REEVALUATION OF *TRICHOGRAMMA* RELEASES FOR SUPPRESSION OF HELIOTHINE PESTS IN COTTON

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Abstract

Field studies were conducted at 3 sites over 2 years to reevaluate the feasibility of releasing *Trichogramma* wasps for suppression of heliothine pests (*Helicoverpa zea* + *Heliothis virescens*) in cotton. Overall, releases of *T. exiguum* significantly increased egg parasitism and reduced bollworm egg hatch in release plots. However, there was not a proportional reduction in number of instar 5 larvae or boll damage, and upon final assessment, there was no significant difference in yield between release and control plots. Analysis of 1996 bollworm data revealed that this lack of suppression may have been in part due to the interaction of density-dependent egg and larval mortality factors (compensatory mortality).

Introduction

The use of *Trichogramma* wasps to suppress heliothine pests in cotton has had a checkered history. Results from large- and small-scale studies (e.g. Stinner et al. 1974, Johnson 1985, King et al. 1985, Lopez and Morrison 1985) have been variable and for the most part poor, and several authors (e.g. Knipling & McGuire 1968 and King et al. 1985) suggested that *Trichogramma* spp. were not good candidates for use in biological control programs in cotton.

However, no evidence was provided to explain why these past efforts failed. One possible reason may have been the use of poor quality *Trichogramma*. Keller & Lewis (1985) demonstrated high brachyptery (non-functional wings) and low longevity in *T. pretiosum* Riley reared and released in a large-scale pilot program conducted in North Carolina and Arkansas cotton fields (King et al. 1985). Another possible reason may have been improper species selection. Past (Keller 1985) and our own recent surveys (unpublished data) indicate that *T. exiguum*, not *T. pretiosum*, is the most predominant *Trichogramma* species in North Carolina cotton fields. Other reasons for failure may include inadequate release rates, untimely releases, questionable handling and release techniques, and *Trichogramma* exposure to insecticides.

The past decade has brought increased evidence that species selection and quality of laboratory-reared *Trichogramma* are critical to success of augmentation programs (Noldus 1989). Relative quality of biological features such as sex ratio, fecundity, longevity, brachyptery, and host acceptance behavior determine the overall quality (parasitization capacity) of *Trichogramma* spp., and standardized tests have recently been developed to quantify quality (Ceruttii & Bigler 1991, Laing & Bigler 1991, van Bergejik et al. 1989).

The following study was conducted to reevaluate the feasibility of releasing *Trichogramma* wasps for heliothine (*H. zea* + *H. virescens*) suppression in cotton. We released *T. exiguum* Pinto & Platner because of its predominance in North Carolina cotton fields and incorporated extensive quality controls into protocols of this study to eliminate species selection and quality of *Trichogramma* as variables.

Materials and Methods

Trichogramma Source and Quality

Trichogramma exiguum Pinto & Platner were reared from parasitized *H. zea* and *H. virescens* eggs collected in cotton fields located near Plymouth, North Carolina. Species identity was confirmed by John Pinto, University of California at Riverside. Parasitoids were shipped to BIOTOP (Valbonne, France) for mass production and formulated for field release. The formulation consisted of waxed cardboard capsules (approx. 5 cm^3) each containing 600-1400 *T. exiguum* developing inside *Ephestia kuehniella* Zeller eggs. Four small holes made during the encapsulation process were just large enough for adults to escape but small enough to prevent most predators from entering the capsules.

Shipments from BIOTOP to NCSU were made weekly through commercial air freight with each shipment consisting of two cohorts of parasitoids whose development was staggered approximately 45 Celsius degree days apart. For each shipment, a datalogger (HOBO XT Temperature Logger, Onset Computer Corp., 536 MacArthur Blvd., Box 3450, Pocasset, MA 02559-3450) programmed to record temperature hourly was placed alongside capsules to monitor temperature fluctuations. This temperature data allowed estimation of degree day accumulation during shipping and handling so accurate predictions of adult emergence could be made. Upon arrival, parasitoids were immediately held at 25°C; 80 % RH, and 16:8 L:D period, until required for experimental use.

To ensure that high quality parasitoids were used throughout the experiment, field and laboratory quality control samples were taken for each released cohort. Quality measurements included % field and lab emergence, % female brachyptery, sex ratio, and female adult longevity.

