Field Evaluation of the Egg Parasitoid, *Trichogramma evanescens* West. Against the Olive moth, *Prays oleae* (Bern.) in Egypt

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ABSTRACT

In Egypt, *Trichogramma evanescens* Westwood (TE) is extensively used in inundative releases against a number of lepidopterous pests of several crops and 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 the present study was to evaluate the efficacy of inundative releases of this wasp on fruit yield. The obtained results were encouraging since OM attacks were reduced by 42.9,71 and 69.9% and TE-treated trees yielded significantly greater 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 work, *i.e., T. cordubensis* Vargas and Cabello and *T. euproctidis* Girault.

Key Words: *Trichogramma evanescens*, inundative releases, *Prays oleae*, olive, yield, Egypt.

INTRODUCTION

The olive moth (OM), *Prays oleae* has recently became a serious pest in old and recent established managed olive plantation, in Egypt, causing significant yield loss as well as aesthetic damage (Herz *et al.*, 2005). It is an important pest of olives in the Mediterranean basin (Lopez-Villata, 1999). In Greece, the moth develops three generations per year *vs.* two in arid growing olive zones in Egypt (Agamy, unpublished data). 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, causes most of the damage. The females oviposit on the small fruits close to the stem and the larvae bore into the stone of the olive fruits. When the larva completes its development in September and leaves the fruit, it causes spectacular fruit drop and results 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 early at the next spring (Pelekasis, 1962).

The second and third generations of the moth are relatively difficult to reach 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 be controlled by spraying insecticides targeting the larval stage. The characteristics of the feeding behaviour of this moth species, during its larval stage, require an integrated pest management approach for its suppression (Mazomenos *et al.*, 1999). 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 and Patanita and Mexia, 2004).

Control of the OM is usually achieved by application of insecticides such as dimethoate, methidathion, endosulfan, cypermethrin, carbaryl and trichlorfon (Lopez-Villata, 1999). In general, the use of insecticides and other chemical treatments implies the risk of adverse ecological, toxicological and economic effects. Alternative techniques - mainly biological- include the use of insect predators and parasitoids. Today, *Trichogramma* species (Hym.; Trichogrammatidae) are the most widely used insect natural enemy 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 (Parker and Pinnell, 1972 and Ridgway and Vinson, 1977). Very large numbers of *Trichogramma* adults are required for inundative releases to suppress established populations of moths in field crops or orchards. Methods of producing the large numbers of required *Trichogramma* are always dependent on use of a stored-product moth host, such as the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Grimm and Lawrance, 1975; Morrison *et al.*, 1976 and Hassan, 1981) and on the Mediterranean flour moth, *Anagasta küehniella* (Zeller) (Daumal *et al.*, 1975).

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* 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). Hegazi *et al.* (2004), reported that the combined effect of inundative releases of the egg wasp, *T.evanescens*, 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 the olive moth populations have been evaluated, in Egypt.

MATERIALS AND METHODS

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 along the row and 6 m between rows. No applications of *Trichogramma* releases were previously performed in the farm.

The flight phenology of the OM was monitored by sex pheromone traps 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 weekly counted.

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 commercial available species (TE). In the first plot, four tree patches, each contains 4x4 trees, were selected for *Trichogramma* releases (TR). The distance between patches within or between the second plot was ≤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 3000 wasps/card x 3 cards/tree was applied. In each card, *Trichogramma* of 3 different ages were released to keep searching adults present continuously. Eleven releases were performed/year at 2 week intervals from March 1st to the end of October to cover the first (anthophagous), the second (carpophagous) and the third (phyllophagous) generations (Herz *et al.*2005) of the olive moth (OM).

The efficacy of inundative releases of the TE wasps was assessed by comparing egg parasitism percentage and population size of OM (2004-season), pre-mature fruit fall, damaged 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, 3 neighbouring trees were sampled/tree patch. From each tree, 10 olive shoots (ca 30 cm long) were removed at each direction. In the laboratory, the collected eggs were kept in a climatic chamber (25°C; 70% RH; 16:18 h L:D) until they either hatched or turned black (parasitized). The percentages of parasitism/moth species/sample were recorded.

