J Pest Sci (2010) 83:53–58
DOI 10.1007/s10340-009-0273-x

ORIGINAL PAPER

Field evaluation of the egg parasitoid, *Trichogramma evanescens* West. against the olive moth *Prays oleae* (Bern.) in Egypt

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Received: 6 February 2007 / Accepted: 13 August 2009 / Published online: 4 September 2009

Abstract In Egypt, *Trichogramma evanescens* Westwood (TE) is extensively used in inundative releases against a number of lepidopterous pests of several crops. However, the wasp had not been collected from olive groves. Field trials on the use of commercially available TE against the olive moth, *Prays oleae* (Bern.) (OM) were carried out for three successive years (2002–2004). The objective of this study was to evaluate the efficacy of inundative releases of this wasp on damage reduction. The obtained results were encouraging since OM attacks were reduced by 42.9,71 and 69.9% and TE-treated trees yielded significantly bigger olive fruits by 10.5 and 12.5% than untreated trees in 2002 and 2004 olive seasons, respectively. However, parasitization levels indicated that the wasp is not well adapted to local environmental conditions of olive groves. The suggested measure to improve the quality of released wasps is to mass release of local wasps isolated during the present study, i.e., *T. cordubensis* Vargas and Cabello and *T. euproctidis* Girault.

Keywords *Trichogramma evanescens* · Inundative releases · *T. cordubensis* · *T. euproctidis* · *Prays oleae* · Olive fruit yield

Introduction

The olive moth (OM), *Prays oleae* (Lepidoptera, Yponomeutidae) has recently become a serious pest in old and recently established and managed olive plantation, in Egypt, causing significant direct yield loss as well as aesthetic damage (Herz et al. 2005). The damage caused by this pest is at least 49–63% of production, which equates to 8–11 kg per tree (Ramos et al. 1998; Patanita and Mexia 2004). It is an important pest of olives in the Mediterranean basin (Lopes-Vellata 1999). The moth develops three generations have been reported per year. The first generation of moths appears in April. The females lay eggs on the flower buds. The newly hatched larvae live and feed within the buds and on the flowers at a later stage of their development. The second generation, emerges in early June causing the most important damage. The females oviposit on the small fruits close to the stem and the larvae bore into the stone of the olive fruit. When the larva completes its development in September and leaves the fruit, it causes spectacular fruit drop resulting in major crop losses. The third generation attacks the leaves; the emerging larvae mine in the olive leaves in autumn, hibernate as larvae and complete their development in early next spring (Pelekasis 1962).

The second and third generations of OM are relatively difficult to control with insecticides, because the larvae bore into the fruits and mine in the leaves immediately after hatching. Only the first generation is regarded as rather easy to control by spraying insecticides targeted at the larval stage. The characteristics of the feeding behaviour of OM larvae require the development of an integrated pest management approach because it is superfluous (Mazomenos et al. 1999).

Control of the OM is usually achieved by application of insecticides such as dimethoate, methidathion, endosulfan, cypermethrin, carbaryl and trichlorfon (Lopes-Vellata 1999). In general, the use of insecticides and other chemical treatments implies the risk of adverse ecological,

Communicated by M. Traugott.

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toxicological and economic effects. Alternative techniques—mainly biological—include *Bacillus thuringiensis* (BT), the larvae the first generation (anthophagous) are highly susceptible to commercially available strains (Yamvrias et al. 1986). Of today, *Trichogramma* species (Hym.; Trichogramatidae) are the most widely used insect natural enemies in the world (Li 1994). The *Trichogramma* genus includes about 180 species of minute egg parasitoids of numerous insects, especially Lepidoptera (Pintureau 1990). The use of polyphagous egg parasitoids of the genus *Trichogramma* for the control of various species of orchard and field crop Lepidoptera has received much attention (Usun et al. 1996; Öztemiz and Kornosor 1999; Mills et al. 2000; Wright et al. 2001; Ulrichs and Mewis 2004). For instance, the efficacy of augmentative releases of *T. dendrolimi* and *T. embryophagum* against the codling moth, *Cydia pomonella* in apple orchards (Hassan et al., 1998); *T. platneri* against the avocado pest, *Amorbia cuneana* in avocado orchards (Honda 2005) and *T. bourarachae*, *T. cordubensis* and *T. euproctidis* against the jasmine moth, *Palpita unionalis* and the OM in olive orchards (Hegazi et al. 2007) was tested. The results suggested that releases of *Trichogramma* wasps could improve control of lepidopterous pests on orchards.

