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Application of Inundative Releases of *Trichogramma evanescens* to Control the Olive Moth, *Prays oleae* (Bern.)

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ABSTRACT

Field releases of 900000 wasps/ha/release of *Trichogramma evanescens* were applied against the anthophagous (March 9th, 26th; April 4th, 22nd) and carpophagous (May 13th, 27th; June 10th, 24th) generations of the olive moth *Prays oleae*. A comparable olive grove (0.5 ha) of the same olive varieties (Toffahi and Shamy) was chosen as control site. The flight phenology of the olive moth males was weekly monitored with sex pheromone traps. Estimation of infestation rates by target pest on olive leaves, flowers, fruits and pre-mature fallen fruits as well as parasitism by *Trichogramma evanescens* were weekly recorded. Olive fruit yield of treated and untreated areas was carefully gathered and weighed. Interestingly, male catches in treated sites were greatly reduced from the 3rd release of the first flight to the last release of the 2nd flight of the olive moth. Reduction in male catches in treated plots resulted in decreasing number of eggs of target species on olive shoots in treated sites compared to control site. The maximum percentage of parasitism in *Prays* eggs occurred in May 27th. Results of the efficacy of releasing wasps on the amount of premature fall of olive fruits and fruit yield/tree (weight and number) at treated and control sites offer encouraging data concerning the protection of olive yield by inundative releases of *Trichogramma* wasps. Importantly, a thelytokous strain of *T. cordubensis* was reared for the first time in Egypt from naturally parasitized *P. oleae* eggs and will be tested in the near future.

Key Words: Inundative releases, *Trichogramma evanescens*, *Prays oleae*.

INTRODUCTION

The olive moth *Prays oleae* (Bern) is an oligophagous species, infesting all cultivated varieties of olives, wild species of the genus *Oleae* and even on plant species of different genera in the family Oleaceae, e.g., *Phyllirea*, *Jasminum* and *Ligustrum* (Lopez-Villalta, 1999). It is one of the most important insect pests of olives in the Mediterranean basin. The moth develops three generations per year (Pelekasis, 1962). In Egypt, 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 May and causes most damage. The females oviposit on the small fruits and the larvae penetrate into the stone of the olive fruit. When the larvae complete their development in June-July and leave the fruit, they cause spectacular fruit drop and result in major crop losses. The third generation appears in mid-August and attacks the mature fruits and leaves. The larvae make mines and hibernate in autumn and complete their development early the next spring (Hegazi and Khafagi, 2003). The characteristics of feeding behavior of the second and third generations of *P. oleae* larvae make them difficult to be reached with insecticides, because the larvae bore into the fruits or make mines in fruits or leaves immediately after hatching (Pelekasis, 1962).

Various methods are used for suppression of the moth population. The larvae of the first generation are susceptible to standard strains of *Bacillus thuringiensis* (var. *kurstaki*) (Yamvrias *et al.*, 1986). For the second and third generations, the control measures should be concentrated on the adult stage.

During the last three decades, egg parasitoids have been widely used against several pests on agriculture and forestry (Wajnberg, 1994). It is estimated that over 32 million hectares have been treated annually with *Trichogramma* in more than 30 countries allover the world (Li Li-Ying, 1994). These beneficial agents have been used, on a particular large scale, on corn, sugarcane, cotton, fruit trees and vegetables (DeBach, 1974; Stinner, 1977; DeBach and Rosen, 1991 and Li Li-Ying, 1994), and researches are still carried out in several laboratories in order to develop new biological control programs with these wasp parasitoids.

Egg parasitoids, however, are almost exclusively used through inundative releases, wherein the crop is "flooded" with mass-reared parasitoids in order to increase the parasitization rate sufficiently to reduce crop damage (Wajnberg, 1994). In such a case, estimating economic impact of a biological control program has to

be based on a lot of different indicators including, among others: (1) reduction of the pest population size, (2) increase in crop yield, (3) increase in production, (4) increase in farm revenue or receipts, (5) cost saving, (6) increase in product value, and even (7) social gain (Huffaker *et al.*, 1976 and Tisdell, 1990). However, there are no data concerning biological control programs against pests of olive trees. The main objective of this study is to release *T. evanescens* in order to reduce pesticide load on the crop and also to reduce associated problems such as pesticide residue, resurgence of pest and increasing cost on pesticide use in general.