Premature fruit drop caused, in part, by OM larvae was recorded by spreading 2 plastic nets covering the area of projection of 2 tree canopies/treated tree patch from time of fruit setting to the harvest. All fallen fruits/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/tree. At each sampling time, 200 fruits/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 was characterized by mines on fruit surface. At harvest time, fruit yield was assessed by selecting 5 to 6 trees/tree patch and the total weight of fruit harvest/tree was determined.

Data analysis

Data were analyzed for normality and mean values were separated by Student's t-test. Percentages of data were transformed to arcsin square root of proportions before statistical analysis, but the untransformed means ±SD were presented for comparison (SAS Institute, 1989).

RESULTS AND DISCUSSION

Monitoring of the OM was performed for the three successive olive seasons. Only, the results of the 3rd year are shown in Fig. 1. Based on the number of captured OM males, the first adults' appearance was on March 24th when the inflorescence reached stage "D", *i. e,* before bloom (Arambourg and Pralavorio, 1986). Peak catches of the first flight (anthophagous generation) reached 53.8±36.1 moths/week/trap on April 21st coincided with 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 May 5th (tree phonological stage "G", *i.e*, fruit setting). Trap catches of the third (phyllophagous) generation were recorded from August 4th to mid-September. The number of adult moths in the traps was extremely low. The followings were detected:

1) the first (anthophagous) generation closely overlapped with the second (carpophagous) one,
2) the OM population was significantly greater in the high fruiting years (2002 & 2004) than in the low fruiting year (2003, data not shown), and
3) monitoring with pheromone traps 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 evaluation of egg-laying activity on flower and fruits, *i.e.*, a good method for determination of the most suitable time for *Trichogramma* releases.

The females of the anthophagous generation oviposit on the newly formed fruits and larvae of the second carpophagous generation bore into the stone of the olive fruit in May-June. When larvae complete their development in July-August and leave the fruit, they cause spectacular fruit drop which result in major crop losses. The economic 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 Figs. 2 & 3. In high fruiting years (2002 and 2004 olive-seasons), significant ($P<0.05$) greater fruit drop occurred on control tree patches compared with those 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 numbers: 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 & 3, respectively).

Besides, both of pre-mature fruit fall caused by feeding damage of the OM during (May-August) and natural thinning, the OM larvae, mid-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 larva (e) attack (s) the fruit inducing mine(s) on the fruit's surface. The results of this type of damage throughout 3 years are shown in Fig. 4. In all the 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 revived no TE wasps compared with those treated with the wasps.

In 2004-olive season, parasitization rates by wasps on treated tree-patches were compared with natural parasitization in non-releases tree-patches (Fig. 5). In control tree-patches, the natural parasitism was very low during the flight period. (March - June) of OM. The maximum percentage of parasitism reached 22.6% late in the season (September 8th). *Trichogramma* wasps emerged

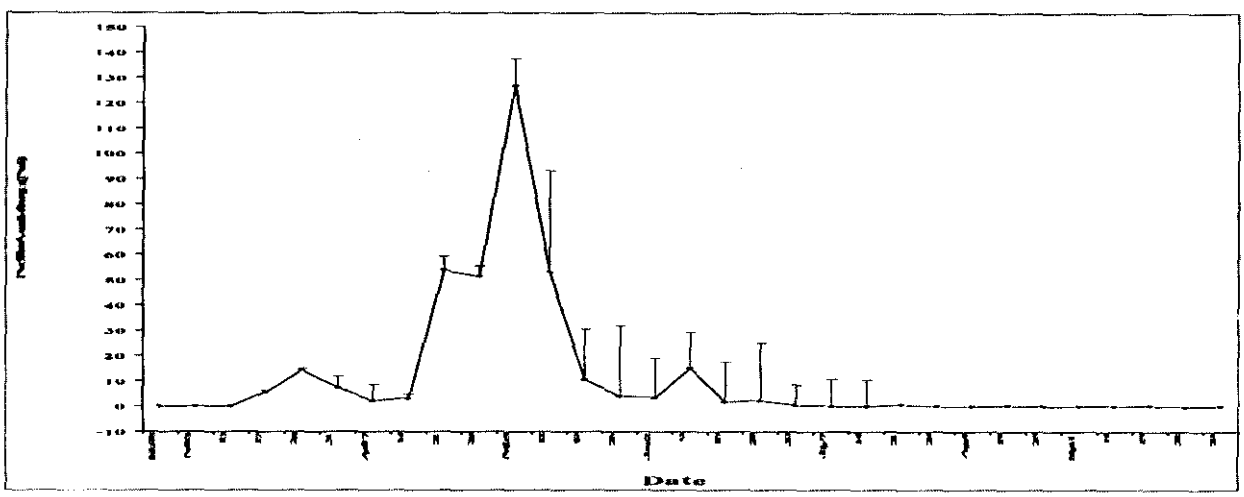

Fig. (1): Weekly mean numbers (±SE) of catches of *P. oleae* males in delta-wing traps.

