Natural Parasitism and Release Efficiency of *Trichogramma evanescens* Westwood in *Ostrinia nubilalis* Hübner Attacking Maize in Turkey[1]

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Abstract The natural parasitism rate and the release efficiency of the egg parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), in the biological control of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), was determined in field plots of maize in the Eastern Mediterranean, Turkey. Parasitoids were released in maize plots as parasitized eggs of laboratory-reared *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The parasitized eggs (n = 150,000) were released twice in a 10-d interval at the beginning of the oviposition period of the third generation of *O. nubilalis* in the second crop of maize in released treatment (without insecticides). Other treatments were an untreated control (without wasps and without insecticides) and an insecticide treatment (Lambda-Cyhalothrin, 50 g l^{-1}, 300 ml ha^{-1}; without wasps). *Ostrinia nubilalis* egg masses, larvae and plant damage were regularly assessed until crop harvest. Parasitization of egg masses by *T. evanescens* was determined in each sample. The mean (± SD) percentage of parasitized *O. nubilalis* eggs was 86.2 ± 11.6 (± SD)%. Compared with the control treatment, the number of plants damaged by European corn borer larvae in the release treatment was reduced by 96%, whereas the number of larvae was reduced by 95.2%. Average grain yield was 8,800 ± 15.2 kg ha^{-1} (380.0 ± 1.6 g per 1000 grain weight) in the *Trichogramma* release treatment without insecticide, 7,000 ± 28.8 kg ha^{-1} (314.8 ± 2.9 g per 1000 grain weight) in the control treatment, and 8,533 ± 8.8 kg ha^{-1} (360.4 ± 8.5 g per 1000 grain weight) in the insecticide treatment. The grain yield and 1000 grain weight differences differed significantly ($P \leq 0.01$) between the untreated control and the other two treatments (released treatment and insecticide treatment). Natural parasitization of *O. nubilalis* eggs by *T. evanescens* as observed in control and insecticide-treated plots was 30.2%. These results indicate that biological control of *O. nubilalis* with *T. evanescens* should be developed as an integral control method in integrated management programs for maize grown in Turkey.

Key Words biological control, maize, *Ostrinia nubilalis*, European corn borer, parasitization rate, *Trichogramma evanescens,* egg parasitoid

Maize, *Zea mays* L., is the third most important grain crop in production after wheat and rice for the Mediterranean and Aegean Region of Turkey since the initiation of a second cropping production system. Approximately 800,000 ha maize is currently cultivated in Turkey annually. Because of the increase in maize production, several pests of various population densities have been detected in these new production regions. The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), has emerged as the major pest. The yield loss caused by this pest can reach up

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to 90% even when multiple insecticide applications are used for its control (Kavut et al. 1990). European corn borer larvae damage maize in several ways including: (1) disrupting the nutritient flow within the plant by larval tunnelling within the stalk; (2) causing an increase in disease incidence; (3) promoting stalk breakage and ear drop, and; (4) directly damaging kernels and hence reducing the yield. Because of the stem-tunnelling behavior of the larvae, chemical control is possible only if the treatment is applied during the brief period after hatching and before larvae tunnel into the plant. However, the control achieved is typically unsatisfactory due to poor timing, dosage, and spraying difficulties. Furthermore, the usage of pesticides is not cost-effective and poses an environmental threat. Therefore, it is crucial to develop effective alternative management methods such as biological control.

The egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), are the most widely augmented species of natural enemy in the biological control of the European corn borer. Trichogramatids have been mass-produced and released in production fields for almost 70 yrs. Worldwide, over 32 million ha of agricultural crops and forests are treated annually with *Trichogramma* spp. in 19 countries (Li 1994, Wang et al. 1999, Wright et al. 2002). Among these species, *T. evanescens* Westwood (Hymenoptera: Trichogrammatidae) is the most widely used species in biological control programs in several countries including France (Voegele et al. 1975), Switzerland (Suter and Babler 1976), and Germany (Neuffer 1986). Olkowski and Zhang (1989) reported that 9 species of *Trichogramma* are produced commercially for use in augmentation programs in various parts of the world. Hunter (1994) listed 78 private commercial suppliers of *Trichogramma* spp. in North America (8 in Canada, 22 in Mexico, and 48 in the United States). In addition, the former Soviet Union and the Peoples Republic of China are known to be major producers and users of *Trichogramma* (Greenberg and Nikonov 1988, Flippov 1992).

