EVALUATION OF THE BIOSPRAYER FOR THE APPLICATION OF TRICHOGRAMMA TO COTTON

Allen Knutson
Extension Entomologist
Texas Agricultural Extension Service
Texas A&M University
Dallas, TX

Abstract

A tractor mounted machine, termed the 'biosprayer', was evaluated for the application of Trichogramma wasps to cotton for bollworm control. Passage of Trichogramma host eggs through the biosprayer reduced wasp emergence by 22%. Additional mortality resulted from predation, primarily fire ants. In the absence of predators, rain and dew, 88% of the applied host eggs were retained on cotton leaves for three days. Most (79%) of the host eggs retained by the cotton canopy were deposited in the plant terminal. Application of 100,000-200,000 Trichogramma pupae per acre twice a week did not consistently increase parasitism of bollworm eggs in field plots.

Introduction

Trichogramma are very tiny wasps that parasitize and kill eggs of bollworms and budworms in cotton. Several species of Trichogramma occur naturally in many cotton growing regions. However, these beneficial insects are often not abundant enough to significantly reduce bollworm numbers. Several companies rear Trichogramma in large numbers and sell them for release in cotton for control of bollworms and budworms. While research studies have shown that it is technically feasible to control certain caterpillar pests of some crops under optimum conditions with Trichogramma, the release of Trichogramma in cotton is not commonly practiced in the U.S. (King et al., 1995). Major constraints to the use of Trichogramma include high cost of production, sensitivity to insecticide drift, a very short application interval, variable shelf life, highly variable level of pest control and the difficulty in application. (Ridgeway et al. 1996).

This study addresses one of these constraints, the lack of efficient application technology. Trichogramma are sold as pupae within host (moth) eggs. Host eggs can be distributed within the field as loose eggs or as eggs glued to cards. Egg cards can be placed in or fastened to crop plants but this is labor intensive and not cost effective for commercial agriculture. Methods to broadcast Trichogramma pupae by aircraft have been developed (Bouse and Morrison, 1985). Technology to apply Trichogramma by ground was recently developed by Louis Tedders at the USDA ARS Fruit and Tree Nut Research Laboratory in Georgia. Originally designed to apply lacewing eggs to pecan tree foliage, the machine can also apply host eggs containing Trichogramma. This ARS invention, herein termed the biosprayer, is being commercially developed to apply several species of beneficial organisms to a number of field and tree corps.

The objective of this study was to evaluate the use of the biosprayer for applying Trichogramma to cotton for bollworm control.

Materials and Methods

All studies were conducted in 0.5 acre plots of cotton at the Texas A & M Research and Extension Center at Dallas, Texas. The study field was planted to 'Delta Pine 50' cotton on 36 inch row spacings with a final plant population of 51,000 per acre. Plots were treated with insecticides for early season pests but received no insecticide after mid-July. Trichogramma were reared on eggs of the tobacco moth, *Ephestia elutella,* and shipped overnight from the commercial insectary. Moth eggs, termed host eggs, containing Trichogram-ma pupae were mixed in a 1:8 solution of "biosticker" and water and were then applied to cotton foliage with the biosprayer at a rate of 80 mls of spray solution per 100 row feet (2.4 gals./acre). The biosprayer was mounted on a three point hitch behind a tractor traveling at 3.4 mph. The spray solution containing Trichogramma pupae was dispersed into an airstream which carried the solution onto the cotton canopy. Adhesives in the "biosticker" held the pupae on the leaf after the spray solution dried.

Results and Discussion

Effect Of The Biosprayer On Wasp Emergence

The effect of passage through the biosprayer on wasp emergence from host eggs was measured using three different methods on three different dates. In each method, a sample of loose eggs, termed "after application" was mixed with the "biosticker" and water (1:8) and applied through the Biosprayer. Eggs were collected and held for adult emergence. A second sample of eggs was taken from the same shipment but was not applied through the Biosprayer. This sample, termed "before application" was also held for adult emergence and served as a control.

In test A, loose eggs from the "before application" sample were placed on individual discs of cotton leaf using a fine brush wetted in the spray solution. The "after application" sample was obtained by collecting the spray solution from the Biosprayer into a graduated cylinder and then removing the eggs to individual discs of cotton leaf with a fine brush. Eggs from both samples were placed individually in egg trays, covered with a glass lid, and held at room temperature in an air-conditioned laboratory.

