MOVEMENTS BY TRICHOGRAMMA PRETIOSUM (HYMENOPTERA: TRICHOGRAMMATIDAE) RELEASED INTO COTTON[1]

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ABSTRACT

Qualitative studies of the flight behavior of Trichogramma pretiosum Riley, released into cotton for the control of Heliothis spp., were carried out using sticky traps. Most flight was detected within the cotton canopy. Dispersal from the field does not appear to have been responsible for the lower than desirable levels of pest control achieved.

INTRODUCTION

Trichogramma spp. are released each year on an estimated 200,000 ha or more in the United States (Ridgway et al. 1981) and are the most widely augmented entomophagous insect in the world today (King et al. 1984). In spite of this widespread use, attempts to experimentally demonstrate effective pest control using Trichogramma have been only partly successful (Stinner 1977, Ridgway et al. 1981). Dispersal from fields was among the factors listed by Ridgway et al. (1981) as reducing the efficacy of Trichogramma releases. Past work on Trichogramma dispersal has examined movements away from point release sites. Movements by these parasitoids occur in all directions, but at times are greater downwind (Schread 1932; Kot 1964; Hendricks 1967). Dispersal of radioactively labelled Trichogramma has been observed over distances of up to 600 m in one day (Stern et al. 1965). Allen and Gonzalez (1974) observed nonrandom movement away from a point release site; egg parasitization was lower around the release point than at nearby sites. The impact of dispersal on the efficacy of released Trichogramma has been postulated but has not been previously examined in conjunction with a large augmentation experiment.

We undertook a qualitative study of movements by Trichogramma pretiosum Riley released in cotton fields. Our research was conducted in conjunction with a U.S. Department of Agriculture pilot study to test the effectiveness of Trichogramma release technology, the timing of releases, and to evaluate the resulting level of control of Heliothis spp. Our study focused on flight behavior within and away from cotton fields, and was the first attempt to directly measure dispersal of released Trichogramma after inundative releases dispersed through a crop field.

[1]Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

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MATERIALS AND METHODS

Studies were carried out in four cotton fields near Clinton, NC during July and August 1983 (Table 1). Two fields were selected for controls and T. pretiosum was released into the two other fields at rates of 400,000/ha/release as a part of the U. S. Department of Agriculture pilot test program (King et al. 1984). Trichogramma pretiosum reared from Sitotroga cereallela (Olivier) were released as pharate adults from a small airplane in the early morning between 800 and 900 h.

TABLE 1. Field Sampling Plan.

Treatment	Code	Field size (ha)	Cotton variety	No.of sticky traps In field	Perimeter
Control	1C	1.1	Coker 310	8	10
	3C	1.6	Coker 315	8	12
Trichogramma release	1T	5.3	Coker 310	8	12
	3T	4.6	Coker 315	8	13

Sampling of Trichogramma spp. was conducted with sticky traps and egg cards. Sticky traps were made from 5 cm diam. x 20 cm long white PVC drain pipe. An area 16.7 cm h was marked on the surface of the pipe with a knife such that a 100 cm^2 cross-sectional area faced the wind. Stickem Special®, diluted with chloroform, was painted on the pipe surfaces. Sticky traps were held on 2 x 4 cm wooden poles with two large binder clamps (no. 10, Charles Leonard, Inc., Glendale, NY), one clamp above and one below each trap. In cotton-fields, three sticky traps were mounted on each supporting pole with trap centers at 1/2 cotton canopy height, at canopy height, and at 0.5 m above the canopy. Traps around the field perimeters were mounted at maximum cotton canopy height, and at 0.5 m above maximum canopy height. The traps were placed at eight random locations inside cotton fields and were spaced evenly around the perimeters of fields, usually three traps/field side (12 total). Due to irregular field shapes, field 1C had 10 perimeter traps and field 3T had 13 perimeter traps.