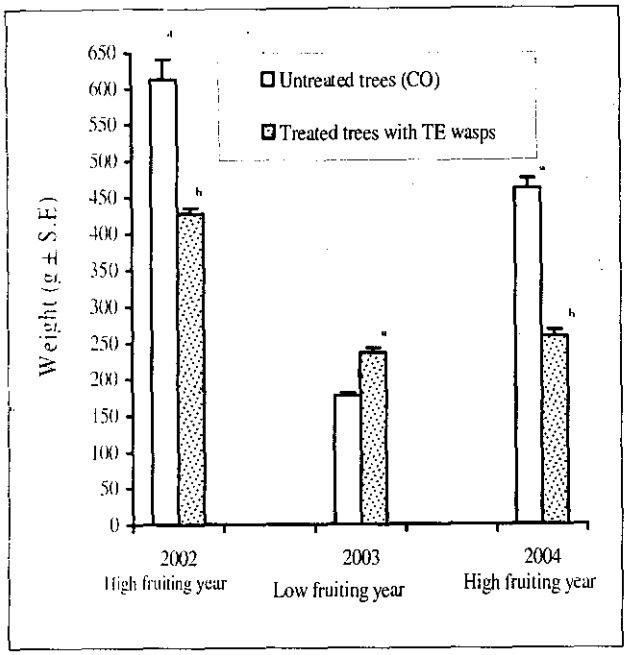

Fig. (2): Total weight of premature fruit fall/tree (May-August) of untreated and treated trees with *T. evanescens* (TE).

Means (±SE) of bars per sampling season with different letters are significantly different at P<0.05

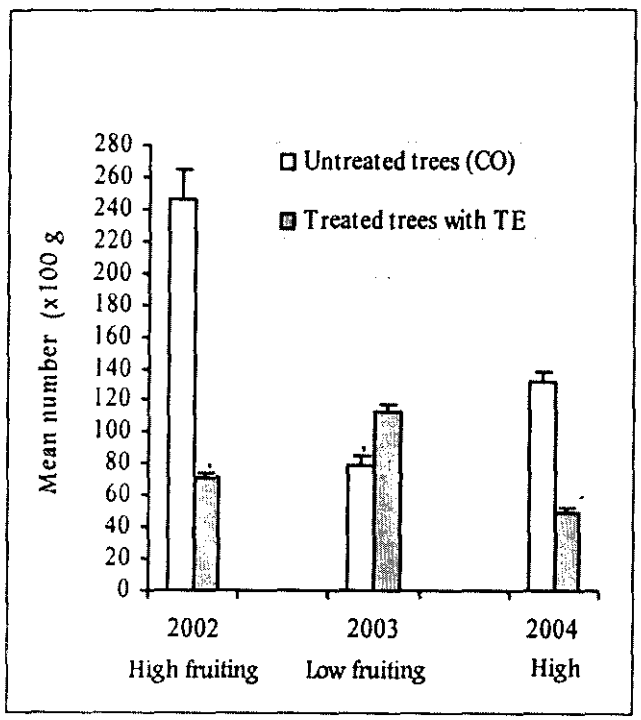

Fig. (3): Total numbers of premature fruit fall/tree (May-August) of untreated and treated trees with *T. evanescens* (TE).

Means (±SE) of bars per sampling season with different letters are significantly different at P<0.05.

from naturally parasitized eggs showed that 60.8 and 38.0% were *T. cordubensis* Vargas and Cabello (TC) and *T. euproctidis* Girault (TEU). On the other hand, olive tree-patches received TE releases showed different ranges of parasitism. Parasitism levels ranged from 0.2% on April 7th to 54.2% on September 15th 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.

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. 6). 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 (Fig. 7), respectively.