The study reported herein was conducted to determine the natural parasitism rate of the egg parasitoid, *T. evanescens,* and its release efficiency against the European corn borer in the second cropping of maize in the Eastern Mediterranean, Turkey.

Materials and Methods

Parasitoid production. *Trichogramma evanescens* collected from Adana, in the Eastern Mediterranean of Turkey, were reared on eggs of a laboratory host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), in conditioned rooms adjusted to 25 ± 1°C temperature, 65 ± 10% RH, and a 16:8 h (L:D) photoperiod (Hassan 1981, Hoffmann et al. 2001).

Release of *T.* evanescens. This study was conducted in Ceyhan-Adana, in the Eastern Mediterranean region of Turkey, in the second crop maize after harvesting wheat in 2004. *Ostrinia nubilalis* produce two generations in the first crop maize from April to September and two generations in the second crop maize from June to November in the region. The third generation occurs in the second crop maize (Stein 1987). The study was planted on 26 June and harvested on 2 November and was composed of 3 treatments: parasitoid release without insecticide, a control treatment without wasps and insecticide, and an insecticide treatment without wasps. The distance between the release and control plots was 600 m because of the passive but high spreading potential of the parasitoid by prevailing winds. Lambda-Cyhalothrin (Karate™, Syngenta Crop Protection, Wilmington, DE; with a dosage of 300 ml ha^{-1}) was applied to the insecticide-treated plots with the first application in early August and the second application in early September. Treatments were arranged in a completely randomized experimental design with 3 replications per treatment. Plots were 2 ha in size.

Adult *O. nubilalis* populations were monitored using a Robinson light trap which was located near the study area in Ceyhan-Adana to determine the timing of the release for *T. evanescens*. The first adult was captured in the light trap on 11 August. *Ostrinia nubilalis* eggs were counted at 20 locations within each plot by visually inspecting 5 plants at each location (total 100 plants per plot). When oviposition was observed, *T. evanescens* adults were released twice (11 and 21 August) in a 10-d interval with 75,000 wasps per ha for each release (80% female). The distance between the 2 release sites was 14 m. The parasitoids were released with a releasing bag including 1500 eggs of *E. kuehniella* parasitized by *T. evanescens* (Hassan and Heil 1980). After each release, 100 plants (5 plants at 20 different locations) were visually inspected for *O. nubilalis* eggs. Parasitized egg masses were recorded. Unparasitized egg masses were marked and observed periodically until the eggs either turned black or larvae of *O. nubilalis* emerged. Egg masses were counted twice a week from a week prior to the first release until the plots were harvested. The control treatment and the insecticide treatment were monitored for egg masses twice a week until harvest.

Efficiency of *Trichogramma* parasitism was assessed by calculating the percentage of *O. nubilalis* egg masses parasitized throughout the growing season (Rawensberg and Berger 1986) and counting the number of damaged plants, the number of larvae, and yield data in all treatments. In addition, 100 plants from each plot were randomly selected and examined for larvae, pupae and entrance holes at harvest (Tran and Hassan 1986). Reductions in the number of larvae and damaged plants were corrected using Abbott (1925) to account for natural mortality (Karman 1971). Data were statistically analyzed using analysis of variance (ANOVA) (Zar 1999) and the least significant difference (LSD) test (Fischer 1954, Zar 1999) to statistically separate treatment means where significance was indicated by ANOVA (Tables 1, 2).