Experimental Plots

The experiment was designed as a randomized complete block with two treatments and one check, three replications,

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and field sites acting as blocks. All field sites were located near Plymouth, NC. The treatments were *T.exiguum* release, foliar bollworm insecticide, and control (not treated with *T. exiguum* or bollworm insecticides). Within each site, *Trichogramma* release plots were located downwind (based on prevailing wind direction for that area) approximately 300 m from control plots to reduce possibility of parasitoid dispersal into control plots. Release and control plots were approximately 0.4 ha in size. Because availability of land was restricted, six outer rows of the control plots (approximately 0.03 ha) were used for insecticide treatment plots.

***T. Exiguum* Release and Insecticide Application**

In 1996, nine releases (spaced 3 to 4 days apart) were made in each release plot at an average rate of 108,357 females/ha/release. Each release contained a single cohort of *T. exiguum* housed in capsules with adults programmed to emerge within 12-24 hours after field release. The capsules were hand-placed at 42 release sites evenly spaced throughout each release plot. Each release site had one waxed cone-shaped paper cup, placed 0.5 m above soil surface in the cotton canopy, into which capsules were placed. In 1997, six releases (spaced one week apart) each containing two developmentally staggered cohorts (in separate capsules) of *T. exiguum* were made in each release plot at an average rate of 139,000 females/ha/cohort/release. One set of capsules contained adults programmed to emerge within 12-24 after field release and the other 72-84 hours later.

Insecticide-treated plots were sprayed when North Carolina State Univ. Cooperative Extension Service egg or larval thresholds were met or exceeded (Bacheler 1996). When scouting suggested treatment, lambda cyhalothrin (Karate 1EC, Zeneca, Inc., Wilmington, DE) was applied @ 0.04 kg ai/ha. This resulted in three applications spaced 10-14 days apart in both years.

Evaluation of *T. Exiguum* Releases

Pheromone trap catches (*H. zea* and *H. virescens*) and heliothine egg densities (eggs/100 terminals) in each release and control plot were recorded every 3 to 4 days throughout the ovipositional period. Daily rainfall measurements were also recorded throughout this period.

The level of parasitism in *T. exiguum* release and check plots was determined by collecting 100 heliothine eggs throughout each plot, and holding them under laboratory conditions. In order to standardize parasitism, only tan colored eggs oviposited in the top 30% of the cotton canopy were collected. Eggs were checked daily and hatched larvae removed. After 6 d, eggs were checked for parasitism (indicated by shiny black appearance of egg chorion). Two collections, spaced one week apart, were made each year.

Individual cohorts of naturally and freshly oviposited heliothine eggs (30 or 75 eggs/cohort/plot) were tagged and fate followed in each *T. exiguum* release and control plot. Eggs were categorized as either hatched, preyed-upon, dislodged from plant, or parasitized. Four cohorts, spaced 7 days apart, were followed in 1996, and two cohorts, also spaced 7 days apart, were followed in 1997.

Heliothine larval infestation and fruit damage were assessed in all plots on a weekly basis. For each sampling date, 100 squares and bolls were checked in each plot for damage and presence of larvae. Final yield was estimated by machine-harvesting a known area in each plot and converting this data to a per hectare basis. Lint cotton yield was estimated using 38% gin-out.

Results and Discussion

***Trichogramma* Quality**

High quality *T. exiguum* were released throughout the experiment in both years (Table 1). Thus, species selection and quality of released parasitoids were not an issue in our study.

Evaluation of *T. Exiguum* Releases

The occurrence of the F_3 heliothine ovipositional period and timing of *T. exiguum* releases varied from year to year (Fig. 1). However, initial releases in both years were made approximately one week prior to the onset of the F_3 heliothine ovipositional period. Based on a cotton microclimate study (unpublished data), we found that all areas in cotton fields were suitable (temperature-wise) release sites for *T. exiguum* capsules. However, suitability on the soil surface heavily depended on level of canopy enclosure, cloud cover, and soil moisture content.

