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Dispersal and field progeny production of *Trichogramma* species released in an olive orchard in Egypt

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Abstract Dispersal ability and field progeny production of augmentative released biological control agents depend on ecological adaptations of the particular species or strains used. Four species of the egg parasitoid genus *Trichogramma* were compared aiming to select suitable candidates for control of lepidopteran olive pests. Three of them (*T. bourarachae* Pintureau and Babault, *T. cordubensis* Vargas and Cabello, *T. euproctidis* Girault) had been previously collected from olive groves, whereas the commercially available strain used (*T. evanescens* Westwood) was originally isolated from sugarcane fields. During five consecutive field releases in an olive orchard near Cairo, dispersal and/or progeny production of these species was monitored using sentinel eggs placed at different heights in the release tree canopies as well as in neighboring trees ("distance effect"). The cardinal direction of dispersal was random for *T. euproctidis* and *T. evanescens*. Significant higher parasitism occurred on sentinel eggs placed on the middle part of tree canopy and highest parasitism was observed in trees where wasps had been released. Field progeny production was highest for *T. bourarachae*, followed by *T. euproctidis* and *T. cordubensis*. *T. evanescens* propagated less under field conditions. Inter-tree dispersal of all species except *T. bourarachae* was limited and, for biological control, releasing material should therefore be distributed on each olive tree, preferably also at different levels of the canopy.

Keywords *Trichogramma bourarachae* · *Trichogramma cordubensis* · *Trichogramma euproctidis* · *Trichogramma evanescens* · Inundative release · Egg parasitoid · Olive pest control

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Introduction

Biological control is one of the most promising alternatives to the reliance on pesticides in pest management. Although traditionally it has been focused on introduction and permanent establishment of natural enemies, greater effort is now being directed toward augmentative, particularly toward inundative, biological control (Mills 1990; Parrella et al. 1992).

An inundative biological control strategy involves the release of mass-reared natural enemies at regular intervals during the season, timed to coincide with periods when the host is susceptible to attack. Studies on natural enemies have demonstrated the importance of their dispersal in inundative releases (McDougall and Mills 1997; Saavedra et al. 1997) and in the successful establishment when introduced for insect pest control (McDougall and Mills 1997). Biological control has been successful in a number of cropping systems and with a range of natural enemy pest combinations (DeBach and Rosen 1991; King 1993).

Trichogramma species are among the most commonly used groups of natural enemies because they are relatively easy to culture and, being egg parasitoids, they kill the host before crop damage occurs. However, the effectiveness of *Trichogramma* inundation has been variable (Li 1994). One reason may be the parasitoid release without proper knowledge of the dispersal ability of the selected species. Wright et al. (2001) observed rapid dispersal of *T. ostriniae* over distances of 35–230 m after an inoculative release of ~1 million wasps from a central release point. In addition, they found that parasitism of *Ostrinia nubilalis* (Hubner) (Lep.: Crambidae) egg masses occurred up to 1–2 ha around a central release point. Babendreier et al. (2003) have shown that 3.1% of *Chrysoperla carnea* eggs were parasitised by *T. brassicae* significantly less than the observed parasitism rate of *Ephestia kuehniella* egg clusters (64%) under field conditions and also that the dispersal of *T. brassicae* was found close to the release point.

Members of the Trichogrammatidae family are very small (≤1 mm) which makes experimental manipulations challenging when monitoring their spatial dispersal. Dispersal of natural enemies after release has been measured most frequently using sentinel hosts. However, sticky traps (Hendricks 1967) and radio-active markers (Stern et al. 1965) have also been used. The conventional methods of marking are not feasible for these minute wasps. Direct applications of fluorescent powders are likely to have severe negative impacts on survivorship and behavior (Corbett and Rosenheim 1996).

Lepidopteran pests are important constraints to olive production in Egypt, in particular the olive moth, *Prays oleae* (Bern.) and the jasmine moth, *Palpita unionalis* Hübner, which cause direct yield loss as well as aesthetic damage (Balashowsky 1972; Herz et al. 2005). The present study was conducted as a precursor to field release of three local and one commercial available *Trichogramma* species for inundative biological control of aforementioned pests. The objective of this study was to assess the dispersal of parasitoids and sentinel egg parasitism after inundative releases and determine the relative production of parasitoid progeny under field conditions. An understanding of dispersal and searching abilities of the parasitoid can assist in the optimization of parasitoid releases in olive orchards.

Materials and methods

Origin and mass production of *Trichogramma* species

Four indigenous species of *Trichogramma* spp. were obtained from field collections, done in two olive orchards from early spring to late summer (and occasionally fall) of the years 2002, 2003 and 2004 (Hegazi et al. 2005), and were identified using morphological and molecular methods (Herz et al. 2007) according to Pinto (1999) and Silva et al. (1999). The first three species *T. cacoeciae* Marchal, *T. cordubensis* Vargas and Cabello and *T. euproctidis* Girault were collected at the olive orchard "Paradise Park" at the desert road near Cairo (see below), where no *Trichogramma* releases had been performed before, and the fourth species *T. bourarachae* Pintureau and Babault was collected at the olive farm "Bourg El-Arab" at the coast near Alexandria. These species were tested in the laboratory for host preference (Herz and Hassan 2006). *T. bourarachae, T. cordubensis* and *T. euproctidis* were mass-reared at the Biological Control Laboratory (Alexandria University, Egypt) on fresh eggs of *Sitotroga cerealella* Olivier, supplied by the International Company of Bioagriculture (Giza, Egypt). The quality of the mass rearing strains was maintained by regularly introducing feral individuals taken from the field into the rearing process (Bigler 1994). The mass rearing procedure followed the standard protocol developed by Hassan (1981).

