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Dispersal Ability and Parasitization Performance of Egg Parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) in Field and Storage Conditions

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Abstract: In this study we evaluated the dispersal ability of *Trichogramma evanescens* in field and storage conditions. In field studies we tested the effects of plant structure on the dispersal ability of *T. evanescens* from release points. Both in a corn field and on grapevines the level of parasitism was negatively correlated with distance when the host eggs were located away from the release point. The parasitization rate on grapevines and corn plants at the release point was greater than that away from the release point. Parasitization rates were significantly higher at the highest wasp density in cages. When 1000 wasps were released, the percentage of parasitized host eggs was 11.41%, and this rate increased to 29.75% and 62.06% when 2000 and 3000 wasps, respectively, were released. A similar trend was observed in plastic bags and increasing parasitoids caused a reduction in adult pest emergence.

Key Words: *Trichogramma evanescens*, release, parasitization, dispersal ability

Arazi ve Depo Koşullarında Yumurta Parazitoidi *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)'in Etrafa Dağılma ve Parazitleme Yeteneğinin Araştırılması

Özet: Bu çalışmada yumurta parazitoidi *Trichogramma evanescens*'in salıverilme noktasından farklı uzaklıklardaki parazitleme yeteneği araştırılmıştır. Mısır ve asma bitkilerinin her ikisinde de salıverme noktasından uzaklaştıkça parazitleme miktarının azaldığı görülmüştür. Depo koşullarında en yüksek parazitleme oranı salıverme noktasında gözlenmiş ve salıverme noktasından daha yüksek mesafelerde parazitleme azalmıştır. Parazitoid yoğunluğu arttıkça parazitleme miktarında da artış olmuştur. Kullanılan parazitoid sayısı 1000 olduğunda parazitleme oranı % 11,41 olarak hesaplanmışken, 2000 ve 3000 parazitoid salıverildiğinde bu oran sırasıyla % 29,75 ve % 62,06 olarak bulunmuştur. Yine depo koşullarında içerisinde un bulunan plastik kovalar kullanıldığında parazitoid yoğunluğuna bağlı olarak konukçu ergin çıkışında kontrole göre önemli bir azalma görülmüş ve 500 adet parazitoid salıverildiğinde kontrolün % 22,86'sı kadar konukçu ergin çıkışı gözlemlenmiştir.

Anahtar Sözcükler: *Trichogramma evanescens*, salıverme, parazitleme, dağılma yeteneği

Introduction

Egg parasitoids of the genus *Trichogramma* are considered efficient biological control agents and are widely used commercially for the control of lepidopterous pests on many crops (1,2). The short generation time of *Trichogramma* wasps and the fact that they can be reared on factitious hosts allow these wasps to be produced quickly and affordably, relative to other parasitoids (1,3). Dispersal ability and the parasitization rate of parasitoid wasps in field and storage conditions are desired characteristics for biological control programs. Determining the dispersal ability of *Trichogramma evanescens* Westwood in field and storage conditions is important for developing effective release techniques and for predicting the efficiency of such releases. A Potential biocontrol agent should have pronounced host searching and dispersal ability. Dispersal ability not only ensures that insects become well distributed within release areas, but can also reduce the workload by reducing the number of release points per unit area (4).

After an appropriate *Trichogramma* species has been selected, the next step is to determine adequate release strategies, including the introduction rate, frequency, and density of release points (5). Progress has been made in establishing the appropriate spacing of release points for given introduction rates in field crops, forest stands, and fruit orchards, based on studies of the dispersal ability of *Trichogramma* from release points following inundative introductions (6-8). The use of *Trichogramma* has been further established for the control of storage pests. *T. evanescens* is used against the Indian meal moth, *Plodia interpunctella* (Hübner), in stored cereal products. Other target pests in storage environments are *Ephestia kuehniella* Zeller and *Sitotroga cerealella* (Olivier). *Trichogramma* species have been evaluated for the control of the common cloth moth, *Tineola bisselliella*, (Hummel) under laboratory conditions. The range of target pests for *Trichogramma* egg parasitoids has, therefore, increased considerably in Germany (9).

The present study aimed to test the dispersal ability and parasitization performance of *T. evanescens* in field and storage conditions. We chose to evaluate dispersal ability as a parameter because of its importance to field and storage efficacy, and due to the fact that it is still poorly studied in the laboratory (10). The data obtained in this study may be of use in estimating the number of parasitoids needed to accomplish parasitization in field and storage conditions.