MATERIALS AND METHODS

Experiments were carried out in 2001 olive season. An olive farm (7-8 y old) in olive producing zone between Alexandria and Cairo, 177 km south Alexandria, was selected. The farm is divided into 88 isolated olive plots (each 2.5-3.5 ha) by windbreak trees. The vast majority of farm plots combine 2 cultivars (ca. 450 trees/cultivar); 3 lines (30 trees/line) of one cultivar are grown alternately by other 3 lines of the second cultivar. A large plot of 5 ha of Toffahi and Shamy varieties was divided into three small plots for *Trichogramma* releases (site TR). A control plot of 5 ha (CO) was left without treatment. The TR and CO sites are at least 300 m apart. The parasitoid was mass reared on *Sitotroga cerealella* eggs. On each tree of TR sites, 3 cards/tree (3000 wasps/card) were installed. Four releases were done against each of the first (March 3rd, 25th, April 8th, 22nd) and second (May 13th, 27th, June 10th, 24th) generations of the olive moth.

To evaluate the actual releasing doses of *Trichogramma* wasps, 5 cards from each releasing date were separately kept in the lab under standard conditions of 25±1°C, 70% R.H. and 16:8 L/D. The numbers of non-emerged wasps from parasitized eggs, non-parasitized eggs and daily and fortnightly numbers of emerged wasps were carefully recorded. These estimates were also fortnightly recorded on 5 other cards collected from the field to assess the number of already emerged wasps under field conditions.

The attraction of traps to the olive moth was monitored with pheromone traps. At each plot, a delta-wing trap (baited with (Z)-7-tetradecenal) was installed. The pheromone dispensers of the traps were changed every generation. The distance between traps was more than 60 cm from each other. The traps (3/ha) were served once a week from March 2nd to end of August. The phenological stage of olive trees was carefully recorded. Estimation of infestation rates by target pests on olive leaves, flowers and fruits, egg parasitism by *T. evanescens* and fallen fruits were weekly recorded. Olive harvest of the treated area (site TR) and control "site CO" was carefully gathered and weighed.

Data were analyzed by the analysis of variance procedure and by Duncan's (1995) multiple range test and Student's t test for mean separation at 0.05 level of significance.

RESULTS AND DISCUSSION

As shown in Fig. 1, four releases were performed against each of anthophagous (RI_1-RI_4) and carpophagous (RII_1-RII_4) generations. Importantly, it was recommended to apply doses of 900000 wasps/ha/treatment. The releasing doses calculated from lab and field data are shown in Fig. 1. The following could be concluded: 1) the used dose/release was significantly lower (640-2691 wasps/releasing card) than the proposed one (3000 wasps/releasing card). 2) Differences in the proportions of reduction of number of emerging wasps were greatly higher under field than under lab conditions. This reduction was greater in releases performed against the carpophagous generation (Fig. 1, RII_1-RII_4) than those done against anthophagous one (Fig. 1, RI_1-RI_4).

T evanescens was multiplied on *Sitotroga cerealella* Olivier eggs in order to carry out inundative release experiments against the olive moth, *P. oleae*. The reduction of number of emerging wasps in the field compared with corresponding figures in the lab (Fig. 1) could be because of poor adaptation of the species used to local environmental conditions. Mass rearing can influence the parasitoid's biological parameters (Van Lenteren, 1991 and Bigler, 1994). This may indicate the needs of more adapted candidate of *Trichogramma* to control the olive moth in hot weather. Fortunately, a thelytokous strain of *T. cordubensis* was reared from naturally parasitized, *P. oleae* eggs and will be tested in near future.