The commercially available species *T. evanescens* Westwood, reared by the International Company of Bioagriculture for use in other crops (corn, vegetables) was also tested in field releases in comparison with the aforementioned indigenous species. This strain had been originally isolated from eggs of *Chilo agamemnon* Bles in sugar cane (Ahmed and Kira 1960; Abbas 1990).

Releasing cards were prepared by gluing approximately 2,500–4,000 parasitized eggs of *S. cerealella* on small cardboard cards (1.5 × 3.5 cm). The number of eggs depended on the expected sex ratio of each species i.e. *T. evanescens, T. bourarachae* and *T. euproctidis* are arrhenotokous species with a female-biased sex ratio of 60–70% females. *T. cordubensis* is a thelytokous species, producing only females when temperatures are moderate (<30°C, Pintureau et al. 1999). Thus, fewer amounts of parasitized host eggs were used for thelytokous species and larger amounts of host eggs were used for the arrhenotokous species. Eggs contained parasitoids close to emerge to ensure their emergence during the first two days of their presence in the field. Exact application rates and emergence pattern of each of the four *Trichogramma* species was monitored under semi-field conditions for quality control of produced cards (Hegazi et al. 2007). The releasing cards wrapped up the parasitized eggs completely and were stapled on the sides to protect them from foraging ants, which have been observed in previous studies to remove considerable amounts of the release material or sentinel eggs shortly after installation in the field (Hegazi et al. 2005; Herz et al. 2005). Moreover ants were prevented to access experimental trees from the soil by applying rat glue (Temo rat, AM-Advanced Industries Co., Egypt) on the base of the tree, 15 cm above ground before each release (Fig. 1).

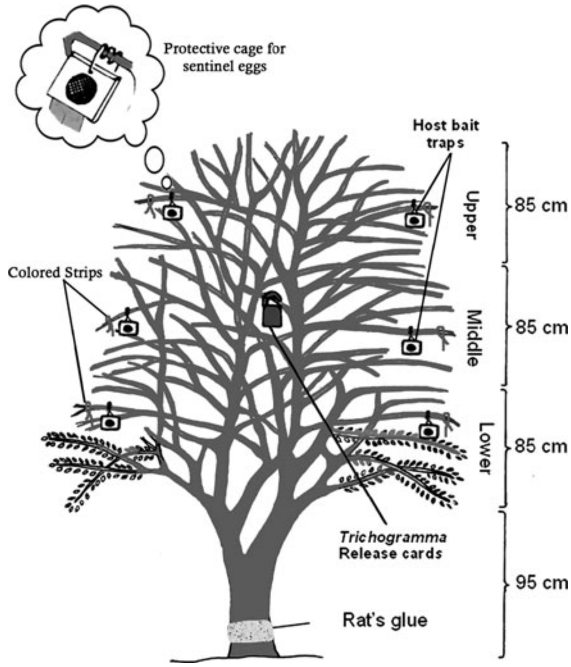

Fig. 1 Schematic diagram of a standardized release tree showing plant divisions and location of cages with sentinel eggs, colored strips, release cards and place of rat's glue

Site description

All studies were carried out in a large olive orchard ("Paradise Park", size of 240 ha), located in the arid olive growing area (E 30° 51′ 21″; N 30° 08′ 27″) between Alexandria and Cairo, 177 km south of Alexandria. The Paradise Park farm is divided into 88 isolated olive plots (each 2.3–3.5 ha), separated by windbreaks planted with *Casuarina stricta*. Olive trees were 15 years old, approximately 3–4 m high. They were planted at a density of 336 trees ha^{-1}, 5 m along the row and 6 m between rows, and were drip-irrigated. During 2005, the mean annual rainfall was 9.4 mm. The rainy season occurs from November–December to January–February. The mean monthly minimum temperature varied from 10.2°C in January to 23.9°C in August and the mean monthly maximum temperature varied from 18.9°C in January to 35.6°C in July. The mean RH varied from 47.8% in April to 63.8% in January. Wind was not measured at the release sites. Nearby airport readings and personal observations indicated that wind velocities were low throughout the experiments.

Experimental set-up

Mass-reared *Trichogramma* had never been released before 2002 when small-scale experiments started using the commercial strain *T. evanescens* (Hegazi et al. 2004). In 2005, three plots (3.3 ha each) which were semi-isolated and consisted with olive trees of Shamy and Toffahi varieties, were used for the experiment. On each plot (replicate), four patches of 5 × 5 trees (480 m^2) were selected for releases of the commercial species *T. evanescens* and the indigenous species *T. bourarachae, T. cordubensis* and *T. euproctidis*