Based on collection of tan-colored heliothine eggs, the overall level of heliothine egg parasitism was significantly higher in *T. exiguum* release plots compared with control plots (Table 2). In 1996, parasitism levels ranged from 62 to 86% in release plots and 16 to 64% in control plots. In 1997, parasitism levels ranged from 71 to 94% in release plots and 8 to 81% in control plots. In general, parasitism levels in control plots were substantially low during the initial F_3 heliothine ovipositional period, but steadily increased throughout the heliothine ovipositional period. Thus, our *T. exiguum* releases had more of an impact on heliothine eggs laid early in the ovipositional period than on eggs laid later in the season. In a separate study (unpublished data), we found that cotton plant size (e.g. height, no. of nodes, and leaf surface) did not significantly impact parasitism levels incurred by natural or high released populations of *Trichogramma.*

Unfortunately, estimates of % parasitism often do not accurately reflect impacts of parasitoids on pest populations. Therefore, we examined the fate of naturally oviposited heliothine egg cohorts in release and control plots, to accurately assess the impact of *T. exiguum* releases on heliothine egg populations in the presence of other egg mortality factors. Our egg fate study revealed that, overall (1996 and 1997), the number of heliothine eggs lost to parasitism was increased by 84% in release plots (Table 3). We also found a strong correlation between rainfall and parasitism (Fig. 2). During periods of rain, the % of heliothine eggs lost to parasitism was substantially reduced with the amount of reduction dependent on the intensity and duration of rainfall. Despite the negative impact of rainfall, overall (1996 and 1997), heliothine egg hatch was reduced by 60% in release plots compared with control plots.

However, upon final assessment, there was not a proportional reduction in number of instar 5 heliothine larvae or boll damage, and there was no significant difference in yield between release and control plots (Table 4). Analysis of 1996 heliothine population data revealed a very strong correlation between relative larval mortality (difference between hatched egg density and fifth instar larval density) and neonate (hatched eggs) density (Fig. 3). In other words, density-dependent larval mortality factors impacted the lower neonate densities in release plots to a lesser extent.

Conclusions

Our study showed that releases of high quality *T. exiguum* significantly increased bollworm egg parasitism and significantly reduced bollworm egg hatch. However, reduction of bollworm egg hatch did not result in a proportional reduction in instar 5 larval populations or boll damage. This lack of suppression may have been related to the very high bollworm population in 1996, and/or the resulting interaction between egg and density-dependent larval mortality factors. In this study, *H. zea* was the predominant heliothine species, and it is likely that density-related larval mortality factors such as cannibalism and predation had an offsetting effect on the additional egg mortality produced by *Trichogramma* parasitism. This interaction (compensatory mortality) may partially explain the lack of boll damage suppression reported in this study as well as in past *Trichogramma* release studies in cotton. Because of the occurrence of compensatory mortality, the egg stage may not be an appropriate target for biological control of heliothines in cotton.

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Table 1. Overall mean (± SD) quality measurements for released cohorts of *T. exiguum*. Plymouth, NC, 1996 and 1997.

	Lab quality [a]				Field quality
Year	% lab emergence	% females	% female brachyptery	female longevity (days)	% field emergence
1996	92 ± 7	62 ± 5	3 ± 2	15 ± 4	97 ± 2
1997	95 ± 2	69 ± 4	2 ± 1	24 ± 3	94 ± 3

[a] *Trichogramma* reared at 25 °C, 80% RH, and photoperiod of 16:8 (L:D) h.

Table 2. Overall mean (± SD) % parasitism of naturally oviposited heliothine (*H. zea* + *H. virescens*) eggs in release and control plots. Plymouth, NC, 1996 and 1997.

	% parasitism			
	T. exiguum		Control	
Year	Mean (±	Range	Mean (± SD)	Range
1996	75 ± 10 a	62 - 86	40 ± 18 b	16 - 64
1997	82 ± 9 a	71 - 94	44 ± 31 b	8 - 81

Within a row, values followed by different letters are significantly different by Fisher's Protected LSD (P=0.05).

Table 3. Overall fate of heliothine (*H. zea* + *H. virescens*) eggs oviposited in *T. exiguum* release and control plots. Plymouth, NC, 1996 and 1997.

		Percentage of bollworms that were:			
Year	Treatment	Hatched	Preyed upon	Dislodged [a]	Parasitized
1996	*T. exiguum*	5 ± 4 a	15 ± 7 a	44 ± 23 a	35 ± 23 b
	Control	13 ± 10 b	24 ± 14 a	45 ± 19 a	18 ± 15 a
1997	*T. exiguum*	9 ± 4 a	14 ± 12 a	43 ± 11 a	34 ± 8 a
	Control	23 ± 8 b	12 ± 8 a	44 ± 15 a	20 ± 14 a

Values in a column within each year followed by different letters are significantly different by Fisher's Protected LSD (P≤0.05).