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Test B, conducted August 4, was similar except host eggs from both samples were placed individually in small glass vials tightly sealed with a rubber stopper. Leaf discs were not included.

In Test C, conducted August 9, the "after application" eggs were applied with the Biosprayer to cotton plants in the field. Treated plants were searched for host eggs which were then removed on leaf discs and placed in egg trays. The "before application" egg sample was placed on discs of cotton leaf as in Test A.

In all three tests, passage through the biosprayer reduced adult emergence by 20-22 % (Table 1). Wasp emergence from host eggs prior to application averaged 81-88% compared to an average of 56-66% after application through the biosprayer.

These results suggest host eggs suffered some injury while passing through the biosprayer, resulting in fewer adults emerging from parasitized eggs. Modifications of the sprayer are underway to reduce this effect. Depending upon the success of these modifications, release rates may need to be increased slightly to compensate for the reduced emergence if the reduction is consistent and known.

<u>Fate Of Host Eggs On Cotton Foliage</u>

Trichogramma pupae within host eggs face several hazards once deposited on the cotton leaf. Pupae may die from adverse environmental conditions (high temperature, low humidity) on the leaf surface, host eggs may be fed upon by insect predators such as fire ants before wasps emerge, or the host egg may fall from the plant and the Trichogramma pupa within die when exposed to high soil temperatures or other factors. Several studies were conducted to determine the retention time of host eggs on the leaf, wasp emergence from host eggs, and predation on host eggs.

<u>Egg Retention and Adult Emergence</u>

On August 10, host eggs were applied through the biosprayer and collected into a graduated cylinder. Parasitized (black) eggs were removed from the spray solution with a fine brush and placed on a the upper surface of a terminal cotton leaf. A total of 25 eggs were placed on one leaf of five different plants spaced along 4 different rows for a total of 20 leaves (500 eggs). Host eggs remained in the spray solution for up to 90 minutes before placement on the leaf. A small arrow was drawn with a marker near each egg on the leaf to identify the initial position of each egg. The petiole of each leaf with eggs was circled with Stickum to prevent access to the eggs by fire ants, lady beetles and other predators. A sample of 4-6 leaves with host eggs was collected after 1,3,4 and 5 days. Leaves with host eggs were placed individually in large plastic plates with moist filter paper and held in the laboratory for 12 days to allow parasite emergence. The number of retained eggs per leaf and final adult emergence from host eggs was determined.

Most (82%) of the host eggs applied with the brush remained on the leaf for five days (Table 2). Weather conditions were hot and dry during the study with no dew or rain. The "biosticker" is water soluble so rainfall or heavy dew would likely increase loss of host eggs from foliage.

Emergence of wasps from host eggs after one day in the field was 63 % Table 2), which confirmed results from the previous study (Table 1). Adult emergence appeared to decline with increasing time in the field as emergence after 3, 4 and 5 days was 52, 26 and 48%, respectively. Low relative humidity and high temperatures on the leaf surface may have contributed to mortality of pupae in host eggs exposed for several days.

The number of days after application required for adult emergence under field conditions was estimated to be no more than five days in this study. Results demonstrate the importance of applying host eggs which are within 1-2 days of yielding the adult Trichogramma to reduce field mortality.

<u>Impact Of Predation On Host Eggs In Cotton</u>

Lady beetle adults and larvae, tarnished plants bugs, big eyed bugs and fire ants were observed feeding on egg cards placed on cotton in the study plots. Studies were conducted to measure predation on host eggs applied on cards and as loose eggs applied with the biosprayer.

In the first study, cards of host eggs 0.5 X 1 inch were stapled individually to a terminal leaf on 60 different plants. These cards were termed "open" cards as they were accessible to predators. Plants with eggs were spaced one row apart along three transect of twenty plants each across the center of the plot. A second group of cards, termed "closed cards", were placed inside 1 X 2 inch heavy paper sleeves to exclude predators. Sleeves were cut from penny coin holders and 10-15 holes were punched through the sleeve with a push pin to allow adult wasps to escape. The egg card was placed inside the sleeve and both ends were stapled shut. These "closed" egg cards were stapled individually to 60 different plants arranged along three transects of 20 plants each in the same plot as the "open" egg cards. Both "open" and "closed" cards were placed in the field 2-3:00 p.m. on July 25. Five hours later, each card was examined in the field for the presence of insect predators. One and two days later, every other card of each type was collected and returned to the lab. Each egg card was examined to estimate the percent of the eggs consumed or removed by fire ants and other predators.