Sticky traps were chosen to meet the objectives of the experimental design. Circular traps were selected because they capture small insects from all directions. Trap catch increases as wind speed increases (Johnson 1950). Assuming all traps were exposed to virtually the same winds in the field, variations in wind speed should have affected all traps equally at a given height with respect to the cotton canopy. Traps were placed in cotton to detect the heights where flight by released T. pretiosum was most frequent relative to wild Trichogramma in control fields. Winds tend to be greater above than inside a plant canopy. Since trap catch varies with wind speed, traps above the canopy would generally catch more Trichogramma than traps inside the cotton canopy if aerial densities are the same at both heights. Therefore, direct comparisons of trap catch at different heights are not possible. Sticky traps were placed around the perimeter of cotton fields to catch dispersing individuals. Increases in trap catch around release fields without similar increases in catch around control fields would indicate dispersal of released T. pretiosum, assuming that no wasps were released outside of the boundaries of fields.

Samples were taken once prior to releases and in conjunction with three T. pretiosum field releases (Table 2). Since releases were made from a plane, traps placed in the field prior to releases would have caught T. pretiosum at the time of release and confounded trap catch results. Therefore, sticky traps were put out in the morning after wasps were released and were retrieved in the early morning before subsequent releases were made. After field exposure, traps were examined under a dissecting microscope and the number and sex were recorded of Trichogramma spp. caught. Assignment of males to morphological species groups was done by examining the length of antennal setae. Individuals with setae ca. 3X the width of the antennal flagellum were considered to be in the "T. pretiosum group" while individuals with setae ca. 2X the width of the flagellum were assigned to the "T. exiguum group." Samples of Trichogramma taken from these fields during releases confirmed that these were the two most common species in the experimental area (Hung et al. 1985). Differences among fields in numbers of Trichogramma caught/sticky trap were tested using analysis of variance for traps at each height and location (in-field or perimeter). The data were transformed (log 10 (n+1)) to stabilize variances in all analyses.

TABLE 2. Sticky Trap Sampling Dates.

Release date	Fields sampled	Sampling dates
Prerelease	1C, 1T	July 20-23
	3C, 3T	July 19-22
July 26	1C, 3C	July 26-28
	1T, 3T	July 26-27 & 27-28
July 28	All fields	July 28-31
August 3	All fields	August 3-6

Egg cards were made by gluing a piece of cheese-cloth (1 x 2 cm) holding 10 or more naturally deposited Heliothis zea (Boddie) eggs onto a piece of green index paper (2.5 x 3.8 cm). Cards were held flat on cotton leaf surfaces in the upper 2/3 of the canopy with a wire twistem passed through a hole in the card. Ten cards were put out at each location within fields corresponding to sticky trap locations. Egg cards were put out on July 20 (fields 1C, 1T) and July 27 (all fields). After 1 day of field exposure, egg cards were returned to the laboratory, isolated, and held for observations of parasite emergence.

To check for parasite longevity, a sample of T. pretiosum was collected prior to release in a 1 oz. plastic cup, allowed to emerge, and transferred to 9 mm X 50 mm petri dishes ca. 3 h after the onset of emergence. Either H. zea or S. cerealella eggs were provided as hosts and 1/2 of the individuals were provided with a droplet of 10% honey as a food source. These T. pretiosum were held in a screened enclosure adjacent to field 3T and checked daily for mortality.

RESULTS

Similar Trichogramma activity levels were observed among three of the four fields sampled prior to releases. Egg card samples revealed low levels of Trichogramma exiguum Pinto and Platner activity in fields 1C and 1T on July 20 (Table 3). In-field sticky traps caught similar numbers of Trichogramma in fields 1C, 1T, and 3T (ca. 0.1 wasp/trap/day; Fig. 1). Trap catches in field 3C were low; no Trichogramma were caught on traps within or at canopy height. Sparse cotton in the interior of field 3C, caused by high early-season seedling mortality, may have resulted in overall reduction in insect density throughout this field. Perimeter traps caught more Trichogramma around release than around control fields (Middle traps: $F(1,42) = 11.84$, $P < 0.001$; upper traps: $F(1,42) = 0.65$, $P > 0.40$). Initial differences in perimeter trap catches between release and control fields may have been due to differences in the vegetation surrounding each field. Field 1C was surrounded on three sides by woods and on one side by a watermelon field. Border vegetation around field 1T included a soybean field, a weedy roadway, and woods on