Figure 2 shows the captures of *P. oleae* males during the first and second flight of olive moth adults in plots treated with *T. evanescens* and untreated ones. The time of first treatment for the 1st flight was on March 9th where the population of *P. oleae* adults in treated and untreated sites was very low. Interestingly, male catches in TR sites was greatly reduced from the 3rd release of the first flight to the last release of the 2nd flight of olive moth. Reduction in male catches in TR sites resulted in decreasing number of eggs of

target insects on olive shoots in TR sites compared to CO site (Fig. 3). The differences in reduction of proportions of eggs of target pests between TR and CO sites were highly significant in samples of late May, June 3rd and June 17th

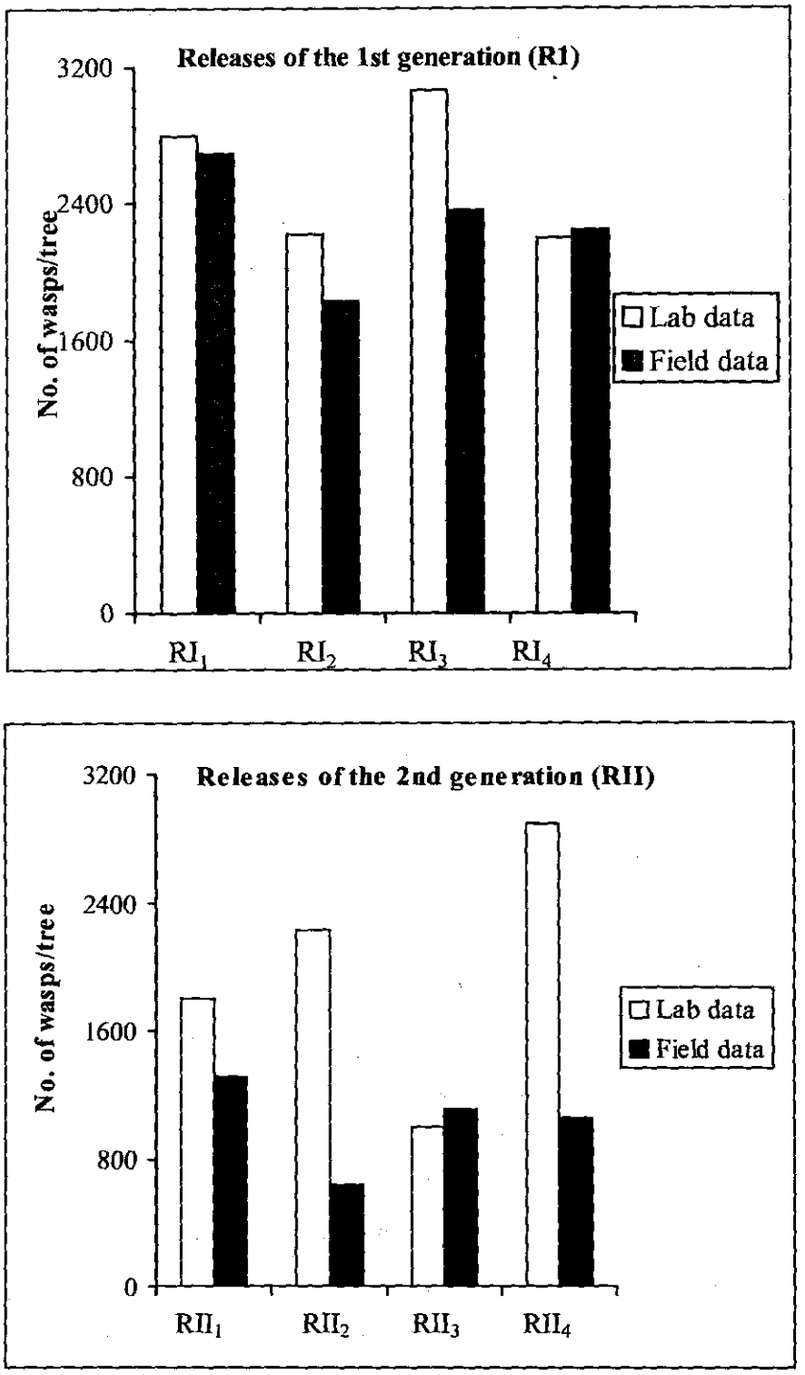

Fig. 1: Numbers of emerged *Trichogramma evanescens* wasps per tree for controlling the 1st and 2nd generations of the olive moth, *Prays oleae*. (RI1-RI4: releases of 1st generation; RII1-RII4: releases of 2nd generation).