Predators, primarily fire ants, were present on 22 % of the "open" cards and 27 % of the "closed" cards five hours after placement in the field (Table 3). After one day in the field, predators had destroyed 24-50% of the host eggs. In a similar study conducted in the absence of fire ants (Garden

City, TX), only 3% of the host eggs on open cards were removed by predators after 2 days in the field.

Placing egg cards inside the coin holders did not protect the eggs from preda-tors (Table 3). The staple on each end of the coin tube did not completely seal the tube from entry by fire ants. Also, fire ants enlarged the pin holes, either before or after entry, which permitted ready access to the egg cards by more or larger ants. Results suggest any type of release device must be designed to protect host eggs from fire ants when these predators are present.

In the second study, host eggs were applied on August 9 with the biosprayer to 0.5 acre plots of cotton. Immediately after application, plants in the center two rows of each plot were searched for host eggs. Once a host egg was found, a small arrow was drawn on the leaf indicating the position of the host egg. A red ribbon was then tied to the leaf petiole and numbered to assist in relocating the host egg. A total of 33 host eggs were located on individual plants in each plot. Eggs were examined daily for 6 consecutive days.

In the absence of predators, about 80 % of the eggs were retained on the leaves after one day (Table 4). Retention declined to 35% after 3 days and 17 % after 6 days.

Host egg loss was due to both predation and failure of the spray solution to adhere the egg to the leaf (detachment). The relative contribution of predation and detachment was estimated using this and the previous study (Table 2). Predation was estimated to be 13, 53% and 53 % after 1, 3 and 4 days exposure in the field, and egg detachment was estimated to be 7%, 12% and 18% (Table 5).

Distribution Of Host Eggs In Cotton Canopy With Biosprayer

This study was designed to determine the distribution of host eggs in the canopy after application with the biosprayer. Experience had shown that host eggs applied with the biosprayer would adhere to white paper index cards. Host eggs were much easier to see on index cards than cotton leaves, and thus were used to simulate cotton leaves positioned at different levels in the canopy. Plants were about 32 inches high so a single card was placed at 10, 20 and 30 inches above the soil in each of 54 plants located in the center ten rows of a 0.5 acre plot. Cards were positioned flat against a leaf and stapled to the leaf at four corners. The top leaf was fastened to a terminal leaf. The crop had reached its maximum leaf surface area at this time and had terminated new growth.

Host eggs were applied at a rate of 800,000 host eggs per acre to the 0.5 acre plot with the biosprayer on August 11. The plot was treated twice on this date (total 1.6 million host eggs per acre) to increase the opportunity to detect eggs on the index cards. Tractor speed was 3.4 mph, blower speed was 40 mph, and sprayer output was 80 mls/100 row feet or 2.4 gals/acre. The biosprayer was equipped with two spray nozzles, each positioned 4-6 inches above the center of a single row of cotton. Eggs were applied from 3-4 p.m. Conditions were 95 F with a wind speed of 5 mph.

As expected, most of the host eggs were deposited in the terminal of the plant (Table 6). Sixty percent of the cards located in the terminal had one or more host eggs and 79 % of the 364 eggs recovered on cards were on the cards located in the top (terminal) of the plant. Placement of host eggs in the terminal is presumably preferred because bollworm eggs are usually deposited in the plant terminal. However, Trichogramma pupae in host eggs placed in the terminal may experience greater mortality if exposed to lower relative humidities and higher leaf surface temperatures than those placed lower in the canopy.

Parasitism Of Bollworm Eggs In Plots Treated With The Biosprayer

Host eggs containing Trichogramma pupae were applied to 0.5 acre cotton plots with the biosprayer at the Texas A&M Research and Extension Center at Dallas Texas. Plots were established in each of the four corners of a 28 acre field of 'Delta Pine 50' cotton. Two plots received Trichogramma while the remaining two plots were left untreated as a check. Upon receipt of the host eggs, the number of black eggs was counted in four 0.01 grams samples. Results found an average of 359 black eggs per 0.01 gram, compared to the insectary estimate of 400 eggs (40,000 per gram). A total of 1.4 grams (50,000 black eggs) of loose eggs was them applied to each of the 0.5 acre plots to achieve a release rate of 100,000 Trichogramma pupae per acre. Eggs were applied to the spray tank with a 1:8 solution of "biosticker" and water. Spray output was 2.4 gals/acre (80 mls/100 row ft) at a tractor speed of 3.4 mph and blower speed of 33-40 mph. High temperatures ranged from 93-105 during the study period.