TABLE 3. Parasitism of Heliothis zea Eggs on Egg Cards.[a]

Date	Release date	Field	Parasitism n	Parasitism %	Identity n	Identity Species
July 20-21	Prerelease[b]	1C	8	4%	2	T. exiguum
		1T	8	1%	1	T. exiguum
July 27-28	July 26[c]	1C	8	0%	0	-
		3C	8	0%	0	-
		1T	8	18%	12	T. pretiosum
		3T	8	15%	12	T. pretiosum

[a]Ten egg cards were put out at each of eight randomly selected locations in each field.
[b]Mean % parasitization not significantly different ($t(14) = 1.13$, $P < 0.33$).
[c]Mean % parasitization significantly different between Trichogramma release fields and control fields ($F(1,21) = 6.07$, $P < 0.02$).

the remaining two sides. Field 3C was surrounded by grass lawns, a soybean field, and exposed soil along a roadway. Tobacco and corn fields were separated from field 3T by a hedgerow along one side, while a pond, a roadway, and a residence occurred on the remaining sides. Each type of border vegetation has a different insect fauna, and therefore would be likely to have different Trichogramma population levels (Keller 1985).

For each release of T. pretiosum, sticky traps in release fields caught significantly more Trichogramma at all heights than control fields (Fig. 1; lower traps: $F(1,89) = 29.75$, $P < 0.0001$; middle traps: $F(1,89) = 15.94$, $P < 0.0001$; upper traps: $F(1,89) = 7.97$, $P < 0.005$). Egg parasitization in release fields was low following the July 26 release.

Trichogramma pretiosum parasitized 18% of egg cards in release fields compared to no parasitism in control fields on July 27-28. Similarly, collections of naturally occurring tan (>1 day old) Heliothis spp. eggs revealed 7.0-12.5% parasitization in release fields and no parasitization in control fields (Lopez and Morrison 1985). Higher rates of parasitization of tan Heliothis eggs were observed following the releases on July 28 and August 3, 26.7-82.9% for release fields vs. 3.0-37.1% for control fields (Lopez and Morrison 1985). Sticky trap catches for these later releases had a different vertical profile of Trichogramma activity than those of the July 26 release. In particular, relatively more Trichogramma were trapped at canopy height in later samples compared to trap catch for July 26-28. Analysis of variance indicated a (Trichogramma release treatment) X (release date) interaction for middle traps ($F(2,89) = 6.57$, $P < 0.002$) suggesting that the behavior of released Trichogramma leading to capture at canopy height was different for the July 26 release.

A check of samples taken from releases on July 26 revealed an unusual incidence of brachyptery among these Trichogramma (Table 4). Perhaps superparasitism (Salt 1940) or unusual handling caused this condition and resulted in altered flight behavior and reduced levels of parasitization.

Samples taken on the 2 days following the July 26 release in cotton fields revealed a substantial reduction in Trichogramma flight activity on the second day following release (Table 5). There are three possible explanations for these data. (1) Significant movement from the field or (2) mortality may have reduced trap catch on the second day. Alternatively, (3) fewer of the remaining Trichogramma may have been flying after 1 day; these wasps may have spent more time resting or walking on the foliage while foraging for eggs rather than flying. Sticky trap catch on perimeter traps also declined on the second day following the July 26 release suggesting that movement from the field was at least in part responsible for the reduction in in-field trap catch.

Since the levels of egg parasitism were uniformly low following the first release, a switch to foraging while walking on the second day after release appears to be unlikely. There were no data that could reveal the relative level of mortality between days, but if brachypterous wasps are unable to find sufficient food, then mortality would be high after 1 day (see below).

Releases after July 26 produced higher levels of egg parasitization. Sticky trap catch following the July 28 release indicated high levels of Trichogramma activity within the cotton canopy and at canopy height in release fields (Fig. 1). Parasitism of naturally laid eggs following this release was high, up to 82.9% in field 1T (Lopez and Morrison 1985). Following the August 3 release, trap catch was relatively low in release fields, but still higher than catch in control fields (Fig. 1).