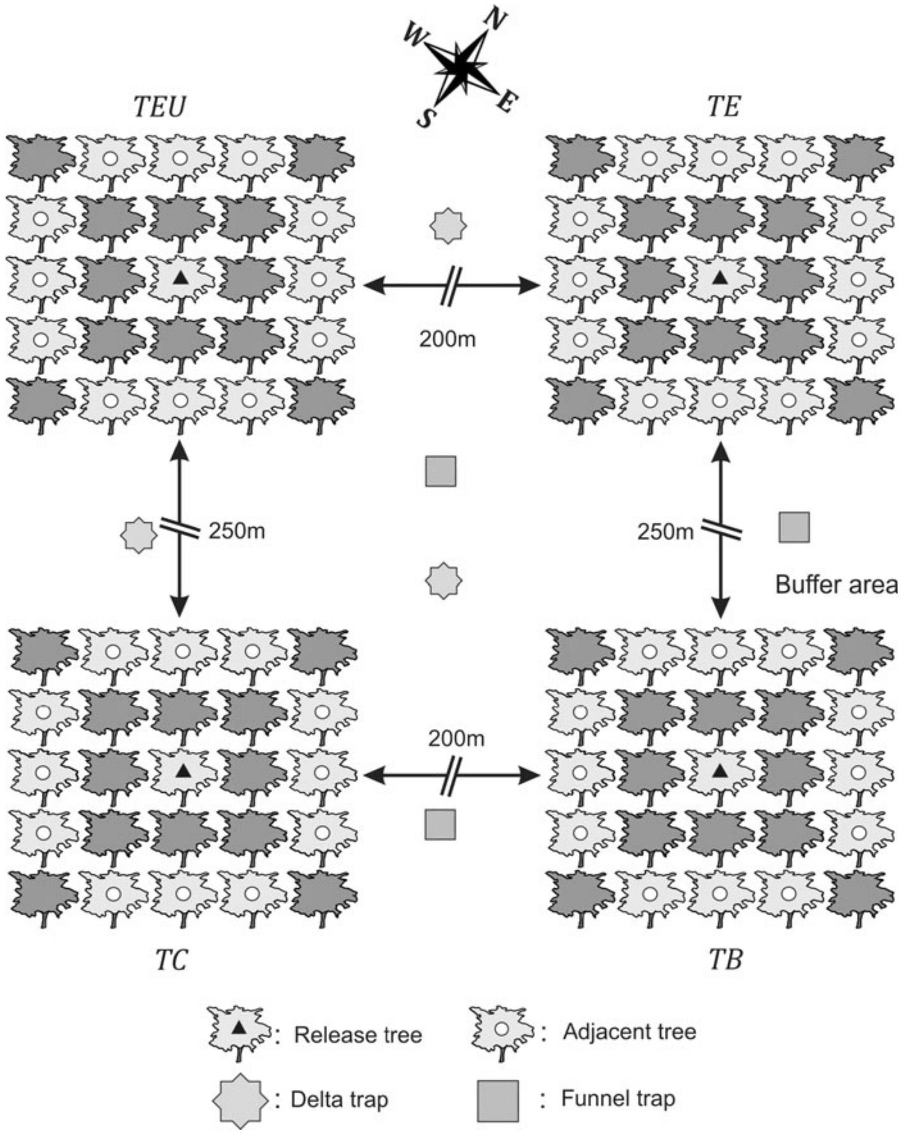

Fig. 2 Schematic diagram of the plot arrangement for monitoring the dispersal of several *Trichogramma* species (TEU: *T. euproctidis*, TC: *T. cordubensis*, TB: *T. bourarachae*, TE: *T. evanescens*), using sentinel eggs, within the release tree and to adjacent trees, 10–12 m far from the release site. The first appearance of olive and jasmine moths were checked by delta and funnel traps, respectively

(Fig. 2). The patches were separated by 200–250 m buffer areas with trees that were left untreated. Three comparable plots (3.3 ha each), cultivated with the same olive varieties (Shamy and Toffahi), 500 m away from nearest *Trichogramma* plots were used as control (CO). Four patches of 5 × 5 trees in each plot were selected for sampling. No insecticide treatments had been applied in the plots during the previous three years and during the present trial. The release tree in each tree patch was in the middle (Fig. 2). The release trees were standardized in shape and height by pruning. Each pruned tree (ca. 3.50 m height) was arbitrarily stratified into linear sections, each consisting of a third of the tree canopy (designated lower, middle and upper third, Fig. 1). Releases were made under sunny conditions, range of temperature of 20–24°C and range of wind speed of 0.6–1.8 m s^{-1}.

For each of the *Trichogramma* species, a series of five separate weekly releases were made at 7:00 am in February/March 2005, coinciding with the olive tree phenological phase B–C, in the absence of native *Trichogramma* species and target lepidopteran pests whose flight periods were surveyed by pheromone traps. The releases were conducted, to estimate the dispersal of the examined species and a parasitization pattern on the release and the adjacent trees. Parasitoid performance was not observed directly, but was inferred from the number of parasitized sentinel eggs. Each release represents a trial which was repeated for five successive weeks.

Trichogramma within-tree dispersal was studied by stapling three releasing cards at the centre, upper, lower, centre and upper part of the release trees during the 1–5th release weeks, respectively. On the release tree, an application rate of approximately 9,000 female wasps per tree was applied (about 3,000 female wasps per card × three cards per tree) for each release. The number of parasitized eggs per card were ca. 3,000 and ca. 4,300 for thelytokous and arrhenotokous species, respectively. Sentinel eggs were exposed on the trees immediately prior to parasitoid releases and were replaced after seven days. The interval between releases in the same tree-patch was seven days providing adequate time for all parasitoids to disperse and die. Dispersal of released wasps was monitored by evaluating parasitism of exposed fresh eggs of *S. cerealella*. The sentinel eggs were glued on paper (550–600 eggs per paper) and inserted in a small protective cage which could be fixed on olive twigs (Sakr et al. 2000; Hegazi et al. 2005). Colored strips were fastened near the cages, as optical cues, to facilitate their collection after exposure. Sentinel eggs were placed at the top, middle and lower in each quadrant of the tree canopy (120 cages per tree; 30 per quadrant). Sentinel eggs were replaced by fresh ones weekly, followed immediately by the release of wasps in the centre of each release tree. *Trichogramma* inter-tree dispersal was tested in a distance of 10–12 m from a particular release tree, where three trees at each of northern, southern, western and eastern sides were selected and ten cages containing sentinel eggs were fixed in each of these trees (Fig. 2). Sentinel eggs were replaced once per week.

Recovered sentinel eggs were stored separately in small glass tubes in an incubator at 25°C before scored for parasitism by *Trichogramma* wasps which developed solitary on these eggs. Emerged wasps were identified by morphological methods and counted. Egg parasitism was calculated as percentage of egg masses with at least one parasitized egg (Honda 2005).

