Parasitization Rate of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) Eggs After Field Releases of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) in Cotton in Cukurova Region of Turkey

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**ABSTRACT:** The control of the noctuid *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) with *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on a cotton (Malvaceae) farm in Turkey is reviewed. *Helicoverpa armigera* has five generations in a year, but only three attack cotton in the East Mediterranean region of Turkey. Releases of *T. evanescens* are made twice against each of the first three generations of *H. armigera* in the years of 2004–2005. In each release, 120,000 parasitoids ha−1 were released, resulting in 62.9% and 71.6% parasitism and the numbers of larvae of *H. armigera* were reduced by 76.8% and 80.6%, respectively. In fields where insecticides were applied, the numbers of larvae of *H. armigera* were reduced by 57.1% and 77.1%, respectively. Furthermore, it was observed that population of natural enemies was increased in released parasitoid plots, with an average of 33 to 39 *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) were encountered in 25 plants. Pollen-generating plants (maize, okra, weeds, etc.) probably increased the density of the predator. The cultivation of okra as a trap plant can be recommended especially in cotton fields where parasitoid release was conducted. The results of biological control of the noctuid *H. armigera* by *T. evanescens* appear promising.

**KEY WORDS:** Cotton, *Helicoverpa armigera*, *Trichogramma evanescens*, Biological Control, Turkey
et al., 2005). The amount of land devoted to cotton growing varies from year to year depending on conditions such as, market demand, pricing policy, and changes in prices of cultivation and manpower.

One of the most important entomological problems that cause yield loss in cotton is the bollworm, Helicoverpa armigera Hübner (Lepidoptera: Noctuidae). Helicoverpa armigera is a polyphagous pest that directly affects cotton yield due to feeding on generative organs. Larvae of H. armigera feed on flowers, bolls, and above all, combs. Damaged combs fall, flowers do not transform into bolls, and damaged bolls do not open and will ultimately wither and decay. Therefore, this pest may cause a considerable amount of yield loss if not controlled.

Chemical control is common against this pest in the Mediterranean region. The cost of control of the pest is around US$46.60 per hectare. On the other hand, the cost of chemical control of all pests in cotton is US$247.00 per hectare and the number of insecticide treatments varies between 3 and 7 per season (Anonymous, 2001). Chemical control is expensive. Furthermore, when harmful aspects of chemical control are considered, alternative control methods may be more cost effective in the long run. The foremost of these is biological control. One of the commonly used natural enemy groups in biological control is egg parasitoids. They have a special significance in pest control on account of killing the pest inside the egg without damaging the plant, being easily reared in laboratories, and their easy release in the field. Trichogramma are one of the most widely used egg parasitoid groups; they parasitize around 400 species of pests, particularly pest species of economic value belonging to the Lepidoptera group. During the past 35 years, Trichogramma spp. have been used against pests in maize, cotton, sugarcane, vegetables and fruit trees in 30 countries (Li, 1994). Currently, 18 species of Trichogramma are reared and used in biological control of lepidopterous pests in maize, cotton, sugarcane, rice, soy bean, sugarbeet, cereals, vegetables, and fruit and forest trees (Hassan, 1993). Trichogramma spp. were found to be the most effective parasitoid group for controlling of H. armigera in cotton (Bournier and Vaissayre, 1977; Cai et al., 1997). Suh et al. (1997) reported that as many as 59% of bollworm eggs in cotton were parasitized by natural populations of Trichogramma wasps and argued that bollworm egg parasitism in cotton could be substantially increased with augmentative releases of Trichogramma wasps.

The purpose of this study is to determine the release efficacy of Trichogramma evanescens in biological control of H. armigera in cotton in the context of the Mediterranean Region.