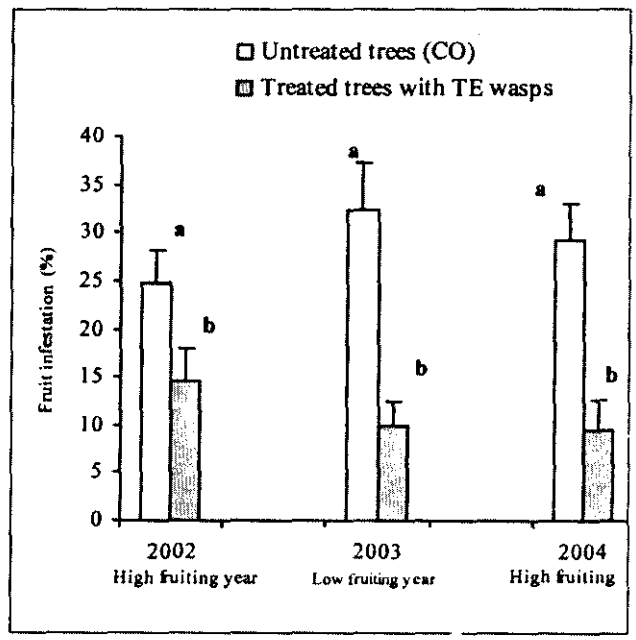

Fig. (4): Damaged fruits/tree by *P. oleae* larvae at mid-late season period on untreated and treated trees with *T. evanescens* (TE).

Means (±SE) of bars per sampling season with different letters are significantly different at P<0.05.

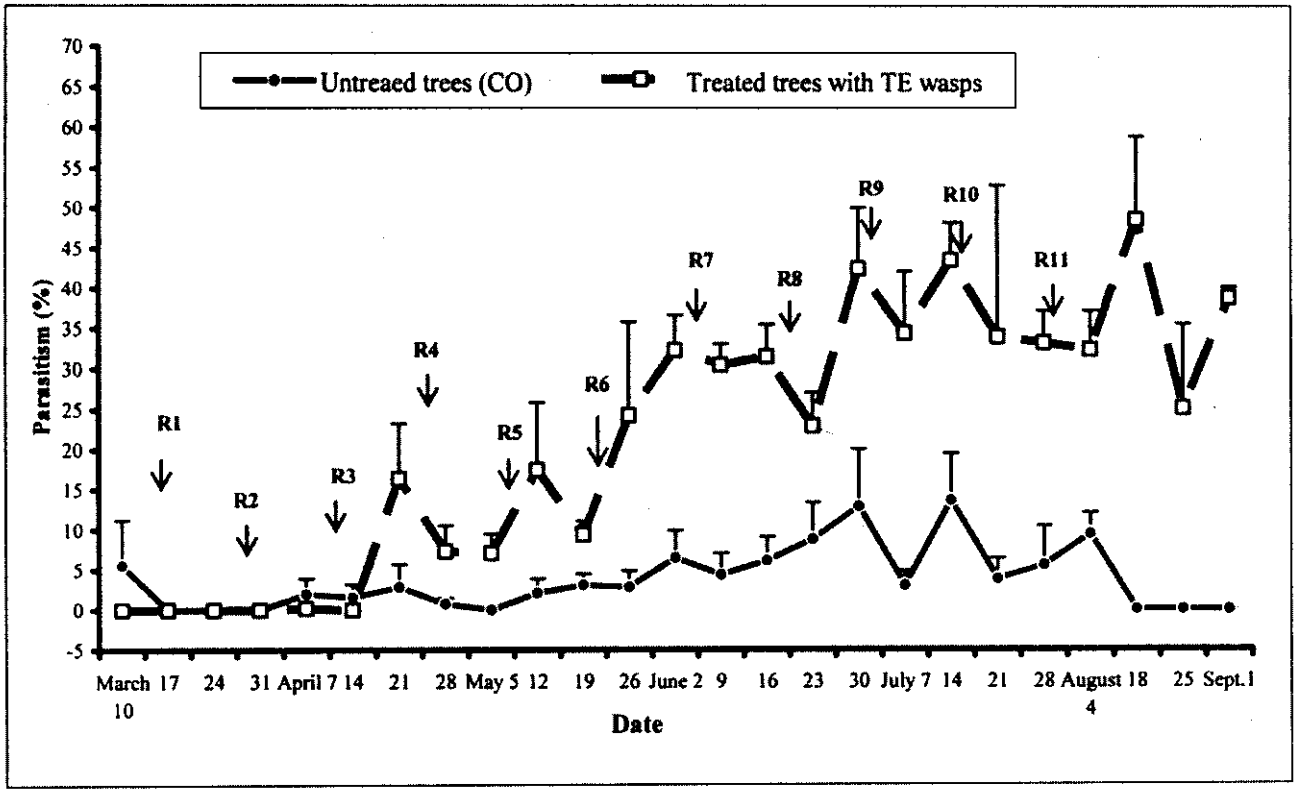

Fig. (5): Weekly means (±SE) of percentage of natural parasitism in non-release tree patches (CO) and tree patches treated with *T. evanescens* (TE) releases (R1-R11), 2004-olive season.