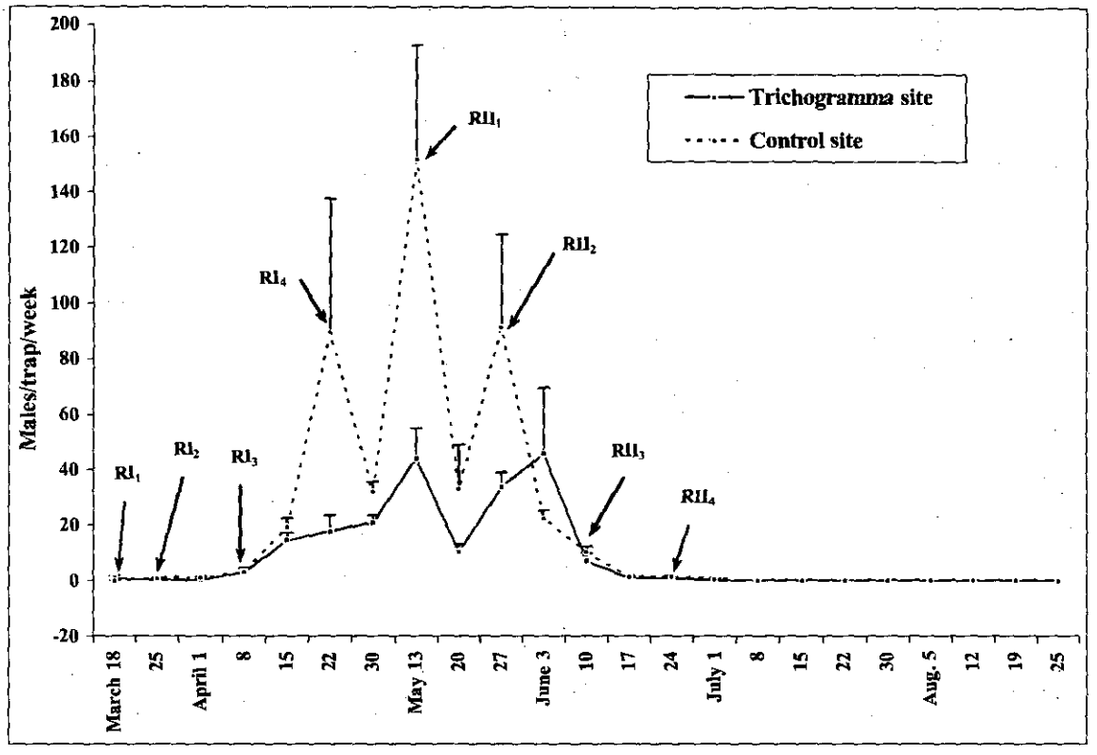

Fig. 2: Pheromone trap catches of *Prays oleae* males in plots treated with *Trichogramma evanescens* and untreated plots. The arrows indicate dates of treatments by *Trichogramma* wasps