Natural parasitism rate. In an additional study, 3 subregions (1st Subregion: Ceyhan, Kozan, Kadirli, Osmaniye; 2nd Subregion: Yuregir, Karatas; 3rd Subregion: Tarsus, Mersin) were selected for sampling to determine the natural parasitism rate of *T. evanescens* in the East Mediterranean Region of Turkey. Maize in each subregion was surveyed weekly during the growing season. For each sample, 100 plants were sampled inspected. Egg masses of *O. nubilalis* were collected from the upper and lower surfaces of leaves by cutting the leaf and transporting back to the laboratory where the number of egg masses and the number of individual eggs were counted to determine the

Table 1. Mean parasitism rate of *Trichogramma evanescens* on the eggs of *Ostrinia nubilalis* in response to parasitoid releases, insecticide applications, and an untreated control

Treatment	Total No. Eggs	No. Parasitized Eggs	% Parasitism
Parasitoid Release	63.4 ± 15.8 a	54.6 ± 14.9 a	86.2 ± 11.6
Insecticides	24.2 ± 4.2 b	0.0 ± 0.0 b	0.0 ± 0.0
Untreated	83.2 ± 14.7 a	6.0 ± 4.1 b	7.2 ± 7.9

*Means within the same column and followed by the same letter are not significantly different (LSD, $P \leq 0.01$).

Table 2. Production level in the plot with *Trichogramma* release, untreated plot and plot with insecticide treatment in the Eastern Mediterranean, Turkey

	Grain Yield (kg ha $^{-1}$)	1000 Grain Weight (g)	No. of Larvae	No. of Damaged Plants
Parasitoid Release	8,800 ± 15.2a	380.0 ± 1.6 a	1.0 ± 0.5 c	1.0 ± 0.5 c
Insecticides	7,000 ± 28.8 b	314.8 ± 2.9 b	21.0 ± 1.0 a	25.6 ± 1.2 a
Untreated	8,533 ± 8.8 a	360.4 ± 8.5 a	7.0 ± 0.5 b	9.0 ± 0.5 b

Means within the same column and followed by the same letter are not significantly different (LSD, $P \leq 0.01$).

percentage of parasitism. Masses were placed individually in glass (140 x 25 mm) vials and were checked daily for egg eclosion or parasitoid emergence. Parasitism rates were calculated for each area surveyed.

Results

The first *O. nubilalis* egg mass detected (11 August) in the *T. evanescens* study contained 13 eggs, none of which were parasitized. Following the first release of *T. evanescens* parasitoids, 39 eggs were observed and egg parasitism was 69.2%. After the second release, egg parasitism increased to a mean of 87.7%. This level of parasitism was observed in the release plots for the remainder of the study, even reaching 100%. During this study, 507 eggs were counted with 437 parasitized. The overall mean parasitism rate for the release treatment was 86.2 ± 11.6% (Table 1, Fig. 1). A total of 666 eggs was counted in the control treatment plots. Only 48 of those eggs were parasitized with a mean parasitism rate of 7.2 ± 7.9% (Table 1). In the insecticide-treated plots, 194 eggs were counted, but no parasitism was observed (Table 1, Fig. 1).

The yield and 1000 grain yield differences were statistically different ($F = 24.7$ and 39.4, df = 8, $P \leq 0.01$) between the untreated control and the other two treatments (released treatment and insecticide treatment). Mean grain yield was 8,800 ± 15.2 kg ha^{-1} in the release plots, 7,000 ± 28.8 kg ha^{-1} in the control plots, and 8,533 ± 8.8 kg ha^{-1} in the insecticide-treated plots, whereas 1000 grain weights of harvested grain were 380.0 ± 1.6 g in the release plots, 314.8 ± 2.9 g in the control plots, and 360.4 ± 8.5 g in the insecticide-treated plots. In comparison with the control plots, the number of damaged plants in the release plots was reduced by 96% ($F = 225.0$; df = 2,6, $P \leq 0.01$), the number of observed larvae at harvest was reduced by 95.2% ($F = 189.6$; df = 2,6, $P \leq 0.01$), the number of larval tunnels was reduced by 92%, and the tunnel lengths were reduced by 93%. In comparing the control with the insecticide treatments, the number of damaged plants was reduced by 64%, the number of larvae at harvest was reduced by 69%, the number of larval tunnels was reduced by 60%, and the tunnel lengths were reduced by 64% in the insecticide treatment (Table 2).