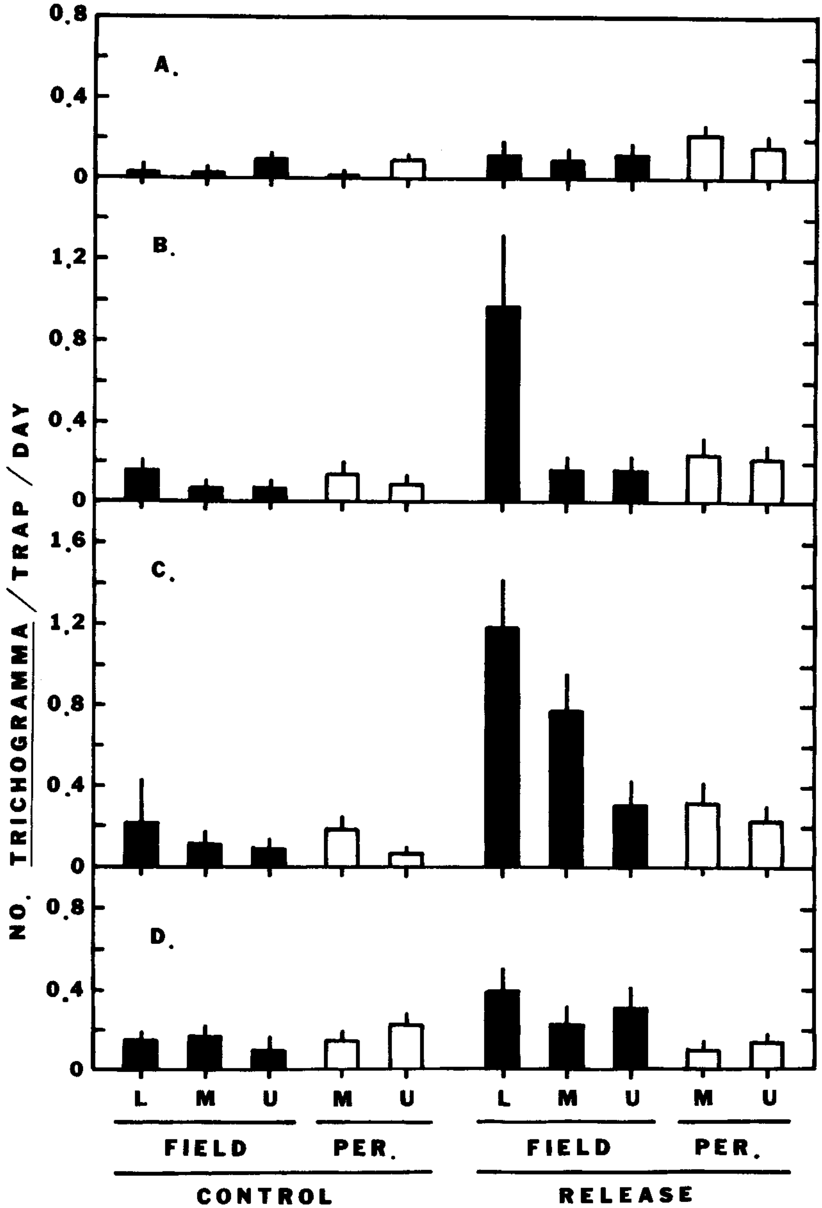

FIG. 1. Sticky trap catch of *Trichogramma* spp. in and around the perimeter (per.) of cotton fields following releases of *T. pretiosum*. Traps were mounted at 1/2 canopy height (L - lower), at canopy height (M - middle), and at 0.5 m above the cotton canopy (U - upper). A. Prerelease samples taken July 19-23. B. Postrelease samples taken July 26-28. C. July 28-31. D. August 3-6.

The level of parasitization of naturally laid Heliothis spp. eggs was intermediate, 26.7-55.6%, at this time vs. 3.4-37.1% in control fields (Lopez and Morrison 1985). The relatively low trap catch following the August 3 release cannot be adequately explained, but suggests low levels of flight activity.

TABLE 4. Proportion of Released T. pretiosum With Normal or Brachypterousa Wings.