The egg parasitoid, *Trichogramma evanescens* Westwood is extensively used in inundative releases against a number of lepidopterous pests in Europe. Among the cereal crops, successful attempts to control the European corn borer, *Ostrinia nubilalis* Hbn. with *T. evanescens* Westw. have been reported from Germany, France, Switzerland, West European countries and to control Asian corn borer, *O. furnacalis* Guenee from the Philippines (Tran and Hassan 1986). Successful attempts to control the grape moth *Lobesia botrana* in vineyards in Egypt have been reported by El-Wakeil et al. (2008). Hegazi et al. (2004) reported that the combined effect of inundative releases of egg wasps, *T. evanscens*, with mating disruption technique was successful and could provide a model for control of lepidopterous pests of olive trees.

The use of commercial strain of *T. evanescens* (TE) as biological control agent for suppression of the olive moth populations is being evaluated in Egypt.

Materials and methods

Field trials

Field experiments were conducted for three successive years (2002–2004) in a commercial olive farm located in the arid olive grove area between Alexandria and Cairo, 177 km south of Alexandria. The farm 'Paradise Park' is divided into 88 isolated plots (each 2.3–3.5 ha). Olive trees were planted in early 1996 at a density of 336 trees/ha. Trees were approximately 3–4 m height, planted at 5-m distance along the row and 6-m distance between rows. No applications of *Trichogramma* releases were previously performed on the farm.

The flight phenology of the OM was monitored by sex pheromones supplied by Prof. B.E. Mazomenos (Chemical Ecology and Natural Products Laboratory, NCSR 'Demokritos', Greece). Delta-wing traps (2/ha), baited with polyethylene vials loaded with 1.0 mg Z7-14: Ald, were used for OM. Fresh dispensers were used at the end of each generation. All traps were controlled weekly.

Two olive plots, each 1–1.5 ha, cultivated with the same olive varieties (Shamy and Toffahi) were selected to evaluate the efficacy of the commercially available species (TE). In the first plot, four patches, each contains 4 × 4 trees, were selected for *Trichogramma* releases (TR). The distance between patches within or between the second plot is <50 m. Patches of the second olive plot were used as control, i.e., without wasp releases (CO). Mass production of TE wasps was carried out by the International Company for Bioagriculture (ICB), Egypt. At each release, a dose of 3,000 wasps/card × 3 cards/tree was applied. In each card, *Trichogramma* of three different ages were released to keep searching adults present continuously. Eight-eleven releases were performed per year at 2-week intervals from 1 March to the end of October to cover the first (anthophagous), the second (carpophagous) generations and the whole seasonal presence of the jasmine moth, *Palpita unionalis* (Herz et al. 2005) of the olive moth (OM).