[a] Dislodged indicates that egg was removed from plant by action of predators, rain or wind; includes eggs that may have been parasitized prior to turning black.

Table 4. Mean (± SD) larval infestation, boll damage, and lint yield upon final assessment in *T. exiguum* release, control, and insecticide-treated plots. Plymouth, NC, 1996 and 1997.

Year	Treatment	Infestation [a]	% damaged bolls [b]	Lint yield [c] (kg/ha)
1996	*T. exiguum*	17 ± 6 a	39 ± 18 a	573 ± 38 b
	Control	20 ± 11 a	44 ± 11 a	506 ± 163 b
	Insecticide	1 ± 1 b	1 ± 1 b	857 ± 136 a
1997	*T. exiguum*	2 ± 2 a	13 ± 12 a	1214 ± 102 a
	Control	3 ± 2 a	22 ± 10 a	1123 ± 81 a
	Insecticide	0 ± 0 b	1 ± 1 b	1305 ± 97 a

Values in a column within each year followed by different letters are significantly different by Fisher's Protected LSD (P≤0.05).

[a] Number of instar 5 larvae found in 100 dime- and quartered-sized bolls per plot.

[b] Based on 100 quartered-sized bolls per plot.

[c] Based on machine harvest and 38% gin-out.

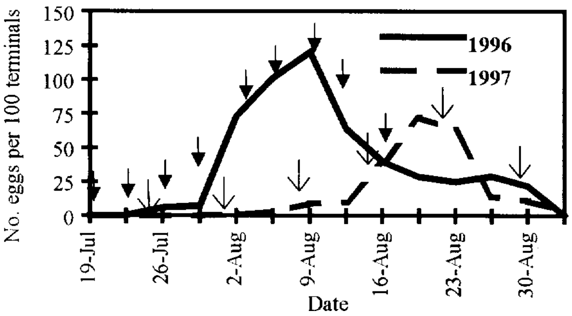

Figure 1. Average heliothine (*H. zea* + *H. virescens*) egg densities in release plots with timing of *T. exiguum* releases in 1996 and 1997 . Plymouth, NC.

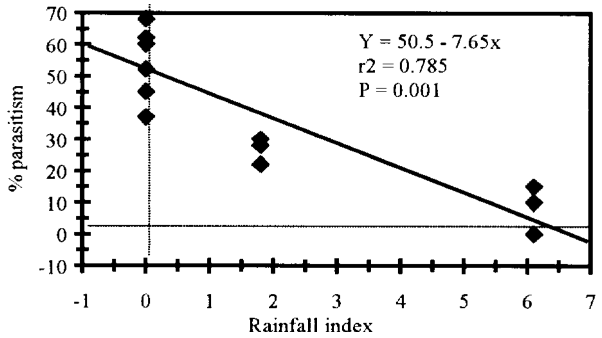

Figure 2. Relationship between rainfall and parasitism of bollworm eggs by *Trichogramma* wasps. Plymouth, NC, 1996. (Rainfall index = amount of rainfall (cm) during a 3-day period times the no. of days rain occurred during those 3 days.

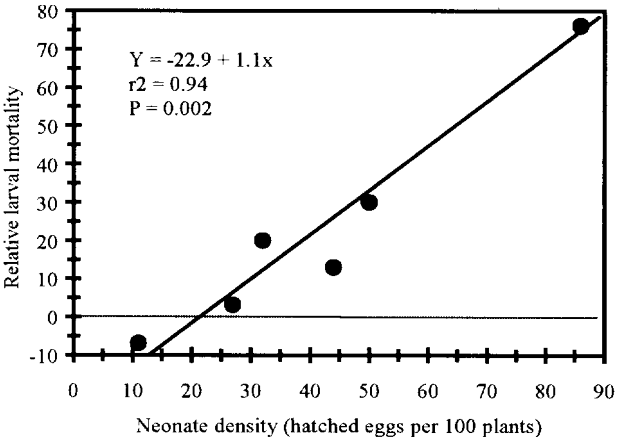

Figure 3. Relationship between density-dependent larval mortality and neonate density. Plymouth, NC, 1996. (Relative larval mortality = difference between neonate and fifth instar larval density).