Materials and Methods

Dispersal Ability of *T. evanescens* on Corn and Grapevine Plants

In a corn field, dispersal ability was monitored during 4 days with *E. kuehniella* sentinel egg cards (on every 250 irradiated eggs at each distance (0, 75, 100, 150, and 200 cm)) that were changed every 24 h. A 4 × 4-m plot in the corn field was selected and the center area of the plot was determined as the release point. In all, 2000 *T. evanescens* pupae were released as blackened host eggs in a waxed card folded 3 times from which adult *Trichogramma* could emerge at the sides, which were stapled at a height of 45 cm in the center of the plot. Host eggs were protected from direct sun light and rain by a waxed cardboard roof, and from predators by a 0.755-mm mesh screen, allowing free access to wasps. After 24 h of exposure each egg card was collected and incubated at 27 °C (70% r.h.), with a photoperiod of 14:10 h (L:D). Parasitism was assessed by checking for the presence of blackened eggs, and the number of parasitized eggs was counted on each egg card. A similar experimental design was also applied using grape plants, but there was an approximately 2-m space between plants. As such, each plant was selected as a plot and dispersal ability was tested at 25-cm intervals, up to a maximum 1-m distance from the release point. Each treatment was replicated 4 times.

Dispersal ability of *T. evanescens* in Glass Cages

In cage studies 1000, 2000, and 3000 parasitoids were released as blackened host eggs from release points, and parasitism was tested according to height and parasitoid density. Cube-shaped glass boxes (1 × 1 × 1 m) were selected as release cages. The central bottom of each cage was determined as the release point. Egg cards containing 200 UV-irradiated sentinel host eggs were placed at different heights and locations relative to the release points. Blackened host eggs were than transferred to glass tubes and counted under a stereo microscope. Each experiment was replicated 4 times.

T. evanescens Release in Plastic Bags

A miniaturized release cage was used to examine the efficacy of the parasitoids in storage conditions. In this experiment 20-l plastic bags were used as release cages. The bottoms of the cages were covered with 10 cm of wheat flour and 50, 100, 150, 250, and 500 *T. evanescens* pupae were put inside the cages a day before adult emergence. Additionally, 10 pairs of unmated *E. kuehniella* adults (< 24 h age) were also released into each cage for oviposition. The cages without parasitoids were selected as the control group. The unparasitized eggs oviposited by host adults were incubated until adult emergence in order to determine the level of pest control. The number of emerged adults was recorded until the end of the experiment. Each treatment was replicated 3 times.

Statistical Analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS for Windows v.11.0. Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test.

Results

Dispersal Ability of *T. evanescens* on Corn and Grapevine Plants

Decreases in the parasitization rate were observed, which were dependent on the distance from release points. The majority of parasitization was performed by the parasitoids at the release point. In the corn field, parasitization at the release point was 10.04%, which decreased to 7.10%, 5.98%, 3.46%, 2.98%, and 0.46% at distances of 50, 75, 100, 150, 200 cm, respectively. The daily parasitization rate also decreased, depending on parasitoid age. In the corn field most of the parasitization was observed on the first day at all distances from the release point (Table 1).

T. evanescens demonstrated similar dispersal ability on grapevine and corn plants, but there was a 2-m space between the grape plants and many of the parasitoids stayed on the plants; therefore, more parasitization was observed on grapevines than on corn plants. In total, 37.67% of the host eggs were parasitized by *T. evanescens* at the release point when released on grapevines, but this rate decreased as the distance from the release point increased (Table 2).

Dispersal Ability of *T. evanescens* in Glass Cages

For the parasitoids released in glass cages the rate of parasitization decreased with distance from the release points. A great amount of parasitization occurred near the release points, as in the field experiments. The rate of parasitization decreased with distance, from bottom to top. When 1000 parasitoids were released into the glass cages, the decrease in parasitization up to 30 cm from the release points was significant ($P < 0.0001$), but the parasitization rate did not significantly change from 30 to 100 cm ($P = 0.098$). The same decrease in parasitization was also observed when 2000 and 3000 parasitoids were released (for 2000 parasitoids, $P < 0.001$ and for 3000 parasitoids $P < 0.001$). Increased parasitoid density resulted in dispersion from release points and the distribution of parasitism was more even when a higher number of parasitoids were released.

At the release points parasitization was 56.16%, 30.98%, and 19.38% when 1000, 2000, and 3000 parasitoids were released, respectively. The results show that parasitization at the release points was inversely related to the number of parasitoids released and that the dispersion of parasitoid adults was directly related to the number of parasitoids. As the number of parasitoids released increased, parasitization at the higher parts of the cages increased.