Trichogramma were applied July 18, 21, 25 and 27 at a rate of 100,000 pupae/acre on each date. Rates were increased to 200,000 per acre on July 28 and August 1 and 5. The seven applications totaled 1 million host eggs per acre during the 3 week study. The cost of Trichogramma alone was $6/100,000 host eggs for a total cost of $60/acre.

Bollworm eggs were collected from plants in both the release and check plots every 1-2 days. Eggs were aged on the day of collection and only brown eggs were used to estimate percent parasitism. Bollworm eggs were held in the lab in individual cells until they either hatched, died for unknown reasons or turned black due to Trichogramma parasitism. (Knutson et al. 1995).

The seven releases appeared to increase parasitism of bollworm eggs above the check on some dates (July 21, 22, 27 and 28) (Table 7). However, of the 535 bollworm eggs collected during the study, overall parasitism in the release plots was only 20% compared to 10% in the check or no-release plots.

Quality control samples found about 90 % of the adults were female, which is desirable for high levels of egg parasitism. Earlier results (Table 4) estimated about 80% of the applied host eggs were present on the cotton foliage one day after application. Results also indicated about 65% of these host eggs would yield an adult Trichogramma (Table 1). Thus, a release rate of 100,000 black host eggs per acre would yield about 52,000 adult Trichogramma per acre (.8 X .65 X 100,000), or about 1 wasp per plant.

Density Of Adult Trichogramma In Release And Check Plots

Three techniques were tested to measure density of adult Trichogramma in the release and check plots described above. The first method involved cutting off terminals of cotton plants and immediately plunging them into a l liter container of 50% alcohol and 50 % water where they were vigorously shaken to wash off any adult Trichogramma. Twelve samples each of 25 terminals were collected August 12 from 7-8 a.m. from the release plots only. Samples were filtered with a Buchner funnel and the filter papers were examined with a dissecting scope for Trichogramma adults.

Trichogramma had been applied to the plot four days earlier at a rate of 200,000 eggs/acre. However, of the 300 plant terminals sampled, only one adult Trichogramma and 81 black host eggs were collected. As shown above, 1-2 adult Trichogramma should have been present per plant.

The second method involved placing 4 X 6 inch yellow sticky cards within the plant canopy throughout one of the release plots. After 4 days, cards were collected and examined with a dissecting microscope for adult Trichogram-ma. A total of 16 adult Trichogramma were found on 16 cards, with a range of 0-3 adults per card. Although examining the cards required patience and time, this method may be useful in measuring relative difference in adult density between release and non-release plots. However, results could not be used to estimate actual (per plant) density.

The third method involved removing a single, fully expanded leaf from a cotton terminal and immediately placing it a one gallon paper ice cream carton. A total of 50 leaves were collected per carton and 250 leaves were collected from each plot. A small glass vial was place in the top of the container with an inverted funnel. Trichogramma attracted to the light entered the vial and were captured inside. A total of 6 and 5 adult Trichogramma (per 500 leaves) was collected from the no-release and release plots, respectively.

None of these methods indicated adult Trichogramma were common in the release plots in spite of releasing 1 million host eggs over a two week period. This suggests that adults were short lived or left the study plots. If so, this may account for the low and inconsistent levels of bollworm egg parasitism (Table 7). Keller and Lewis (1985) reported dispersal of Trichogramma from the release fields was not the primary factor influencing inadequate control of bollworms.

Conclusions

Host eggs containing Trichogramma pupae on cards are quickly destroyed by fire ants and other predators in cotton. The biosprayer was effective in applying host eggs individually to cotton leaves and reducing losses due to predation during the first day. Most (79%) of the host eggs were deposited in the terminal region of the plant. After one day, about 80 % of the initial host eggs were still present on the leaf. In this study, an estimated 7 % of the eggs detached and another 13 % were removed by predators during the first day. After 3 days, 12 % of the eggs had detached and an estimated 53 % had been removed by predators, primarily fire ants. Application of host eggs through the biosprayer reduced adult emergence by about 22 %. Emergence continued to decline as host eggs remained in the field.