Materials and Methods

Experimental Design

The study was conducted in a 0.5 ha cotton field in Kozan-Adana province of Turkey. There were three different treatments: release (without insecticides), control (without wasps and without insecticides), and insecticide (without wasps, Table 1). The release and control plots were replicated randomly four times (eight different plots total, each plot 0.125 ha) to obtain parasitism rates of the parasitoid. The distance between wasp release and non-release plots was at least 50 m. The insecticide treatment was placed 1000 m away from the release and the non-release plots in a second 0.5 ha plot to minimize adverse effects of many insecticides on
natural enemies. Insecticide treatments were taken into consideration at harvest with release treatment and control to compare the grain yields. Three insecticide applications were carried out in four 0.5 ha plots of the insecticides treatment in 2004, and four times in 2005 against the second and third generation of *H. armigera* in July and August (Table 1).

**Parasitoid Rearing**

The production of laboratory host, *Ephesia kuehniella* Zeller and the egg parasitoid, *T. evanescens* Westwood, were conducted in acclimatized rooms adjusted to conditions of 25 ± 1°C and 65 ± 10% RH, and under permanent dark for *E. kuehniella*, and under 16:8 L:D for *T. evanescens*. *Ephesia kuehniella* was reared on a maize and wheat flour diet (Hassan, 1981; Kayapinar, 1991; Oztemiz, 2001).

**Parasitoid Releases and Pest Monitoring**

*Trichogramma* releases were applied simultaneously in four plots of the release treatment during the first, second and third generations of *H. armigera*.

The bollworm adult population was monitored weekly in pheromone traps in order to determine the parasitoid release time. Four large delta type of traps were used to monitor flight activity of *H. armigera*. One trap was used for each plot in the release treatment. When the first adult was caught in a trap, egg sampling of *H. armigera* commenced and was carried out twice per week by examining all parts of 50 randomly chosen cotton plants at the center of each of the eight plots (Knutson, 1996; Suh *et al.*, 2000). After the first egg was seen, *T. evanescens* releases were executed using 80 parasitoid release bags, each bag containing approximately 1500 parasitized *E. kuehniella* eggs in such a manner that a density of 120,000 parasitoids per hectar was achieved (Huo *et al.*, 1988; Suh *et al.*, 2000). Release bags were placed no closer than 7 m from the edge of the plot, with 10 m between any two release points. There were five parasitoid release points in each release plot (0.125 ha). In each release plot 20 bags, totaling approximately 30,000 parasitoids were released. The parasitoid releases were conducted on 14 and 21 June, 08 and 15 July, 05 and 12 August in 2004; and on 16 and 23 June, 14 and 21 July, and 10 and 17 August in 2005. A total of 6 releases (a total of 720,000 parasitoids ha⁻¹) per year were implemented. Two releases were made for each of the three generations that the pest generated seasonally in cotton (Nasrtdinov, 1978; Suh *et al.*, 2000).

### Table 1. List of insecticides applied to test plots in 2004 and 2005.

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Year</th>
<th>Trade-name</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoxacarb 150 g l⁻¹ SC</td>
<td>2004</td>
<td>Avaunt 450 ml ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Beta Cyfluthrin 25 g l⁻¹ EC</td>
<td>2005</td>
<td>Bulldock EC 025 2000 ml ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Profenofos+Cypermethrin 400+40 g l⁻¹</td>
<td>Thiodicarb %80</td>
<td>Flambo 440 EC 2500 ml ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Profenofos+Cypermethrin 400+40 g l⁻¹</td>
<td>2004</td>
<td>Larvin 80 DF 900 g ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Thiodicarb %80</td>
<td>2005</td>
<td>Flambo 440 EC 2500 ml ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Zeta Cypermethrin</td>
<td>2005</td>
<td>Chen 900 g ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>Zeta Cypermethrin</td>
<td>2005</td>
<td>Fury 1.5 EC 1250 g ha⁻¹</td>
<td></td>
</tr>
</tbody>
</table>
Trap Lure and Trap Placement

The attractant \((Z)-11\text{-tetradeccenyl acetate}\) was used (Gothilf et al., 1979). The delta trap comes with 4 sticky plates, a lure holder and a wire hanger. The trap is suspended within the canopy of the crop. One trap was used for each plot. Each trap has one lure in the center of bottom section of the trap. Traps were placed in the plots. Attractant lure was replaced every three weeks. Traps were monitored twice per week as described above.