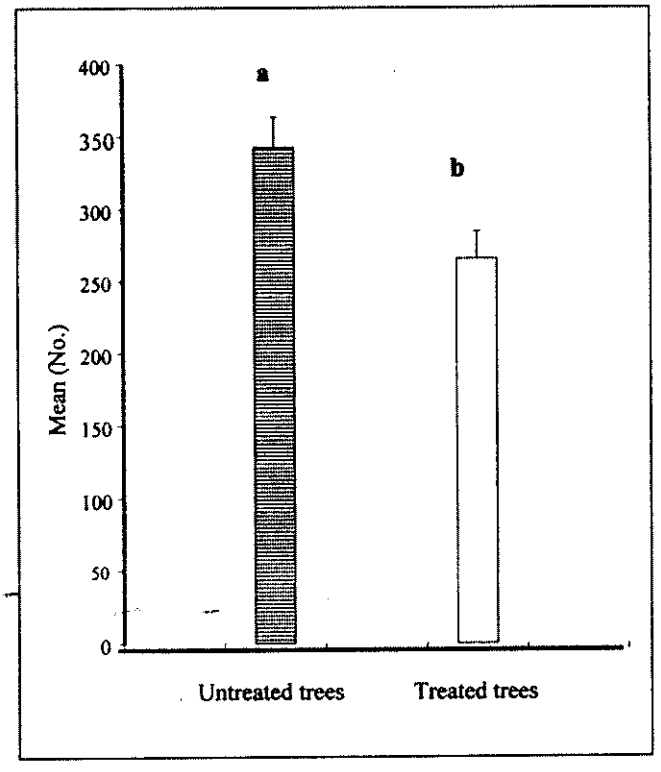

Fig. (6): Full season counts of *P. oleae* larvae/sample/tree on untreated and treated trees with *T. evanescens*.
Means (±SE) of bars with different letters are significantly different at P<0.05.

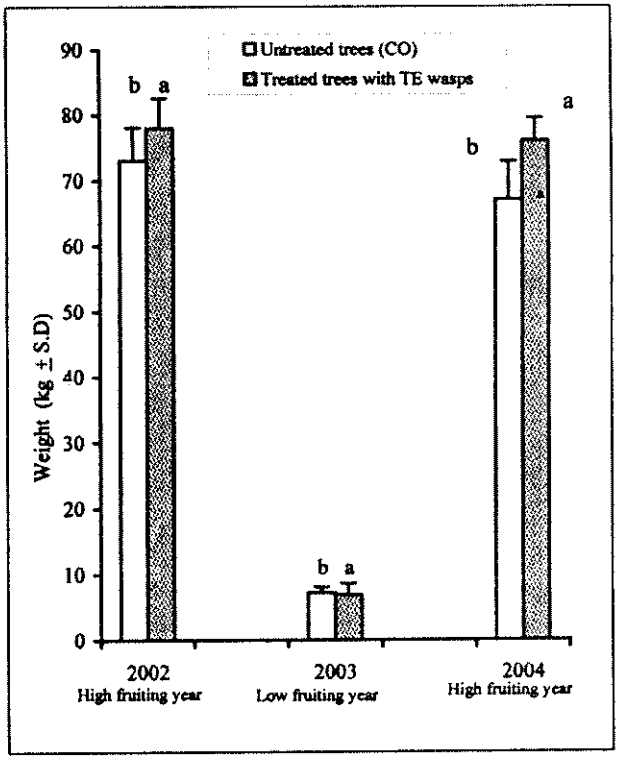

Fig. (7): Weight of fruit harvest/tree of untreated and treated trees with *T. evanescens* wasps (TE).
Means (±SE) of bars per sampling season with different letters are significantly different at P<0.05.