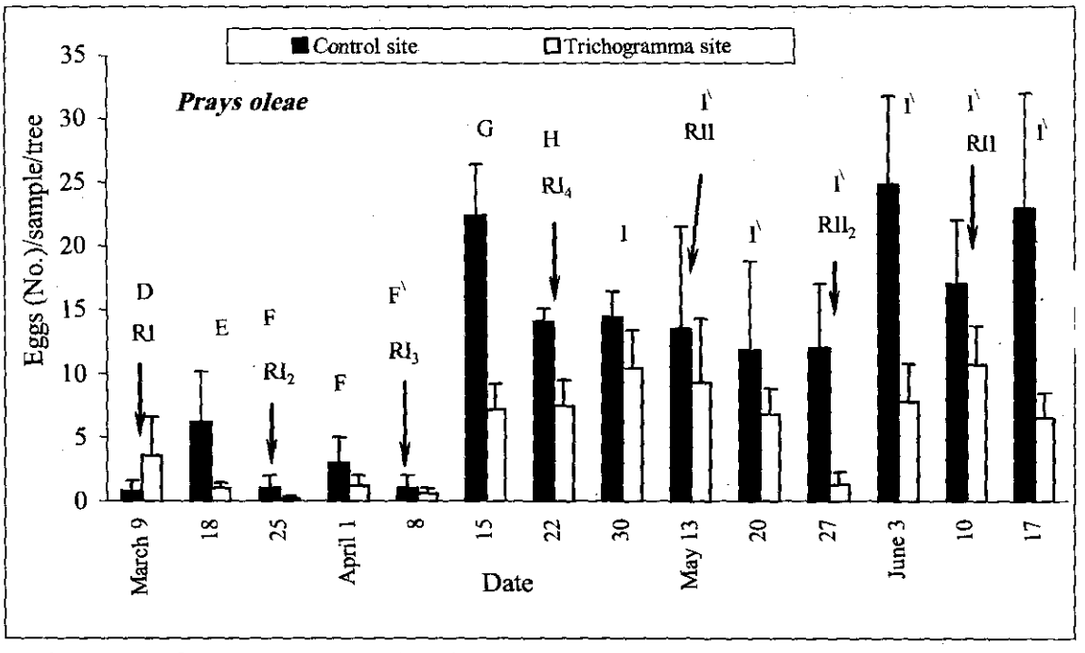

Fig. 3: Weekly counts of *Prays oleae* eggs/40 olive shoots/tree at *Trichogramma* release and control sites. The arrows indicate dates of treatments by *Trichogramma* wasps. (D-I\): plant phenology.

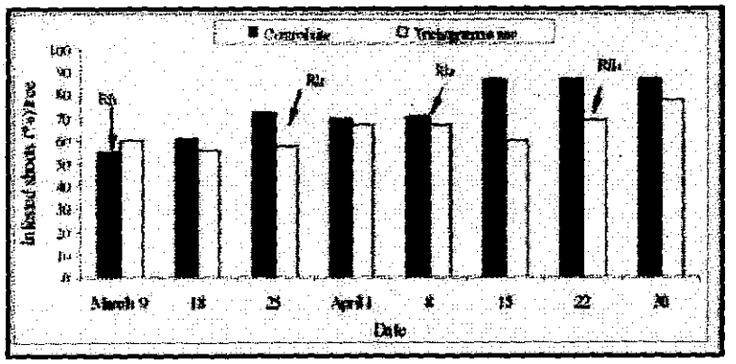

Fig. 4: Weekly infestation rate of olive shoots by *Pray oleae* on *Trichogramma* release and control sites. The arrows indicate dates of treatments by *Trichogramma* wasps.

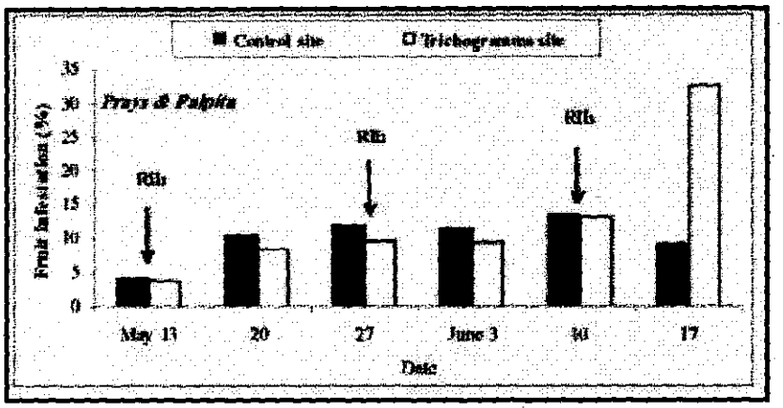

Fig. 5: Levels of fruit infestation of olive trees at *Trichogramma* releasing and control sites. The arrows indicate dates of treatments by *Trichogramma* wasps.