In the survey of the Eastern Mediterranean region in Turkey for natural parasitism of *O. nubilalis* eggs by *T. evanescens, O. nubilalis* exhibited 2 generations which occurred in August and September in second crop of maize. In the first subregion, 1244 eggs were collected of which 498 were parasitized (parasitism rate = 40%). In the

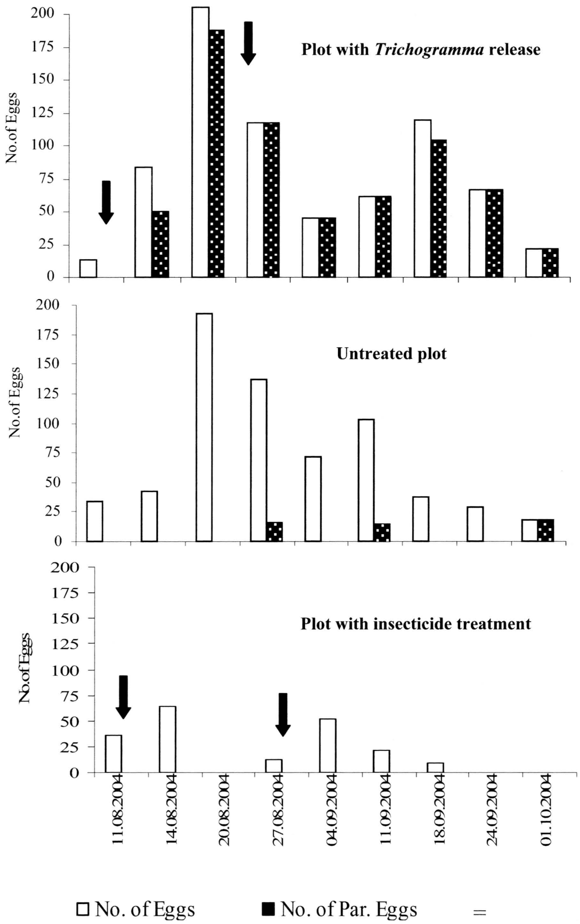

Fig. 1. The parasitization rate of *Ostrinia nubilalis* by *Trichogramma evanescens* in plot with parasitoid release, untreated plot and plot with insecticide treatment (→: treatment).

second subregion, 465 eggs were collected, but no parasitization was observed. This was apparently due to the use of insecticide applications for insect control by these growers. In the third subregion, 633 eggs were collected of which 210 parasitized (parasitism rate = 33.2%) (Table 3). The overall natural parasitism of *O. nubilalis* eggs by *T. evanescens* was 30.2% in the area of Adana, in the Eastern Mediterranean region of Turkey during this study period.

Discussion

The life cycle of *Trichogramma* spp. is 7-10 d from egg through the adult stage. This short life cycle allows for nearly 30 generations of the parasitoids each growing season marked with rapid increases in *Trichogramma* populations. Further, releases of

Table 3. The natural parasitization rate of *Ostrinia nubilalis* eggs by *Trichogramma evanescens* in three subregions of the Eastern Mediterranean region, Turkey

	1st Subregion	2nd Subregion	3rd Subregion	Total
No. of Eggs	1244	465	633	2342
No. of Parasitized Eggs	498	0	210	708
Parasitization Rate (%)	40.0	0.0	33.2	30.2