The efficacy of inundative releases of the TE wasps was assessed by comparing egg parasitism and population size of OM (2004-season), pre-mature fruit fall, damage of mature fruits and fruit yield (2002, 2003 and 2004 seasons) in control and *Trichogramma*-treated trees. Egg parasitism presented on treated trees (TR) and untreated ones (CO) was determined once a week in 2004 growing season. Three sampling points were randomly chosen in the TR and CO sites. On each sampling point, three neighbouring trees were sampled per tree-patch. From each tree, 10 olive shoots (ca 30-cm long) were sampled from each direction for egg counting. In the laboratory, the collected eggs were kept in a climatic chamber (25°C; 70% RH; 16:8 h L:D) until they either hatched or turned black (parasitized) and the emerged wasps were determined to species. When wasps other than TE emerged, fresh *Sitotroga cerelella* eggs (glued on paper) were added to the sample and labelled with a code name. After parasitization, fresh parasitized egg cards were sent to the Institute for Biological Control (Dr. Annette Herz), Darmstad, Germany, for identification. Identification of local species was carried out by using a diagnosis system combining morphological characters (Pinto 1999) with molecular biological attributes

(Silva et al. 1999). The percentages of parasitism by TE/sample were recorded.

Premature fruit drop caused, in part, by OM larvae was recorded by spreading two plastic nets covering the area of projection of two tree canopies per treated tree-patch from time of fruit setting to the harvest. All fallen fruits per plastic sheets were collected and weighed. For each sample, the number of fruits in 100 g/tree was counted to extrapolate the total number of fallen fruits per tree. At each sampling time, 200 fruits per sample were examined to record reasons of dropping.

Fruit damage was assessed in late August to mid-September. Five to six hundred fruits were randomly picked per tree-patch. The mean percentage of fruits with pest damage was computed. OM 'damage' for ripe fruits was calculated from characterized mines on fruit surface. At harvest time, fruit yield was assessed by selecting 5–6 trees per tree-patch and the total weight of fruit harvest per tree was determined.

Data analysis

Data were analysed for normal distribution and mean values were compared by Student's *t* test. Percentages of data were transformed to arcsin square root of proportions before statistical analysis, but the untransformed mean + SD were presented for comparison (SAS Institute 1989).

Results

Monitoring of the OM was performed for the three successive olive seasons. Only, the results of the third year are shown in Fig. 1, as a representative data for all other years. Based on the number of captured OM males, the first adults appeared as representative for all other years on 24 March when the inflorescence reached stage 'D', i.e, before bloom (Arambourg and Pralavorio 1986). Peak catches of the anthophagous generation reached 53.8 + 36.1 moths/week/trap on 21 April coincided with beginning of flower opening, the tree phonological stage 'F'. Then the trap catches progressively increased and the peak of the fruit generation (carpophagous) reached 126.7 + 46.7 moths/week/trap on 5 May (tree phonological stage 'G', i.e., petal fall and fruit setting). No trap catches of the third (phyllophagous) generation were recorded from 4 August to mid-September.

The impact of 8, 11 and 11 TE-wasps releases in 2002, 2003 and 2004, respectively, on the fruit drop of olive trees is shown in Table 1. In high fruiting years (2002 and 2004 olive-seasons), significant ($P < 0.05$) higher fruit drop occurred on control tree-patches compared with those

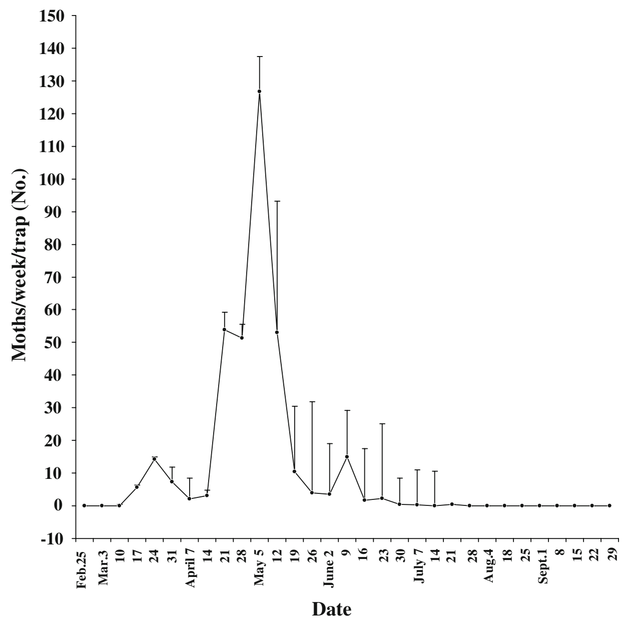

Fig. 1 Weekly mean numbers (±SE) of catches of *P. oleae* males in delta-wing traps