Means (±SE) of bars per sampling season with different letters are significantly different at P<0.05.

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 (Salt, 1935, 1938 and1940). 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, etc.) (Ram *et al.*, 1995), but had not been observed in olive groves. Releases of TE wasps were carried out in an olive farm that represents young and large intensively managed plantations under arid weather conditions 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 9000 wasps/tree (3,000,000 wasps/ha). This dose was fairly larger than what has been used in other crop system. Rate of the parasitoid releases adopted in other several studies ranged from 15000 (Chen and Chiu, 1986) to 450000 adults/ha (Cock, 1985). However, field parasitization levels by TE indicated poor adaptation of TE to local environmental conditions of olive groves. Sithanatham *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 (Fig. 5) and augmentative releases of reared wasps are needed. The strains described in this study could all be easily propagated on factitious hosts (*S. cereallela* and/or *E. kuehniella*), 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 program 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, T.C and *T.* TEU could improve control of lepidopterous pests on olive. The present work points on the need of monitoring local *Trichogramma* species in a particular area before inundative releases of species are conducted for the first time.

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REFERENSES

Arambourg, Y. and Pralavorio, R. (Ed.), 1986. Hyponomeutidae. *Prays oleae* Bern. In: Arambourg, Y. (ed.) Traite d'entomologie oleicole. International Olive Oil Council, Madrid, Spain, pp. 47-91.

Chen, C. C. and Chiu, S. C. 1986. Studies on the field releases of *Trichogramma chilonis* and factors affecting its activity. J. Agric. Res. China 35, 99-106.

Cock, M. J. W. 1985. The use of parasitoids for augmentative biological control of pests in the Peoples Republic of China. Biocontr. News and Inf. 6, 213-223.

Daumal, J.; Voegele, J. and Brun, P. 1975. *Trichogramma.* II- Unity of mass and daily production of a substitute host: *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae). Ann. Zool. Ecol. Anim. 7, 45-59.

Grimm, M. and Lawrence, P. J. T. 1975. Biological control of insects on the world. 1. Production of *Sitotroga cerealella* for mass rearing of *Trichogramma* wasps. J. Agric. West Aust. 16, 90-92.

Hassan, S. A. 1981. Massenproduktion und Anwendung von *Trichogramma.* 1. Produktion des Wirtes *Sitotroga cereallela.* Entomophaga 26, 339-348.

Hegazi, E. M.; Agamy, E.; Hassan, S.; Hertz, A.; Khafagi, W.; Showel, S.; Abo Abd-Allah, L.; Ziton, A.; Hafez, M.; El-Shazly, A.; El-Said, S.; El-Minshawy, A.; Karam, H.; Khamiss, N. and El-Kemny, S. 2004. Preliminary study on the combined effect of mating disruption and

inundative releases of *Trichogramma evanescens* against the olive moth, *Prays oleae*. Egypt. J. Biol. Pest Control, 14O(1), 9-14.

Hegazi, E. M.; Herz, A.; Hassan, S. A.; Khafagi, W. E.; Agamy, E.; Zaitun, A.; El-Said, S.; Abd El-Aziz, G. and Khamis, N. 2007. Field efficiency of indigenous egg parasitoids (Hymenoptera: Trichogrammatidae) to control the olive moth (*Prays oleae*, Lepidoptera; Yponomeutidae) and the jasmine moth (*Palpita unionalis*, Lepidoptera; Pyralidae) in an olive plantation in Egypt. Biol. Pest Control (in press)

Herz, A.; Hassan, S. A.; Hegazi, E.; Khafagi, W. F.; Nasr, F. N.; Youssef, A. A.; Agamy, E.; Jardak, T.; Ksantini, M.; Mazomenos, B. E.; Konstantopoulou, M. A.; Torres, L.; Goncalves, F.; Bento, A. and Pereira, J. A. 2005: Towards sustainable control of Lepidopterous pests in olive cultivation. Gesunde Pflanzen 58, 117-128.

Li, L.Y. 1994. Worldwide use of *Trichogramma* for biological control on different crops. A survey. In: Wajnberg, E., Hassan, S.A. (Eds.). Biological Control with Egg Parasitoids. CAB International, Oxon, pp. 37-51.

Lopez-Villata, M. C. 1999. Olive pest and disease management. International Olive Oil Council, Principe de Vergara, 154-28002, Madrid, 206 p.