In each trial, information was gathered on background parasitism by native *Trichogramma* spp. in the field by control cards. For this purpose, another plot with the same olive varieties and planting distances as the treated plots, located 500 m away from the releasing site was selected and served as control in which no releases were performed. In this plot, sentinel eggs were provided weekly, in the aforementioned manner, for sampling. In order to avoid potential movement of released wasps into the control (no-release) area, all control tree-patches were compared into a separate and distant plot from the treated (release) plots. There is some drawback in this experimental design, because the control patches seem to be not spatially independent. However the control replications were well separated from each other within this plot and ensured sufficient and independent variation of observations.

In all plots, Delta traps (Trece Inc., Salinas, CA, USA) and funnel traps (Agrisence BCS, Pontypridd, UK) were used to monitor the flight activity of olive moth and jasmine moth males, respectively. Traps were set up in groups, each group with three traps/ha. Distances of the traps were 40–50 m within a group and groups were spaced 60 m apart. Traps were hung on poles at 1.5–2 m height close to the external south part of the tree canopy. Each trap was baited with pheromone lures. The sex pheromone components used for monitoring olive moth [(Z)-7-tetradecenal)] and jasmine moth (*E*)-11-hexadecenal (E11-16:Ald) and (*E*)-11-hexadecenyl acetate (E11-16:Ac) were synthesized by Sociedad Española de Desarrollos Químicos (SEDQ, Barcelona, Spain). Traps were checked every day and trials ended when the first moths appeared in the traps.

Statistical analysis

Percentage of parasitism, proportion of emerged wasp and emerged wasp per week were analyzed by one-way analysis of variance (ANOVA) and means were compared by least significant difference (LSD). SPSS 8.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Percentage values were subjected to arcsine square root transformation to increase the homogeneity of variance and normality. The transformed data were normally distributed.

Results

We found no parasitism in the pre-release monitoring of experimental plots, in the control plot and buffer sites between plots, indicating absence of native population of *Trichogramma* wasps during the trials. *Trichogramma* parasitized the sentinel eggs over the experimental period and we never found more than one species per egg-card (except *T. bourarachae*, see below). Of the total amount of sentinel egg cards per release tree, 69.3, 59.2, 45.1 and 35.3% were parasitized by *T. bourarachae*, *T. euproctidis*, *T. cordubensis* and *T. evanescens*, respectively. The parasitoid species exhibited significant differences in percentage parasitism of overall parasitism within the release tree ($F = 11.5$; d.f. = 3, 16; $P < 0.05$). The percentage parasitism of overall parasitism of *T. bourarachae* and *T. euproctidis* was greater when compared with *T. cordubensis* and *T. evanescens* (Fig. 3).

The distance of sentinel eggs from the release tree had a significant influence on the percent parasitism. Percentage parasitism of sentinel eggs on adjacent trees (10–12 m from the release tree, Fig. 2) were 36.4, 30.9, 25.2 and 22.0% for *T. bourarachae*, *T. cordubensis*, *T. euproctidis* and *T. evanescens*, respectively and percentage parasitism for *T. bourarachae* was greater than those for the rest *Trichogramma* species. The wasp species exhibited significant differences in percentage of overall parasitism on the adjacent trees ($F = 7.82$; d.f. = 3, 16; $P < 0.05$). Percentage parasitism of sentinel eggs placed 10–12 m away from the release trees was higher for the indigenous species *T. bourarachae* and *T. cordubensis* or equal for *T. euproctidis* compared to the commercial species *T. evanescens* (Fig. 3, $F = 16.894$; d.f. = 3, 16; $P < 0.05$).

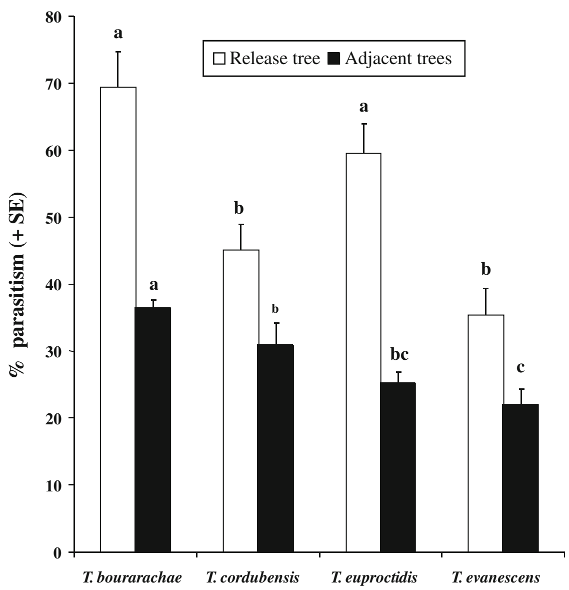

Fig. 3 Mean percentage (+SE) of parasitism of sentinel eggs on release and adjacent trees (10–12 m far from the release site) by each of four parasitoid species. For each set of trees, bars headed by different letters are significantly different by ANOVA followed by least significant difference (LSD = 6.4 for release tree, 4.4 for adjacent tree) at $P < 0.05$