observed on wasp-treated tree-patches (for total fruit weight: $t = 3.14$ for 2002; $t = 3.7$ for 2004, df = 8 at $P = 0.05$, Fig. 2; for total fruit number: $t = 13.6$ for 2002, $t = 22.5$ for 2004, df = 8 at $P = 0.05$, Fig. 3). However, reverse results were recorded in low fruiting year (2003 olive season, $t = 4.9$ for total weight, $t = 13.6$ for total number, df = 8, $P = 0.05$) (Figs. 2 and 3, respectively).

Damage due to mining of OM larvae, mid to late in the season, throughout three years are shown in Table 1. In all study years, fruit damage was significantly higher ($t = 3.9$ for 2002, $t = 7.9$ for 2003, $t = 7.3$ for 2004, df = 8 at $P = 0.05$) on tree-patches received no TE wasps compared with those treated with the wasps.

In 2004-olive season, parasitization rates by TE wasps on treated tree-patches were compared with natural parasitization in non-releases tree-patches (Fig. 2). In control tree-patches, the natural parasitism was very low during the flight period (March–June) of OM. The maximum percentage of parasitism reached 13.68% late in 14 July. *Trichogramma* wasps emerged from naturally parasitized eggs showed that 60.8 and 38.0% were *T. cordubensis* Vargas and Cabello (TC) and *T. euproctidis* Girault (TEU), respectively. On the other hand, olive tree-patches received TE releases showed different ranges of parasitism. Parasitism levels ranged from 0.2% on 7 April to 43.5% on 14 July in OM eggs. Emerged wasps from parasitized eggs in the TE tree-patches showed that 21 and 8% of the individuals were TC and TEU, respectively. The TC (thelytokous species) and TEU (arrhenotokous species) were collected several times during the olive seasons.

Table 1 Impact of *T. evanescens* releases on weight (g) and number of premature fall of olive fruits, percentage of damaged ripe fruits and fruit harvest/tree (kg) during 2002–2004 olive seasons

Year	Fruiting	Premature fall/season				Damaged ripe fruits (%)		Fruit harvest/tree (kg ± SD)	
		Weight (g ± SE)		Number (×100 ± SE)		Control	TE-treated trees	Control	TE-treated trees
		Control	TE-treated trees	Control	TE-treated trees				
2002	High	612.9 ± 27.1 a	727.2 ± 7.1 b	246.3 ± 17.9 a	70.7 ± 3.6 b	24.7 ± 3.4 a	14.5 ± 3.5 b	73.5 ± 5.1 b	78.8 ± 4.6 a
2003	Low	176.8 ± 3.9 b	235.2 ± 7.2 a	79.4 ± 5.4 b	112.2 ± 4.9 a	32.3 ± 4.9 a	9.9 ± 2.5 b	7.2 ± 0.9 a	6.9 ± 1.7 a
2004	High	460.2 ± 13.7 a	257.7 ± 9.4 b	131.1 ± 7.0 a	50.12 ± 2.7 b	29.2 ± 3.8 a	9.5 ± 3.1 b	67.3 ± 5.8 b	76.7 ± 3.5 a

In year and set of data, cells with different letters are significantly different at $P < 0.05$