Parasitoid density also affected parasitization levels. While the number of parasitoids increased 3-fold (1000 vs. 3000), the increase in the parasitization rate was approximately 6-fold (11.41% vs. 62.06%) (Table 3).

Table 1. Parasitization of *T. evanescens* at different distances from the release points during 4 days in the corn field.

Distance from release point (cm)	n	Daily parasitism rate				% Parasitism
		1	2	3	4	
0*	1200	244 Aa§	160 Aa#	40 Bab	38 Ba	10.04
50	1200	123 Aabc	91 Aab	84 Aa	43 Aa	7.10
75	1200	126 Aab	53 Ab	78 Aab	30 Bab	5.98
100	1200	83 Abc	40 ABb	14 Bbc	29 ABab	3.46
150	1200	48 Acd	40 Ab	32 Aabc	23 Aab	2.98
200	1200	19 Ad	0 Ac	3 Ac	0 Ab	0.46

* Release point.

§ Means within each row followed by the same upper case letter are not significantly different ($P > 0.05$, ANOVA).

\# Means within each column followed by the same lower case letter are not significantly different ($P > 0.05$, ANOVA).

n: Total number of host eggs at each distance.

Table 2. Parasitization of *T. evanescens* at different distances from the release points during 4 days in the grapevine field.

Distance from release release (cm)	n	Daily parasitism rate				% Parasitism
		1	2	3	4	
0*	1500	1068 Aa§	660 Ba#	304 Ca	48 Dab	34.67
25	1500	356 Ab	292 ABb	247 ABab	149 Bc	17.40
50	1500	221 Abc	156 Abc	136 Aab	118 Ac	10.52
75	1500	201 Abc	168 Abc	124 Ab	89 Abc	9.70
100	1500	168 Ac	81 Ac	125 Aab	39 Aa	6.88

* Release point.

[§] Means within each column followed by the same upper case latter are not significantly different (P > 0.05, ANOVA).

[#] Means within each row followed by the same lower case letter are not significantly different (P > 0.05, ANOVA).

n: Total number of host eggs for each distance.

Table 3. Parasitism by *T. evanescens* according to parasitoid density at different heights from the release point in glass cages.

Height from release point (cm)		Number of parasitized eggs according to parasitoid density		
		1000[¥]	2000[¥]	3000[¥]
0*		410 A[§]a[#]	590 Ba	770 Ca
10		80 Ab	122 Abc	372 Bc
20		21 Ac	209 Bbc	470 Cbc
30		25 Abc	167 Bbc	380 Cc
40		36 Abc	276 Bb	560 Cb
50		46 Abc	200 Bbc	570 Cb
60		62 Abc	239 Bbc	490 Cbc
100		50 Abc	101 Ac	360 Bc
Total	Parasitization	730	1904	3972
	No. of host eggs	6400	6400	6400
	% Parasitization	11.41	29.75	62.06

* Release point.

[¥] Number of released parasitoid pupae.

[§] Means within each column followed by the same upper case latter are not significantly different (P > 0.05, ANOVA).

[#] Means within each row followed by the same lower case letter are not significantly different (P > 0.05, ANOVA).

T. evanescens Released in Plastic Bags

Although the number of moth pairs used was equal for each treatment, the proportion of F_1 adults significantly decreased as the number of parasitoids increased (P < 0.0001) (Table 4). The number of adult pests that emerged was reduced by 77.14% when 500 parasitoid pupae were released. The results also showed that the number of released parasitoids did not significantly change the sex ratio; that is, parasitoids did not differentiate potentially male or female eggs (P = 0.769) (Table 4).

Discussion and Conclusion

One of the most important factors in a *Trichogramma* inundative program is the distance that the emerging parasitoid adults can disperse from a release point (12). Decreases in the parasitization rate of *Trichogramma* were reported to be dependent on the distance from release points in field crops, fruit orchards, and forest stands (5, 7,13-15). In the present study decreases in parasitization were also observed on corn and grapevine plants, as well as in glass cages as the distance from the release points increased.