Figure 4 shows the mean number (±SE) of progeny emerged per week from parasitized sentinel eggs by each parasitoid species, in respect to release and adjacent trees. The emerging wasps were 2216.4 ± 86.9, 1624.2 ± 78.2, 1063.6 ± 98.3 and 924.2 ± 58.4 for *T. bourarachae*, *T. euproctidis*, *T. cordubensis* and *T. evanescens*-release trees, respectively. The mean number of emerging progeny per week from parasitized sentinel eggs differed significantly between *Trichogramma* species on the release trees ($F = 5.29$; d.f. = 3, 16; $P < 0.01$). The overall mean number of emerging wasps per week from parasitized sentinel eggs by each of four parasitoid species also showed significant differences on the adjacent trees ($F = 5.29$; d.f. = 3, 16; $P < 0.01$). Sentinel egg cards on *T. bourarachae*-adjacent trees (Fig. 4) produced more wasps (608.4 ± 115.9 wasps per week) followed by *T. cordubensis*-adjacent trees (475.4 ± 111.9 wasps), *T. euproctidis*-adjacent trees (371.6 ± 89.8 wasps) and finally by *T. evanescens*-adjacent trees (257.8 ± 41.4 wasps). Total progeny production (sum of progeny from release and adjacent trees) was significantly different among the species ($F = 5.29$; d.f. = 3, 16; $P < 0.01$). The emerging wasps per week accounted to: *T. bourarachae* (2824.8 ± 119.9 wasps), *T. euproctidis* (1995.8 ± 100.9), *T. cordubensis* (1539 ± 96.7) and *T. evanescens* (1182 ± 56.7).

Percentage parasitism of sentinel eggs placed at the cardinal directions North, South, East and West of the release trees varied among the four parasitoid species (Fig. 5a). The distribution pattern was random for *T. euproctidis* and *T. evanescens* as the percentage of parasitized sentinel eggs in the four quadrants was not significantly different. The sentinel eggs placed on the release tree to the West for *T. bourarachae* ($F = 5.29$; d.f. = 3, 16; $P < 0.01$) and North and East for *T. cordubensis* ($F = 5.29$; d.f. = 3, 16; $P < 0.01$) had a lower percentage of parasitism than those on the other cardinal directions.

Although the percentage parasitism of the sentinel eggs on the *T. euproctidis* and *T. evanescens*-release trees was not significant in the four quadrants of each

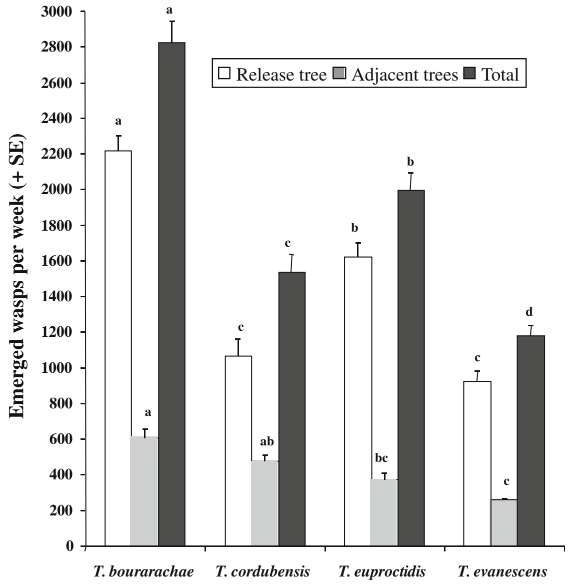

Fig. 4 Mean number (+SE) of emerged wasps per week from parasitized sentinel eggs by each of four parasitoid species, collected from release trees, adjacent trees and total emerged wasps per species. Bars headed by different letters are significantly different by ANOVA followed by least significant difference (LSD = 245.1 at $P < 0.05$ for release tree; 150.1 at $P < 0.01$ for adjacent tree)

tree, the percentage of wasps emerging from the parasitized eggs varied between cardinal directions for *T. euproctidis* ($F = 7.739$; d.f. = 3, 16; $P < 0.01$) or *T. evanescens*-release trees ($F = 28.241$; d.f. = 3, 16; $P < 0.01$). Most wasps originated from sentinel eggs placed at the southern for *T. euproctidis* and eastern and western directions for *T. evanescens* (Fig. 5b). Progeny production at the four cardinal points was also different for *T. bourarachae* ($F = 18.776$; d.f. = 3, 16; $P < 0.01$) and *T. cordubensis*-release trees ($F = 7.355$; d.f. = 3, 16; $P < 0.01$). For *T. bourarachae*, most wasps emerged from eggs placed in the eastern part of the tree and for *T. cordubensis* from eggs placed in the southern and western part (Fig. 5b).

Percentage parasitism on the sentinel eggs was significantly higher on adjacent trees to South of the *T. bourarachae*-release trees ($F = 8.798$; d.f. = 3, 16; $P < 0.01$), to West of the *T. cordubensis*-release trees ($F = 10.37$; d.f. = 3, 16; $P < 0.01$), to North and West of the *T. euproctidis*-release trees ($F = 10.496$; d.f. = 3, 16; $P < 0.01$) and South of the *T. evanescens*-release trees (Fig. 6a). Significantly greater number of wasps was bred from sentinel eggs on these adjacent trees for *T. cordubensis*, *T. euproctidis* and *T. evanescens* except for *T. bourarachae* (Fig. 6b) ($F = 20.15$, 19.926, 5.1 and 27.417 for *T. bourarachae*, *T. cordubensis*, *T. euproctidis* and *T. evanescens* at d.f. = 3, 16 and $P < 0.01$).

Percentage parasitism of sentinel eggs was greater in middle parts (for *T. evanescens* or *T. euproctidis*) or middle and lower part (for *T. bourarachae* or *T. cordubensis*) of the tree canopy than in the other parts of the release trees, respectively ($F = 28.676$; d.f. = 2, 12; $P < 0.01$, $F = 22.102$; d.f. = 2, 12; $P < 0.01$, $F = 11.853$; d.f. = 2, 12; $P < 0.01$, $F = 97.725$; d.f. = 2, 12; $P < 0.01$, for *T. bourarachae*, *T. cordubensis*, *T. euproctidis* and *T. evanescens*, respectively; Fig. 7a).