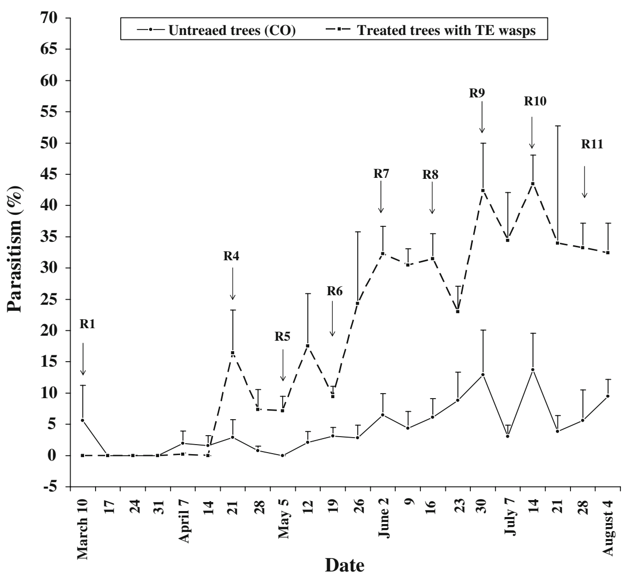

Fig. 2 Weekly mean (±SE) of percentage of natural parasitism in non-release tree-patches (CO) and tree-patches treated with *T. evanescens* (TE) releases (R1–R11), 2004-olive season

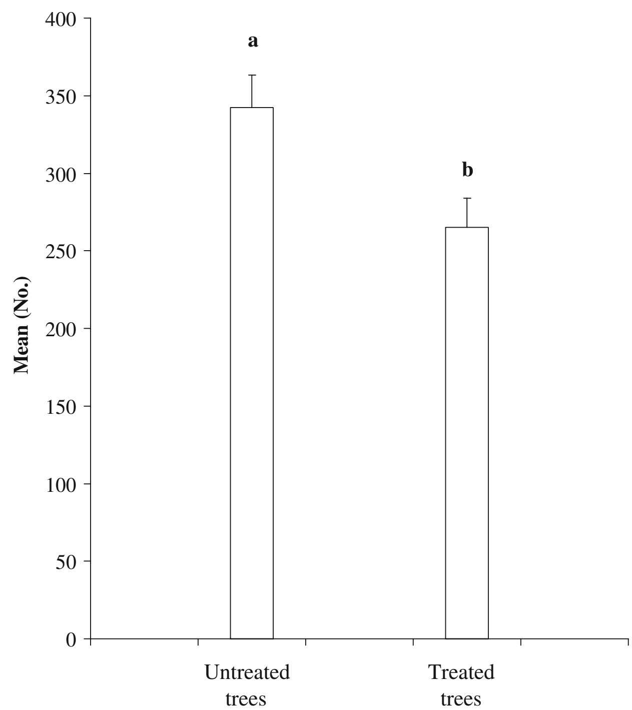

Fig. 3 Full season counts of *P. oleae* larvae/sample/tree on untreated and treated trees with *T. evanescens*. Mean values (±SE) of bars with different letters are significantly different at $P < 0.05$

The effect of inundative releases of TE wasps on the population size of OM larvae was also assessed by recording the full season counts of host larvae/sample/tree on both non-treated and treated tree-patches. Releases of TE wasps caused significant ($t = 6.03$, df = 8, $P = 0.05$) total seasonal reduction of 22.5% in OM population (Fig. 3). As far as fruit harvest/tree was concerned, tree-patches received TE-wasps in only 2002 ($t = 3.3$, df = 8, $P = 0.05$) and 2004 ($t = 5.2$, df = 8, $P = 0.05$) olive seasons yielded significantly greater fruits by 10.5 and 12.5% in 2002 and 2004 than the control trees (Table 1), respectively.

Discussion

The followings were detected: (1) the anthophagous generation closely overlapped with the carpophagous one, (2) the OM density was significantly greater in the high fruiting years (2002 and 2004) than in the low fruiting year (2003, data not shown), (3) monitoring with pheromone trap was not only a good method to detect the presence of adults and to monitor the fluctuation of adult population densities, but also gave useful data for evolution of egg-laying activity on flower and fruits, i.e., a good method for the determination of the most suitable time for *Trichogramma* releases and (4) besides, both of pre-mature fruit fall caused by feeding damage of the OM during May–August and natural thinning, the OM larvae, mid to late in the season, attacked the mature olive fruits especially table varieties. Damaged fruits deprecated the value of table varieties and lower the quality of oils produced from oil varieties.