Release date	Females			Males		
	n	Winged	Brach.	n	Winged	Brach.
July 26	137	0.19	0.81	144	0.53	0.47
July 28	229	0.72	0.28	155	0.60	0.40
August 10	153	0.81	0.29	118	0.75	0.25

[a]Individuals were considered to be brachypterous if one or both forewings were not fully extended.

TABLE 5. Daily Sticky Trap Catch of Trichogrammma spp. Following the Release of T. pretiosum in Cotton on July 26, 1983.

Position[b]	n	No. Trichogramma/trap[a]	
		26 July	27 July
In-field			
Lower	16	1.38 ± 0.60	0.56 ± 0.27
Middle	16	0.25 ± 0.11	0.06 ± 0.06
Upper	16	0.19 ± 0.10	0.13 ± 0.09
Perimeter			
Middle	25	0.36 ± 0.14	0.12 ± 0.07
Upper	25	0.36 ± 0.11	0.08 ± 0.06

[a]Mean ± 1 std. error.
[b]Traps were mounted at 1/2 canopy height (lower), at canopy height (middle), and 0.5 m above the cotton canopy (upper).

In contrast to prerelease samples, perimeter sticky traps did not catch significantly more Trichogramma around release fields than around control fields during releases (middle traps: $F(1,134) = 0.257$, $P > 0.25$; upper traps: $F(1,134) = 2.17$, $P > 0.10$). Apparently, natural populations in surrounding vegetation had increased in density since prerelease samples were collected. The absence of a large difference in perimeter trap catches between release and control fields suggests that few of the released wasps moved from the fields.

The species composition of Trichogramma caught on sticky traps indicated that while T. pretiosum was the most commonly collected species, T. exiguum also was found in significant numbers (Table 6). Since only males were identified, these data may not reflect actual species composition if sex ratios or behavior vary between species. Few T. exiguum were collected in samples of naturally deposited host eggs (Hung et al. 1985) and none were recovered in an egg-card sample on July 27-28. Perhaps the T. exiguum caught on sticky traps reflect movement through the cotton fields without parasitizing eggs.

Female T. pretiosum longevity in the insectary varied between 0.9 and 3.8 days (Table 7). Longevity was greater when either H. zea was provided as a host or when 10% honey was supplied as food. These data suggest that released T. pretiosum had a potential longevity of 1/2 to 4 days in the field.

TABLE 6. Species Composition of Male Trichogramma Caught on Sticky Traps.

Date	Location	Species group: Control	Species group: Release
July 19-23 (pre-release)	In field	1 ex.	-
	Perimeter	1 ex.	1 ex., 1 pr
July 26-28	In field	2 ex.	2 ex., 5 pr
	Perimeter	1 ex.	2 ex., 3 pr
July 28-31	In field	1 pr.	2 ex., 7 pr
	Perimeter	1 ex. 1 pr	1 ex., 3 pr
August 3-6	In field	1 ex.	1 ex., 2 pr
	Perimeter	2 pr.	1 ex., 3 pr
All releases	In field	3 ex., 1 pr	5 ex., 14 pr
	Perimeter	2 ex., 3 pr	4 ex., 9 pr

[a]Trichogramma species groups delimited by the relative lengths of antennal setae were: ex. = T. exiguum, short stout setae; pr. = T. pretiosum, long fine setae.

TABLE 7. Longevity of Insectary-Held Female _T. pretiosum_ When Provided with Droplets of 10% Honey and Either _H. zea_ or _S. cerealella_ as Hosts.

Release date	Host	Food source	n	Longevity (days)[a]
July 26	_H. zea_	10% honey	37	3.7 ± 0.33
July 28	_S. cerealella_	10% honey	10	2.2 ± 0.33
		none	10	0.9 ± 0.16
	H. zea	10% honey	20	3.8 ± 0.43
		none	10	1.4 ± 0.10
August 3	_S. cerealella_	10% honey	10	2.3 ± 0.44
		none	7	1.1 ± 0.20
	H. zea	10% honey	19	2.4 ± 0.29
		none	8	1.2 ± 0.18

[a]Mean ± 1 std. error.