Significant differences in numbers of emerged progeny per week were noted on the three vertical tree divisions in descending order as follows: (1) for *T. bourarachae*-trees middle > lower > upper third ($F = 36.85$; d.f. = 2, 12; $P < 0.01$), (2) for *T. cordubensis*-trees middle = lower > upper third ($F = 33.750$; d.f. = 2, 12; $P < 0.01$), (3) for *T. euproctidis*-trees middle > upper third = lower ($F = 18.491$; d.f. = 2, 12; $P < 0.01$), and (4) for *T. evanescens*-trees middle > lower third > upper third ($F = 22.237$; d.f. = 2, 12; $P < 0.01$) (Fig. 7b). In all *Trichogramma* species, a greater percentage of wasps emerged from sentinel eggs parasitized in the middle part compared with the upper and lower parts of the release trees (Fig. 7b).

Trichogramma bourarachae and *T. cordubensis* tended to disperse and parasitize sentinel eggs outside their releasing target areas. 3.5, 1.8 and 1.5% of sentinel eggs per tree were parasitized by *T. bourarachae* on *T. cordubensis*, *T. euproctidis* and *T. evanescens* trees, respectively. *T. cordubensis* wasps were also bred from 1.2% of sentinel eggs of *T. evanescens* trees. No wasps other than *T. bourarachae* were bred from sentinel eggs of *T. bourarachae*-trees. However, *T. bourarachae* was even found occasionally in sentinel eggs placed on the control plot, 500 m far from the nearest *T. bourarachae* release area.

Discussion

The dispersal and the relative progeny production of three indigenous (*T. bourarachae*, *T. cordubensis* and *T. euproctidis*) and one commercially available (*T. evanescens*) parasitoid species were determined by

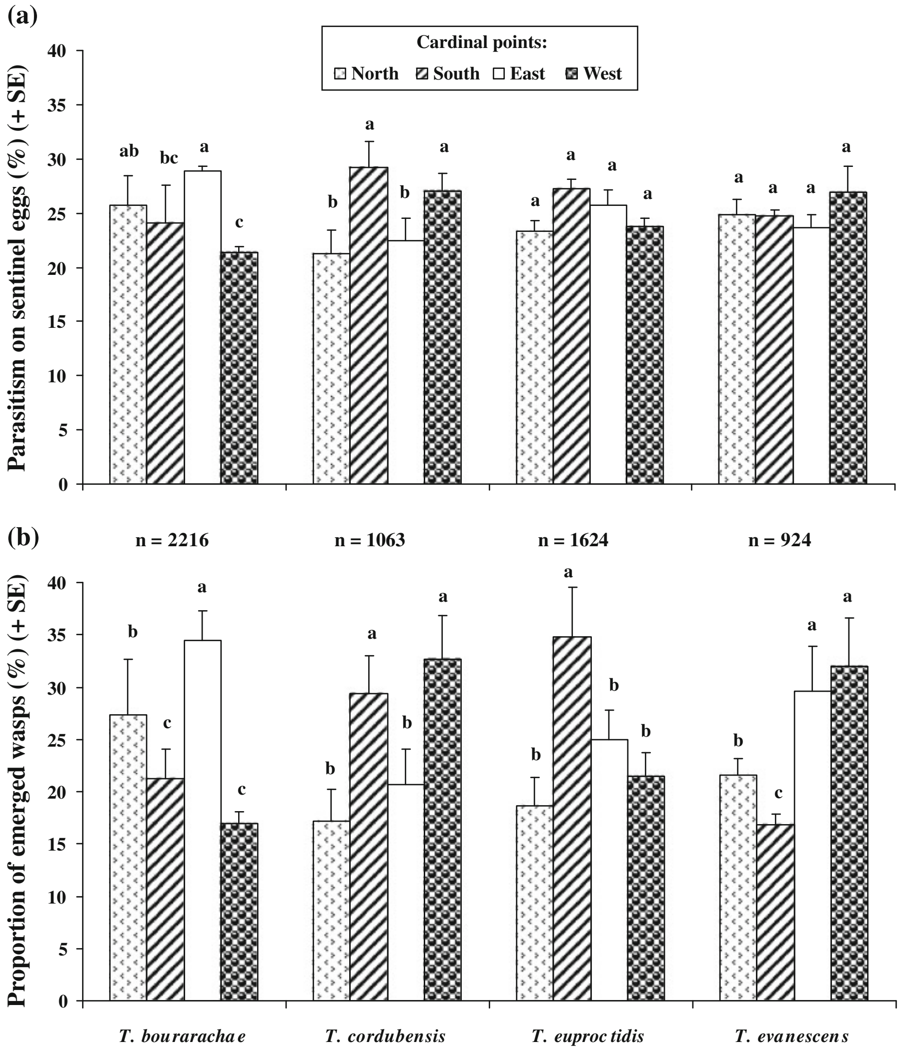

Fig. 5 Distribution of parasitism on sentinel eggs placed in cardinal directions within the release trees of different *Trichogramma* species. **a** Percentage parasitism on sentinel eggs and **b** proportion (%) of emerged wasps from parasitized eggs. For each parasitoid species, bars headed by different letters are significantly different by ANOVA followed by least significant difference (LSD) at $P < 0.05$

monitoring parasitism on sentinel eggs placed on the release tree as well as on neighboring trees. The reason of including *T. evanescens* was to obtain a clear proof of the superiority of local species, adapted in the olive ecosystem, to other *Trichogramma* (Herz and Hassan 2006). All released indigenous wasp species accomplished higher parasitism on sentinel eggs than *T. evanescens* both in release and adjacent trees. This is possibly due to the poor adaptation of *T. evanescens* to the particular environmental conditions of olive groves. *T. evanescens* is extensively used in biological control of a number of lepidopterous pests in several crops (e.g. corn, rice, sugarcane, cotton, fruit trees) (Ram et al. 1995), but had never been collected in our field surveys of the natural *Trichogramma* fauna in olive groves in Egypt, neither in other countries (Herz et al. 2007). Trichogrammatidae are considered to be more habitat-specific than host-specific (Sithanantham et al. 2001; Romeis et al. 2005). Environmental conditions as well as particular traits of the host plant can affect survival and success in host location of these minute egg parasitoids and thus their efficacy to find and parasitize the eggs of important pests in the particular agroecosystem (Gingras and Boivin 2002; Chapman et al. 2009).