The first serious attempts to use *Trichogramma* on olive trees were undertaken by Stavraki (1977, 1985) in Greece, releasing the species; *T. cacoeciae*, *T. dendrolimi*, *T. minutum*, *T. pretiosum*, *T. euproctidis* and two unidentified strains against the carpophagous generation of the olive moth in several field trials. These species/strains had not been collected from olive groves, but some of them were successfully used in other crop systems. TE wasps are known to attack a wide variety of field crop moths (Abbas et al. 1987; Ram et al. 1995; Bayram and Kornosor 1999; Herz et al. 2005). TE parasitoid was extensively used in inundative releases against a number of lepidopterous pests of several crops (e.g., corn, rice, sugarcane, cotton, fruit trees, grape, etc.) (Ram et al. 1995; El-Wakeil et al. 2008), but had not been observed in olive groves. Releases of TE wasps were carried out in an olive farm that represent a young and large intensively managed plantations under arid weather condition and TE free. The obtained results were encouraging since OM attacks were reduced and TE-treated trees yielded significantly greater fruits than the control trees.

Releases were made at a rate of 9,000 wasps/tree (3,000,000 wasps/ha). This dose was fairly larger to what has been used in other crop system. Rate of the parasitoid releases adopted in other several studies ranged from 15,000 (Chen and Chiu 1986) to 450,000 adults/ha (Cock 1985). However, field parasitization levels by TE indicated poor adaptation of TE to local environmental conditions of olive groves. Despite the widespread use of *Trichogramma*, there are relatively few cases where the successful control of a pest can be unequivocally ascribed to releases of these parasitoids. There are many documented failures of *Trichogramma* releases despite a few notable successes (Twine and Lloyd 1982; Smith et al. 1987; Li 1994). Sithanantham et al. (2001) reported that Trichogrammatid parasitoids are more habitat-specific than host-specific. They also reported that when selecting the species to be used, the naturally occurring interspecific diversity and the specialization should be considered. A local species is generally preferred on the basis that it is likely to be better adapted to the ecological conditions than an exotic species (Smith 1996). The obtained results indicated that pest control by naturally occurring *Trichogramma* egg parasitoids was insufficient and local augmentative releases of reared wasps are needed. In olive groves, however, higher levels of egg parasitism were obtained by local strains of TC wasps in OM eggs (up to 59%) in Portugal and in jasmine moth eggs, *P. unionalis* (83%, in Egypt, Herz et al. 2005). Strains described in this study could all be easily propagated on factitious hosts (*S. cereallela*, *E. kuehniella*) (e.g., Ayvaz and Karaborklu 2008; El-Wakeil 2007), thus indicating their potential for mass production, one important prerequisite for their use as biological control agents. Thus, one of the suggested measures to improve the quality of the parasitoid in release programme is to release the endemic wasp species in olive groves, e.g., TC or TEU or both together. Hegazi et al. (2007) suggested that releases of the indigenous TC and TEU could improve control of lepidopterous pests on olive. The present study points on the need for monitoring the local *Trichogramma* species in a particular area before inundative releases of species are conducted for the first time.

Acknowledgements This work was conducted with financial support from the European Commission within the specific programme 'Confirming the International Role of Community Research', contract ICA4-CT-2001-1004 (TRIPHELIO: Sustainable control of lepidopterous pests in olive groves—integration of egg-parasitoid and pheromones). The author would like to thank Dr. Annette Herz, Institute for Biological Control, Darmstadt, Germany, for identifying the local parasitoids and for her technical advice.

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