Mazomenos, D. E.; Ortz, A.; Mazomenos-Pantazi, A.; Stefanou, D.; Stavrakis, N.; Karapati, C.and Fountoulakis, M. 1999. Mating disruption for the control of the olive moth, *Prays oleae* (Bern.) (Lep.: Yponomeutidae) with the major sex pheromone component. J. Appl. Ent. 123, 247-254.

Morrison, R.K.; Stinner, R.E.and Ridgway, R.L. 1976. Mass production of *Trichogramma prectiosum* on eggs of the Angoumois grain moth. Southwest Entomol. 1, 74-80.

Parker, F. D.and Pinnell, R. E. 1972. Effectiveness of *Trichogramma* spp. in parasitizing eggs of *Pieris rapae* and *Trichoplusi ni*. 1. Field studies. Environ. Entomol. 1, 785-789.

Patanita, M. I.and Mexia, A. 2004. Loss assessment due to *Prays oleae* Bern. and *Bactrocera oleae* Gmelin in Moura's region (Portugal) (WWW document). http://pubol.ipbeja.pt/Artigos/Italia.htm.

Pelekasis, C. E. 1962. A contribution to the study of nomenclature, taxonomy, biology and natural parasitization of the olive kernel borer *Prays oleae* (Bern). Annales de l'Institut Phytopathologique, Benaki, (NS) 4, 180-308.

Pintureau, B. 1990. Polymorphisme, biogeography et specificite parasitaire des *Trichogrammes europeens* (Hym.: Trichogrammatidae). Bull. Soc. Entomol. Fr. 95, 17-38

Ram, P.; Tshernyshev, W. P.; Afonina, V. M. and Greenberg, SH. M. 1995. Studies on the strains of *Trichohgramma evanescens* Westwood (Hym.: Trichogrammatidae) collected from different hosts in Northern Maldova. J. Appl. Ent. 199, 79-82.

Ramos, P.; Campos, M. and Ramos, J. M., 1998. Long-term study on the evaluation of yield and economic losses caused by *Prays oleae* Bern. in the olive crop of Granada (Southern Spain). Crop Prot. 17, 645-647.

Ridgway, R.L.and Vinson, S.B. (eds.), 1977. Biological Control by Augmentation of Natural Enemies. Plenum Press, New York, 480 pp.

Salt, G. 1935. Experimental studies in insect parasitism. III. Host selection. Proc. R. Soc. Lond, (B), 117, 413-435.

Salt, G. 1938. Experimental studies in insect parasitism. VI. Host suitability. Bull. Entomol. Res. 29, 223-236.

Salt, G. 1940. Experimental studies in insect parasitism. VII. The effect of different hosts on the parasite *Trichogramma evanescens* Westw. (Hym.: Chalcidoidea). Proc. R. Entomol. Soc. Lond. (A), 15, 81-95.

Sas Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, 4th edn., vol. 1, Cary, NC: SAS Institute Inc, pp. 943.

Sithanantham, S.; Abera, T. H.; Baumgartener; Hassan, S. A.; Lohr, B.; Monje, J. C.; Overholt, W. A.; Paul, A. V. N.; Fanghao Wan and Zebitz, C. P. W. 2001. Egg parasitoids for augmentative biological control of lepidopteran vegetable pests in Africa. Insect Science and its Application 21, 189-205.

Smith, S. M. 1996. Biological control with *Trichogramma*: advances, successes and potential of their use. Annu. Rev. Entomol. 41, 375-406.

Stavraki, H. G., 1977. Results obtained from releases of the oophagous parasites *Trichogramma* sp. against *Prays oleae* Bern. (Lep.: Hyponomeutidae) over a four year period in Greece. Med. Fac. Landbouww Rijksuniv. Gent. 42, 1361-1371.

Stavraki, H. G. 1985. Use of *Trichogramma* spp. against the carpophagous generation of *Prays oleae* (Bern.) in Greece. In: Cavallero, R.; Crovetti, A. (eds.). Proceedings of the CEC/FAO/OBC International Joint Meeting, Pisa, Italy, 3-6 April, 1984, Balkema, Rotterdam, pp. 242-246.

Tran, L. C.and Hassan, S. A. 1986. Preliminary results on the utilization of *Trichogramma evanescens* West. to control the Asian corn borer. J. App. Ent. 101: 11, 18-23.