Data Collection and Statistical Analyses

Three days after each parasitoid release, 50 cotton plants from each plot with 0.125 ha were examined and egg counts of the pest were conducted. Eggs found were marked and re-examined in the next count. This procedure continued until hatching began. Larval counts were conducted twice per week. The economic injury threshold of the bollworm in cotton is two larvae in 3 m of cotton row in Turkey (Yuzbas et al., 2000). The number of damaged generative organs and the number of \(H.\) armigera larvae were recorded by examining all parts of the cotton plants in four randomly selected cotton rows 3 m long in every replication. A total of 100 plants in each of 12 plots was checked for the number of larvae and the number of damaged bolls. Counts were also conducted in the control (no insecticide, no wasp) plots.

The percent efficacy of parasitism was evaluated using Abbott’s formula (Abbott, 1925) to compare the parasitization rate between treatments. These data were transformed (arcsin square root) and analyzed using the Student’s t-test (Gossett, 1908; Zar, 1999) (Table 2).

1) \(\%\) efficacy of control against \(H.\) armigera larvae = \(\frac{(a-b)}{a} \times 100\)

where \(a\) = number of larvae in the release treatment, and \(b\) = number of larvae in the control.

2) \(\%\) efficacy of control in damage in bolls = \(\frac{(a-b)}{a} \times 100\)

where \(a\) = number of damaged bolls in the \(Trichogramma\) release treatment and \(b\) = number of damaged bolls in the control.

At harvest, the counts of damaged bolls and larvae were conducted in a randomized plot experimental design and analyses of variance (ANOVA) (Zar, 1999). The LSD (Least Significant Difference) test (Fischer, 1954; Zar, 1999) was performed to determining differences among averages (Table 3). Also, the results of larval counts were evaluated on the basis of Henderson-Tilton method (Karman, 1971). We used this method to calculate the reduction in the number of larva and the number damaged bolls in the wasp release plots and insecticide treatment plots with
respect to the control (without wasps and insecticides). The formula used in the Henderson-Tilton method is:

\[
\text{Efficacy (reduction \%) = 100 \left[1 - \frac{Ta \times cb}{Tb \times ca}\right]}
\]

where Ta and Tb are the average number of live larvae for insecticide treatments; Ta is after insecticides were applied, Tb is before insecticides were applied; ca and cb are the average number of live larvae in control treatment, ca is after insecticide application, cb is just before the insecticide application.

*Trichogramma* wasps were identified by Bernard Pintureau (UMR 0203 INRA/INSA de Lyon Bâtiment Villeurbanne, France). Voucher specimens have been deposited in the Insect Museum of the Plant Protection Research Institute in Adana, Turkey.

### Results

**Release Efficacy**

Bollworm adult population activity was monitored with pheromone traps to determine the parasitoid release time (Figure 1).

The first bollworm adult capture was seen on June 14, in 2004 and on June 16, 2005. The first releases were done on 14 and 21 June in 2004, and on 16 and 23 June 2005. Two releases were performed within a week’s interval against each of the three generations of the pest per growing season, with a total of 6 releases for each year.

The parasitization rate in the 1st generation of the pest varied between 45.8 and 60.7% in 2004 and 47.4 and 62.5% in 2005.

In our study, counts were conducted in the control plots and parasitoid released plots on the same dates in both years. It was found that 177 of 544 eggs found in the year 2004 were parasitized with a parasitization rate of 32.5%. In 2005, a 131 of 432 eggs were parasitized, with a parasitization rate of 30.3%.

When the release plots and the control plots were statistically compared, a significant difference was founded in respect to the parasitism rate (Table 2).

Compared with the control plot, the decrease in the number of larvae in the release plots for 2004 was found to be 76.8%, and in the insecticide applied plots, it was found to be 57.1%. The decrease in the number of damaged bolls was found to be 75% in the release plot and 61.5% in the insecticides applied plot when compared to the control plots. In 2005, the reduction in the number of larvae in the release plots was 80.7%, and 74.2% in the insecticide applied plots. The reduction in the number of damaged bolls was 79.4% in the release plots, and 76.4% in the insecticide applied plots.

