the longevity of the *Trichogramma* but not as effectively as honey, sucrose or fructose.

The effect of dilution of honey, one of the most effective supplemental food sources, was also tested in the lab, since dilutions would allow the application of supplemental food as a spray without blocking the nozzle. Again there was a very significant effect of diluting honey from 100% down to zero (Fig. 5b). Dilutions of 100%, 50% and 10% all supported *Trichogramma* longevity to the same extent, whereas a dilution of 5% halved the longevity and dilutions of 1% and 0.1% provided no improvement over water.

**CONCLUSIONS**

These data provide evidence of a significant impact of *Trichogramma* augmentation in the control of the codling moth. There are relatively few studies that have monitored the impact of *Trichogramma* releases in the wide variety of crops in which it is used throughout the world. The
data obtained this season, which demonstrates significant differences in the level of nut infestation in replicated field plots receiving different numbers of *Trichogramma* through the oviposition periods of the 3 codling moth generations, are very promising and indicate a good potential for the use of these parasitoids in the control of codling moth in walnuts. In the context of the general literature on *Trichogramma* augmentation this represents one of the few examples where significant data have been obtained from field trials.

The lack of parasitism of sentinel eggs in the control plots provides good evidence that the differences in levels of nut infestation in release plots result directly from the activity of the *Trichogramma* released. Under low pressure from codling moth, as in the orchard at Grimes, a series of 12 releases of 100,000 *Trichogramma* was sufficient to reduce damage by 70%. However, under high pressure from codling moth, as in the orchard at Denair, higher release rates may be necessary to have the desired effect. Unfortunately, the *Trichogramma* releases were not correctly timed in Denair due to the unusual occurrence of a very large 2nd peak to the 1st generation flight and so it is not possible to draw any firm conclusions from the results at this site. This highlights the importance of correct timing of *Trichogramma* releases and the consequences of incorrect timing. In general, however, the results are very promising and indicate the potential of the *Trichogramma* as a tactic for codling moth management, but they also suggest that improvements are needed for this tactic to be used effectively in the control of the codling moth.

The *Trichogramma* feeding tests indicate the very important influence of a carbohydrate food source in promoting the longevity of the adult parasitoids. Surprisingly, molasses failed to support an improvement of longevity and aphid honeydew did not support the parasitoids as well as honey or simple sugars. Honey was also a very effective food source when diluted by as much as 1 in 10, although lower dilutions were useful. These tests suggest that there are several sources of supplemental food for *Trichogramma* which could increase their longevity by as much as 7 fold. Further tests will be required to determine whether aqueous solutions of these supplemental foods can be readily applied to foliage and persist in the field and whether this would allow a reduction in the rates or frequency of *Trichogramma* releases.
Fig. 1. Parasitism of sentinel eggs

(a) Percent parasitism

(b) Tree height in ft

Trichogramma release rate (x 100,000)
Fig. 2. Monitoring the quality of the Trichogramma

Percent parasitized

Percent female

Date


Percent parasitized vs. Percent female graph showing fluctuations over time from April 6 to August 24.
Fig. 3. Canopy samples from Grimes

1st generation, June 14
ANOVA $F(4,14)=3.1$, $P=0.05$

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2nd generation, July 29
ANOVA $F(4,14)=13.5$, $P<0.001$

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3rd generation, September 6
ANOVA $F(4,14)=5.4$, $P=0.008$

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Trichogramma release rate (x 100,000)
Fig. 4. Canopy samples from Denair

Early 1st generation, June 2
ANOVA $F(5,17)=3.2$, $P=0.03$

Late 1st generation, June 24
ANOVA $F(5,17)=6.5$, $P=0.002$

2nd generation, Aug 9
ANOVA $F(5,17)=15.1$, $P<0.001$

Trichogramma release rate (x 100,000)
Fig. 5. Supplemental food for Trichogramma

(a) ANOVA $F(7,72)=64.1$, $P<0.001$

(b) ANOVA $F(6,63)=53.5$, $P<0.001$

Mean longevity in days

Adult food source

Wat Sucr S/Yst Fruct F/Yst Mol Honey Hedew

Dilutions of honey

Wat 0.1% 1% 5% 10% 50% 100%