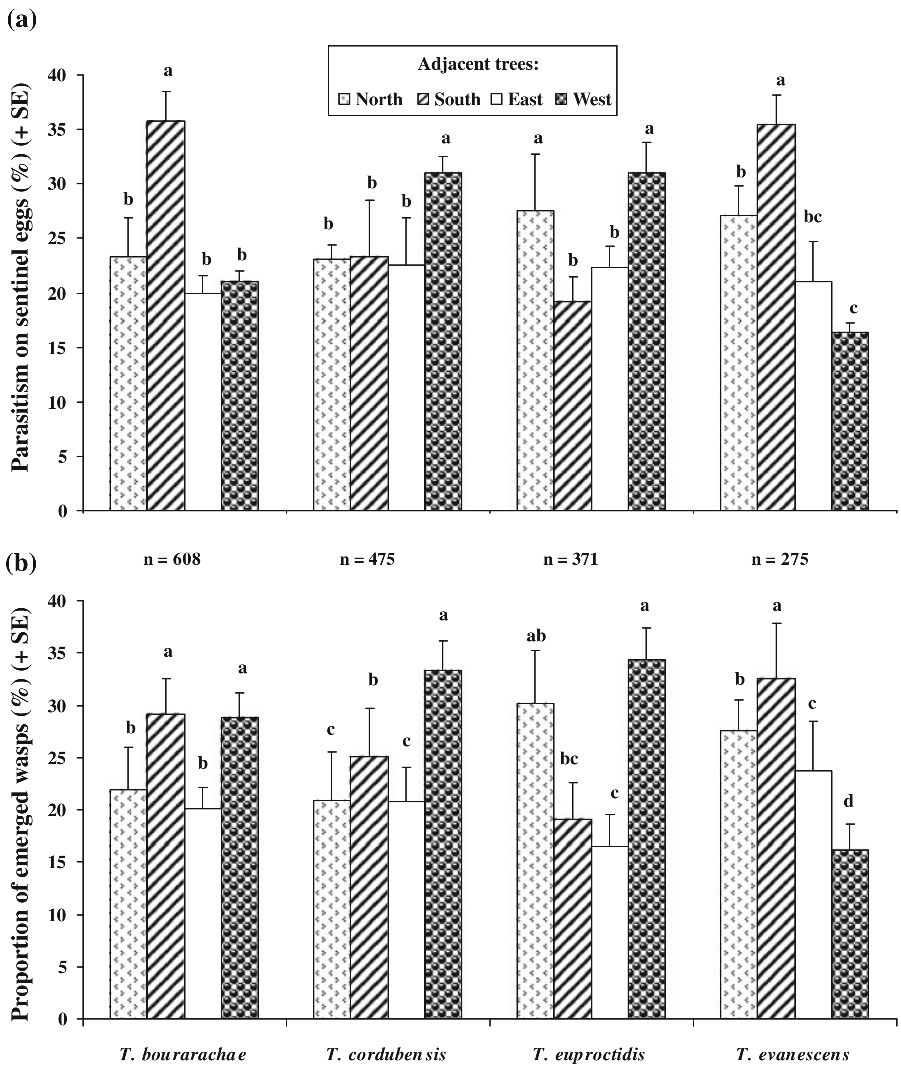

Fig. 6 Dispersal of four parasitoid species from release trees to adjacent trees in a distance of 10–12 m, evaluated by parasitism on sentinel eggs (**a**) and proportion (%) of emerged wasps (**b**). For each wasp species, bars headed by different letters are significantly different by ANOVA followed by least significant difference (LSD) at $P < 0.05$

However, the decline in rate of parasitism on the adjacent trees was species-dependent. McDougall and Mills (1997) similarly found that parasitism of sentinel eggs by *T. platneri* declined from a mean of 62% at the point-source to less than 10% 14 m away during the first three days after release. The four parasitoid species exhibited different tendencies for dispersal to adjacent trees in 10–12 m distance. The importance of the distance from the point of release on the rate of parasitism was also observed for *T. maidis* (Bigler et al. 1988) and *T. brassicae* (Greatii and Zandigiacomo 1995) when eggs of the European corn borer *O. nubilalis* (Hn.) were fastened at different distances from the point of release. To reduce this distance effect in the case of inundative *Trichogramma* release, a high number of point-releases per hectare should be maintained. This strategy might also be able to reduce the wind effect in windy areas.