### Table 3. Mean number of larvae and damaged bolls in the released, control and insecticide treatment plots in Cukurova Region in 2004 and in 2005.

<table>
<thead>
<tr>
<th></th>
<th>Released treatment *</th>
<th>Control treatment*</th>
<th>Insecticide treatment *</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2004</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Larvae</td>
<td>1.62 ± 0.297 b</td>
<td>7.00 ± 0.911 a</td>
<td>3.00 ± 0.408 b</td>
</tr>
<tr>
<td>Number of Damaged Bolls</td>
<td>1.62 ± 0.161 b</td>
<td>6.50 ± 0.645 a</td>
<td>2.25 ± 0.971 b</td>
</tr>
<tr>
<td><strong>2005</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Larvae</td>
<td>1.50 ± 0.288 b</td>
<td>7.75 ± 0.478 a</td>
<td>2.00 ± 0.408 b</td>
</tr>
<tr>
<td>Number of Damaged Bolls</td>
<td>1.75 ± 0.478 b</td>
<td>8.50 ± 0.500 a</td>
<td>2.00 ± 0.577 b</td>
</tr>
</tbody>
</table>

* Means followed by a different letter, differ significantly at \( P \leq 0.01 \) (LSD).
When the effects of the parasitoid release were analyzed by ANOVA, no significant differences were observed between the release plots and the insecticide applied plots with respect to either the number of pest larvae (in 2004, $F = 22.3$, LSD = 2.758, d.f. = 11, $P < 0.0001$; in 2005, $F = 75.5$, LSD = 2.392, d.f. = 11, $P < 0.0001$), or the number of damaged bolls (in 2004, $F = 37.7$, LSD = 1.953, d.f. = 11, and $P < 0.0001$; in 2005, $F = 54.0$, LSD = 1.837, d.f. = 11, and $P < 0.0001$); however, the differences in pest numbers and numbers of damaged bolls among these two treatments and the control treatment was found to be statistically significant (Table 3).
The yield in the release plots averaged 4260 kg ha\(^{-1}\) (2004) and 4470 kg ha\(^{-1}\) (2005), while in the insecticide treated fields the yield in 2004 and 2005 was 4380 kg ha\(^{-1}\) and 4560 kg ha\(^{-1}\), respectively. The control treatment plot yielded 3820 kg ha\(^{-1}\) and 4080 kg ha\(^{-1}\) in 2004 and 2005, respectively. Hence, the yield was increased by 11.5\% and 9.6\% in *T. evanescens* release treatment plots compared to controls, and increased by 14.7\% and 11.8\% in the insecticides treatment plots in comparison to the control treatment plots for 2004 and 2005 respectively. In our experiment, chemical applications increased yield over the release plots by 2.8\% in 2004 and 2.0\% in 2005. In conclusion, the rate of parasitization in *H. armigera* eggs in cotton in the plots where *T. evanescens* was released 2004 and 2005 was an average of 62.9\% to 71.6\% respectively. In the control plots, parasitization rates were 32.5\% and 30.3\% respectively (Table 2). In 2004, the reduction in the number of the bollworm larvae was determined to be 76.8\% in comparison to the control plot, while the reduction in the number of damaged bolls was found to be 75\%. In 2005, the reduction in the number of the bollworm larvae was 80.6\% and damaged bolls was 79.4\%.

Natural enemies were observed in the parasitoid release plots. The most important of these natural enemies is the general predator, *Chrysoperla* (Neuroptera: Chrysopidae) counted on 25 plants. In the parasitoid release plot, averages of 33 to 39 *C. carnea* were encountered per 25 plants. The fact that chemical control was not conducted and that there were pollen-generating plants around (maize, okra, weeds etc.) probably increased the density of the predator.