For grape plants one release point was adequate for achieving a good parasitization rate, but for corn plants several release points must be selected before release for effective parasitization, because most of the parasitoids dispersed from the release points without parasitizing host eggs. In the corn field there were many plants connecting to each other, and so parasitoids could easily cross to untargeted plants. In the grapevine field there was a 2-m space between plants and the parasitoids did not move from the release point; as such, plant structure affected host exploitation by egg parasitoids and more host eggs were parasitized on the grapevines than on the corn plants. In open field conditions the ability of *Trichogramma* to disperse on its own seems to be high within a single plant, but low between plants (6,7,14), which was explained by speculating that parasitoids avoid open areas where they lose their ability of directed flight (3). Most of the parasitism by *T. evanescens* occurred during the first 2 days around the release points in the corn and grapevine fields. In a previous study it was reported that *Trichogramma* females did not disperse at a high rate because they concentrated their search for hosts at the base of the plants close to the release point (16).

It is suggested that there are 3 major components to plant structure relevant to the searching activity of parasitoids: 1) plant size or surface area, 2) the variation among plant parts (structural heterogeneity), such as seed heads, flowers and nectaries, and leaves with heterogeneous surfaces (e.g. glabrous, hirsute), and 3) the connectivity of plant parts or plant form (structural complexity), that is, the way the plant surface area is connected together (17). In the current study the leaf surface area of the grapevines was lower than that of the corn plants. It is suggested that greater plant surface area per host would decrease parasitism rates (18). This inverse relationship between leaf surface area per host and the discovery of European corn borer (*Ostrinia*

Table 4. Control efficacy of *T. evanescens* in small plastic bags.

No. Released parasitoid pupae	Adult moth emergence		Sex ratio of emerged moths (M:F)
	Total	%	
0	6915 a[#]	100.00	0.97:1.00 a
50	5895 b	85.25	1.01:1.00 a
100	5551 b	80.27	1.02:1.00 a
150	4798 c	69.39	1.03:1.00 a
250	2601 d	37.61	1.05:1.00 a
500	1581 e	22.86	0.95:1.00 a

[#] Means within each column followed by the same latter are not significantly different (P > 0.05, ANOVA).

nubilalis (Hübner)) eggs by *Trichogramma nubilale* Ertle and Davis was demonstrated in corn and peppers (19, 20).

In a previous study the vertical distribution of *T. cacoeciae* Marchal, *T. evanescens*, *T. embryophagum* (Hartig), and *T. dendrolimi* Matsumura was tested at the vine canopy (14). It was found that the parasitism rate was the highest at the lowest level of the canopy for the first 3 species, while the forth species preferred the zone > 150 cm high. It was also reported that the vertical distribution of *T. minutum* Riley within apple trees was skewed toward the lower part of the tree canopy (14).

In glass release cages the rate of parasitization near the release points was high, but decreased with distance from the release points. In a previous study when *Trichogramma deion* Pinto and Oatman was released on the floor of a storage container, the parasitization rate was greatest at the lowest level, but when released on the middle shelf *T. deion* was not very effective in killing host eggs on the floor (21). It was suggested that this may be due to early exploitation of the eggs closest to the release point, followed by a lower rate of parasitism and successful larval development on older eggs located farther from the release point. Other studies have shown that host eggs become less attractive and suitable for parasitism by *Trichogramma* species over time as the host embryo develops (22-24). In the current study parasitization at the release points was inversely related to parasitoid density, but the dispersion of the parasitoid adults was directly related to the number of parasitoids. The higher the number of parasitoids released, the higher the rate of parasitization at the higher parts of the cages. The rate of parasitization at the release points was 56.16%, 30.98%, and 19.38%, when 1000, 2000, and 3000 parasitoids, respectively, were released. Although the majority of parasitism was observed at the release points for all 3 parasitoid densities, the rate of parasitism at the release points decreased with increasing density. This suggests that increasing parasitoid density resulted in dispersion from the release points, as there was more even distribution of parasitism when 3000 parasitoids were released than when 1000 or 2000 parasitoids were released. It was reported that increased parasitoid density caused an increase in the rate of parasitism in *Bactrocera correcta* (Bezzi) (25). In the present study increasing parasitoid density also caused a reduction in the rate of moth emergence when both parasitoid and host adults were released together in plastic bags.

The results of this study show that the number of parasitized eggs decreased as the distance from the release points increased. Plant structural complexity reduced the ability of the host to find *Trichogramma*. *T. evanescens* parasitized more host eggs on grapevines than on corn plants. Optimal parasitism was observed when 3000 parasitoids were released in glass cages. Approximately 500 parasitoid adults prevented 77.14% of the host eggs from reaching the adult stage in plastic bags. The results of this study may be beneficial in estimating the number of parasitoids needed to accomplish a desired level of pest control in field and storage conditions.

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