Trichogramma emergence from host eggs could be increased by 1) modifying the biosprayer to reduce impact of host eggs in the spray system which leads to reduced wasp emergence and 2) timing the release of host eggs so that wasps emerge within (preferably) 1 or at most 2 days and thus escape mortality due to predation, low relative humidity, high leaf temperatures and detachment.

The application of 100,000-200,000 Trichogramma pupae per acre twice a week with the biosprayer did not consistently increase parasitism of bollworm eggs in 0.5 acre plots. Additional research is necessary to reduce the cost of Trichogramma and increase levels of bollworm egg parasitism before releasing Trichogramma is economically feasible for cotton production.

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References

Keller, M. A. and W. J. Lewis. 1985. Movements by Trichogramma pretiosum released into cotton. Southwestern Entomologist Supl No. 8:99-109.

King, E. G., K. R. Hopper and J. E. Powell. 1985. Analysis of Systems for Biological Control of Crop Arthropod Pests in the U.S. by Augmentation of Predators and Parasites. p. 201-227 in eds. M. Hoy and D. Herzog. Biological Control in Agricultural IPM Systems. Academic Press.

Ridgway, R. L., M. N. Inscoe, and K. W. Thorpe. 1996. Biologically based insect control products: successes, learning experiences and opportunities. Presentation at Annual Meeting of the Entomological Society of America. Las Vegas, Dec. 1995.

Knutson, A.E., C. Sansone, M. Logan and B. Baugh. 1995. Methods for evaluating the release of Trichogramma for bollworm control in cotton. Proceedings Beltwide Cotton Conferences 2: 1032-1033.

Table 1. Emergence of Adult Trichogramma From Host Eggs Before and After Application With the Biosprayer. Dallas. 1995.

Test	Before Application	After Application	Difference
A	81%	59%	22%
B	88%	66%	22%
C	86%	56%	20%

Table 2. Retention of Host Eggs and Adult Emergence From Host Eggs Applied to Cotton Leaves by Hand After Collection From the Biosprayer. Predators Excluded. Dallas. 1995.

	Days in Field			
	1	3	4	5
Host Eggs Present	93%	88%	82%	82%
Adult Emergence	63%	52%	26%	49%

Table 3. Predation Of Host Eggs On "Open" Cards Exposed to Predation Or "Closed" Cards Placed In Paper Sleeves To Exclude Predation. Dallas. 1995.

		Cards with Predators	Eggs Removed or Destroyed After:	
Treatment	No. Cards	After 5 Hours	1 Day	2 Days
Open Card	60	22%	24%	31%
Closed Card	60	27%	50%	57%

Table 4. Loss Of Host Eggs On Cotton Leaves After Application With The Biosprayer. Predators Present. Dallas. 1995.

	Number of Host Eggs Remaining After Day						
	0	1	2	3	4	5	6
Rep A	33	28	17	14	10	7	4
Rep B	33	25	15	9	9	9	7
Average %	100	80	48	35	29	24	17

Table 5. Estimation Of Host Egg Loss Due To Predation And Detachment From Cotton Leaf. Dallas. 1995.

	Day 1	Day 3	Day 4
Predators Excluded Table 2	93%	88%	82%
Predators Present Table 4	80%	35%	29%
Predation	13%	53%	53%
Detachment	7%	12%	18

Table 6. Distribution Of Host Eggs In The Cotton Canopy After Application With The BioSprayer. Dallas. 1995.

Position of Card in Canopy	Card With Egg(s)	% Of Total Eggs	Ave. No. Eggs/Card
Top	60%	79%	5.7
Middle	37%	14%	0.9
Bottom	20%	8%	0.5

A total of 364 host eggs were recovered on 158 cards

Table 7. Percent Parasitism of Bollworm Eggs By Trichogramma In Field Plots. Host Eggs Applied with Biosprayer. Dallas. 1995.

	Sample Date				
Plot	7/21	7/22	7/23	7/24	7/25
Release	34%	42%	8%	12%	0%
No-Release	0%	8%	5%	6%	6%

	Sample Date					
Plot	7/26	7/27	7/28	8/3	8/6	. Ave.
Release	5%	25%	18%	0%	0%	20%
No-Release	0%	0%	4%	0%	8%	10%

Total Sample of 452 bollworm eggs