Significantly more wasps emerged from samples of sentinel eggs parasitized by the indigenous species. *T. evanescens* was the poorest performer of the four *Trichogramma* species tested. *T. evanescens* is specialized on low-growing plants in irrigated land rather than on trees. Habitat and climatic specialization can

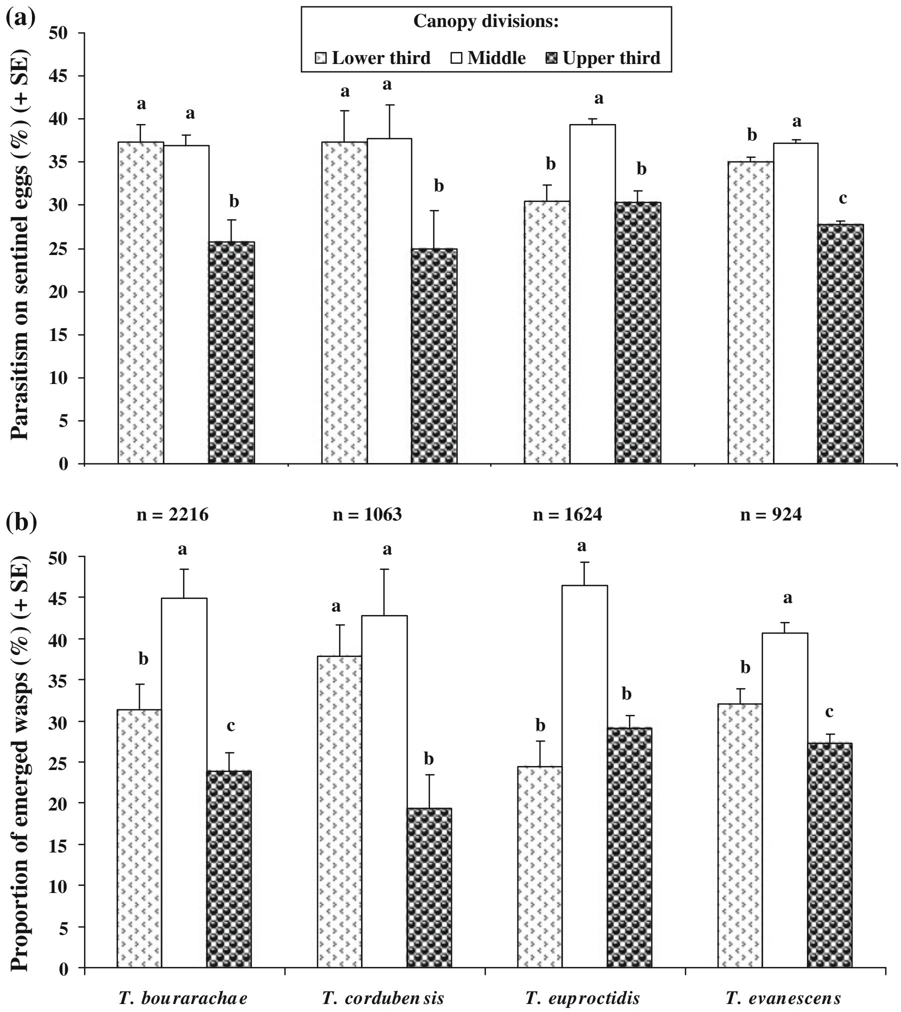

Fig. 7 Vertical distribution of parasitism on sentinel eggs within the release trees of different *Trichogramma* species. **a** Percentage parasitism on sentinel eggs and **b** proportion (%) of emerged wasps from parasitized eggs. For each parasitoid species (release tree), bars headed by different letters are significantly different by ANOVA followed by least significant difference (LSD for **a** = 6.37, 4.6, 4.45 and 155 for wasp species, respectively; for **b** = 9.5, 14.2, 12.8 and 2.8, respectively) at $P < 0.05$

be important criteria limiting the success of biological control agents, if not well adapted. Herz and Hassan (2006) concluded that biological control agents should preferably be isolated from the relevant pest per crop system.

Trichogramma bourarachae was also collected from the releasing areas of the other species, 200 m apart, indicating its efficiency in dispersal and foraging. Other field studies investigating dispersal of *Trichogramma* from a release point which have been conducted in row crops and orchards (Stern et al. 1965; Yu et al. 1984; Greatii and Zandigiacomo 1995; McDougall and Mills 1997) showed that the smallest reported dispersal distance was 8 m for *T. minutum* Riley in cotton (Fye and Larsen 1969) and the greatest distance was 1,050 m for *Trichogramma* species in alfalfa, with the majority of reports indicating a dispersal range of 20–70 m (Stern et al. 1965). Our field data suggested that *T. bourarachae* also dispersed at a great range. The study provided evidence that *T. bourarachae* displays superior dispersal abilities compared to both *T. evanescens* and the other indigenous species. It seems that this parasitoid has some morphological or ecological traits which are responsible of such superiority.

Murdoch et al. (1989) and Tscharntke (1992) reported that parasitoids attacking herbivorous insects show uneven distribution within individual plants. The results obtained in this experiment indicate that the four species of parasitoids exhibited different horizontal and vertical distribution patterns within the olive tree. The rate of parasitism by the four wasp species was significantly influenced by the vertical distribution of the sentinel eggs. The cardinal direction dispersal within the release tree was random in two out of the four species. However, Newton (1988) and Smith (1988) observed no effect of wind direction on parasitism of sentinel eggs. Significantly greater parasitism occurred on sentinel eggs in the middle portion of release tree canopy for all wasps tested. In contrast, dispersal to the upper part of the tree was limited. The wasps probably avoided exposure to direct sunlight at the upper part of the canopy. Weather conditions such as wind and rain can affect the movement of parasitoids and their subsequent levels of parasitism (Chapman et al. 2009).

The present study provided useful hints for developing the design of inundative releases of indigenous, well adapted *Trichogramma* species in olive orchards. Further information is required, e.g. the dispersal of *Trichogramma* species in relation with the distribution of target insects on the tree canopy, the combination of several local *Trichogramma* species and their interaction with the general aim to optimize this strategy for controlling main pests of olive, a major crop in Mediterranean and North-African countries.

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