Trichogramma demonstrate the high potential of parasitizing *O. nubilalis* egg masses (>80%, in some cases) (Knipling and McGuire 1968), and egg parasitism rates of ≥80% are effective in reducing larval populations of *O. nubilalis* (Suter and Babler 1976). In the study reported herein, 80% parasitism rates of *O. nubilalis* eggs were achieved following releases of *T. evanescens* in maize. In this case, 5,000 parasitized *E. kuehniella* eggs were released at the beginning of the oviposition period of the third generation of *O. nubilalis* in the second crop of maize. Similar results have been reported previously. Coskuntuncel and Kornosor (1996) reported that 2 releases of *T. evanescens* made within a 1-wk interval (75,000 parasites per ha) to control *O. nubilalis* on maize resulted in 80.9% parasitism, and the number of damaged plants and the number of larvae were reduced 57.1 and 58.3%, respectively, in the release areas. Wang et al. (1999) achieved 40% parasitism of *O. nubilalis* eggs with 4 releases of 34,000 *T. ostriniae* per ha. Alam Nia (2002) found that the release of approx. 135,000 parasitoid per ha reduced *O. nubilalis* populations by 75%. The low parasitism rate observed in this study (Table 1) is attributed to the passive dispersion power of *Trichogramma* by wind as reported by Yu et al. (1984). Releases of *T. evan*escens are as effective as insecticides in this study; thus, corroborating findings of Kabiri et al. (1990). If the eggs of *O. nubilalis* are parasitized by *T. evanescens* at rates ≥75%, there is no need to use insecticides to control the larval pests. Hassan (1994) reported that if organophosphates and pyrethrum are used, the effectiveness of *Trichogramma* spp. is decreased by 70% and 30%, respectively. One insecticidal application does not eliminate the parasite population; however, if a crop is treated several times, then the population of *Trichogramma* spp. decreases significantly.

Grain yield was increased by 20% by releasing *Trichogramma* in comparison with untreated controls. This shows that the damage by *O. nubilalis* was reduced by the release of the parasitoids and at least 2 applications of insecticides were avoided. Bagar (1997) reported that releases of *Trichogramma maidis* Pint. & Voeg. can increase the yield of maize up to 30%. Several researchers demonstrated that a single inoculative release of *T. ostriniae* (70,000 per ha) could provide season-long parasitism of *O. nubilalis* eggs and reduce ear damage by 50% (Wright et al. 2002).

Release studies in other countries have similar results. The parasitism rates were 89.9-91.8% and 75-93% in Switzerland (Suter and Babler 1976), 63.0-84.3% in Germany (Hassan et al. 1978), 40-76.9% in The Philippines (Tran and Hassan 1986), 80% in Austria (Rawensberg and Berger 1986), and 75-83% in southern Europe (Bigler and Brunetti 1986). The reduction in the number of larvae of *O. nubilalis* was 61-93% in Germany (Hassan 1981), 70% in Switzerland (Bigler and Brunetti 1986), 80-90% in Austria (Rawensberg and Berger 1986), 78.3% in the USA (Orr and Landis 1993), and 64.7-84.0% in the Czech Republic (Bagar 1997). The reduction in the number of damaged plants was 0-60% in grain corn and 51-93% in sweet corn in Austria (Rawensberg and Berger 1986), 80.5-96.8% in Germany (Neuffer 1982), 68.3-95.2% in Switzerland (Bigler and Brunetti 1986), 78.3-95.2% in the Czech Republic (Bagar 1997), and 42% in Poland (Lisowicz and Kot 1999).

The natural parasitism rate observed in the Eastern Mediterranean region of Turkey was 30.2%. Similar results were obtained in other studies of natural parasitism of egg parasitoids in different parts of Turkey. Coskuntuncel and Kornosor (1996) found that natural parasitism by *T. evanescens* was 52.7% and 16.2% in the the Eastern Mediterranean region in 1993 and 1994, respectively. In the Black Sea Region of Turkey, Ozdemir (1981) reported that *T. evanescens* was the most effective parasitoid of *O. nubilalis*. In this region, the rate of parasitism increased to 96% with the lack of insecticide applications.

Results of this study indicate that *T. evanescens* is an effective biological control agent against *O. nubilalis* in maize produced in Adana, in the Eastern Mediterranean, Turkey. The cost of 3 insecticide applications to manage this pest is not economically viable for producers (~ $US 150 per ha). The cost of releasing *Trichogramma* spp. is at least 1/2 more economical than chemical control.

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