DISCUSSION

With release rates of 40 _Trichogramma_/m^2, why were higher rates of parasitization of _Heliothis_ spp. eggs not observed? Factors that might have reduced the efficacy of the released wasps include dispersal from the field, high mortality caused by physical or biotic agents, poor fitness, insufficient numbers of _Trichogramma_ released, and field behavior inappropriate for the goal of pest control. The data presented here suggest that substantial movements from the field by _T. pretiosum_ did not occur. Sticky-trap catch was greatest within the cotton canopy. Since the canopy reduces wind velocity, traps above the canopy would catch more insects than traps within the canopy if aerial insect densities were equal at both heights. Thus _Trichogramma_ flight activity appears generally to have been greatest within the cotton canopy in both control and release fields. Within the canopy, longer distance movements that could carry large numbers of _Trichogramma_ out of a cotton field are probably unlikely since the need for navigation around vegetation would inhibit such long flights. The frequency of _T. pretiosum_ flight at various heights within and above plant canopies has not been well studied. Further research is needed to examine how the frequency of flight at different heights changes with varying conditions of plant maturity and host egg density. Such data, together with information on _Trichogramma_ densities, would enable entomologists to better differentiate localized flights from movements that typically remove individuals from crop fields.

Perimeter sticky trap data also suggest that a large number of _Trichogramma_ did not fly from the fields where they were released. The numbers of wasps caught on perimeter traps around both control and release fields were similar during releases. In contrast, perimeter traps around release fields caught more Trichogramma in prerelease samples than traps around control fields. Unless the native Trichogramma populations declined around release fields during the time of releases, perimeter traps around these fields should have caught more Trichogramma if substantial dispersal had occurred. Such a decline did not take place around the control fields.

If significant levels of movement from release fields did occur, then the Trichogramma must have engaged in behavior that would have allowed them to avoid entrapment on the sticky traps. For example, vertical flights without any substantial horizontal track would have enabled released T. pretiosum to avoid sticky traps. Catches of wild Trichogramma on perimeter traps surrounding control fields and surrounding release fields prior to releases suggest that trap placement was appropriate for sampling the Trichogramma present, assuming that cultured T. pretiosum behave similar to wild populations. Some individuals undoubtedly dispersed from release fields. But dispersal of Trichogramma from release fields was not detected by sampling with sticky traps.

The experimental design of this study assumed that all wasps were released within the cotton field borders. However, aerial releases might have liberated some T. pretiosum outside the field borders. If substantial numbers had been released outside of the study fields, then sticky-trap catch on perimeter traps would have been elevated by releases and confounded the experimental analysis of dispersal. In future attempts to investigate dispersal, the initial distribution of released Trichogramma should be sampled to avoid this problem.

The numbers of Trichogramma caught on sticky traps were low at all locations throughout this study compared to catches on the same traps during other studies. These same traps have caught up to a mean of 13 trichogrammatids/trap/day at canopy height from wild populations in soybeans (Keller, unpublished data). The overall low levels of flight suggested by low sticky-trap catches point to factors other than dispersal from the target areas that might have reduced the efficacy of released Trichogramma. Insectary held females survived for up to 4 days when provided with H. zea eggs and honey. The abundance of Heliothis spp. eggs and availability of cotton floral and extrafloral nectaries suggest that the survival was not limited by the availability of food or hosts. Thus other factors such as brachyptery, field mortality, insufficient numbers of Trichogramma released, or field behavior inappropriate for the goal of increased egg parasitization pest control are factors which could have contributed to the lower than desired levels of parasitization.

CONCLUSIONS

Attempts to colonize or augment beneficial entomophages have met with varying success (Hall and Ehler 1979, Stinner 1977). All projects generally evaluate the % parasitization or changes in crop yield that results from releases of beneficial insects. Future workers, where possible, also should evaluate the behavior and mortality levels of released entomophages in order to better understand the factors that influence success or failure of biological control efforts. In this study, examining the flight behavior of released T. pretiosum suggested that dispersal was not the primary factor influencing inadequate control of Heliothis spp., but more research is needed to establish this thesis. Future work examining the behavior and fitness of released Trichogramma could improve the prospects for successful control of more lepidopterous pests using these entomophages.

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