**Discussion**

The highest rate of parasitization occurred in the 3\(^{rd}\) generation, followed by the 2\(^{nd}\) and 1\(^{st}\) generations in both years, respectively (Figure 2). Especially, at the end of the season, parasitization rate was high. Since the parasitoid generations got mixed up with each other after the releases performed against the first generation because the parasitoids released in the second and third generations reproduced in the field and the supporting releases were continued. Similar results were published in North Carolina by Jones *et al.* (1977), in Moskova (Kovalenkov, 1980), in Arkansas/USA (King *et al.*, 1986), China (Wang and Zhang, 1991), Australia (Scholz and Murray, 1994), Texas/USA (Knutson, 1996). The likely reason for the low parasitization (30.3\%) in the control plot was that the prevalent winds were effective in the spread of species of *Trichogramma* from the location of release (Yu *et al.*, 1984). Likewise, in the other *Trichogramma* release studies, parasitization rate was 33.0\% in the control plot in Australia (Scholz and Murray, 1994) and 18–69\% in North Carolina (Suh *et al.*, 1997, 2000).

In the fields where release was conducted in both years, the number of larvae in 3 m rows was seen to be below the threshold level (1–2 larvae 3 m\(^{-1}\)) and this lends support to the idea that biological control is viable. Most researchers working on cotton biocontrol suggest that there are significant decreases in the *H. armigera* larvae with *Trichogramma* release. For example, Sharafutdinov and Salikhov (1975) found a 21\% reduction in the number of larvae with *Trichogramma* sp. releases against *H. armigera*, while Kovalenkov and Mescheryakova (1986) stated that the reduction in the number of the pest’s eggs was 50 to 62\% with the release of *T. evanescens*. 

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Since no chemical control was applied in the fields where release was performed and there were pollen-generating plants around (maize, okra, weeds etc.) the release area, an increase was observed in the density of natural enemies which were highly active in the field like *C. carnea*. The combination of parasitoids and predators could

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Fig. 2. Parasitism rate of *Trichogramma evanescens* against the cotton bollworm, *Helicoverpa armigera* in released and control treatment plots in Cukurova Region in 2004 and in 2005.
be one approach in integrated pest management programs of *H. armigera* in organic cotton farms (El-Wakeil and Vidal, 2005). The low number of eggs in the first generation of bollworm can be linked to the okra plants on the edges of the field if they acted as a trap crop. In our study we observed the fruit of the okra plant to be dense with *H. armigera* eggs and parasitism seemed to increase as the season progressed. The cultivation of okra as a trap plant can be recommended especially in cotton plots where parasitoid release was conducted (Thotadanya *et al.*, 1978).

Unlike previous years, a significant increase was observed in the *H. armigera* populations in cotton in the Cukurova region in 2004 and 2005. Multiple chemical treatments were conducted against the pest, yet the desired success level in the control attempt could not be attained due likely to chemical resistance. Since the chemical control implemented in Turkey against *H. armigera* produced little effect, the need arose in certain regions for chemical control to be repeated three times. Given that the cost of three chemical control treatments against the pest were uneconomical ($US144.10–154.40 hectare\(^{-1}\)) and considering their harmful effects on the environment and human health, *Trichogramma* releases within the framework of integrated control principle seems viable in the cotton plant. Likewise, Shamuratov *et al.* (1981) reported that the cost of 3 or 4 *Trichogramma* releases were 1/3 more economical than chemical control. Also, Garcia (1990) reported that releases of *Trichogramma* spp. in Colombia had economical and ecological advantages, control costs fell by more than 50% and biological balance was re-established in the agro-ecosystem. In our study, yields in the chemical treatments only surpassed the parasitoid treatments by about 2% in both years. Given the above stated cost differential between chemical and parasitoid treatments, this 2% yield increase appears not to be cost effective.

As a result, the development of an integrated crop production system for cotton in Turkey appears technically feasible and cost-effective, based on this study. The use of natural enemies in cotton fields in IPM strategy will result in the reduction of pesticide use and protection of the environment, and is likely to achieve this at a lower cost to producers than chemical control.

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