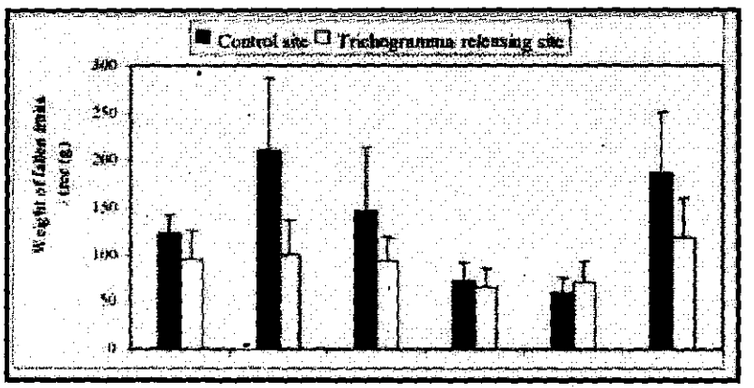

Fig. 6: Weight (in g, upper view) and number (x102, lower view) of fallen fruits/olive tree on control and *Trichogramma* releasing sites

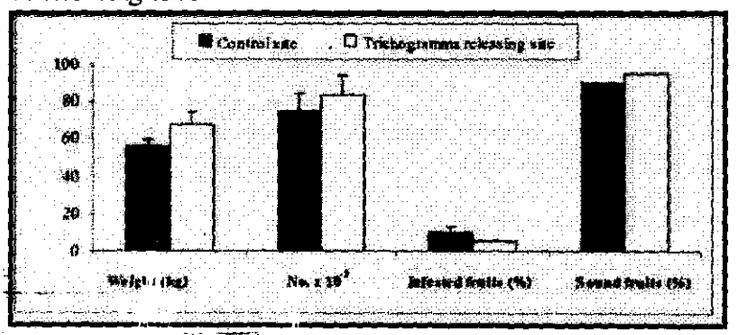

Fig. 7: Fruit harvest/tree (weight & number) at *Trichogramma* releasing site and control site.

Parasitism (%) by *T. evanescens* in eggs of target insects was generally low (data not shown). The maximum percentage of parasitism in *Prays* eggs occurred in May 27th (25%) at TR site and June 10th (21.5%) at CO site. The presence of parasitized eggs of target pests at CO site is probably due to installing of some releasing cards by the owner without our permission to protect his farm. In addition, the egg cards widely differed in their numbers of released wasps. Parasitism by *T. evanescens* was significantly greater in olive moth eggs than the jasmine moth eggs (data not shown).

In the present study, the olive shoot was considered infested whatever one or more eggs or larvae were found on the individual shoot. The illustrated data in Fig. 4 showed that the percentage of infested shoots by target insects at CO site was higher than at TR one.

Figure 5 shows the infestation rate of growing olive fruits by the target insects. Interestingly, infestation rate was unexpectedly higher in TR site on June 17th than CO site. The low percentage of parasitism by the released wasps (data not shown) and relatively higher infestation rate of growing fruits in June by target insects in TR site suggest that the released wasp species was not the right candidate for controlling target lepidopterous insects of olive trees. However, results of the efficacy of releasing wasps on the fallen fruits (Fig. 6) (weight & number)/tree at TR & CO sites offer encouraging data concerning the protection of olive yield by inundative releases of *T. evanescens* wasps. The number and weight of fallen fruits in CO site were higher by 3.34 and 1.4 times than those recorded in TR site. This may further indicate that releasing *Trichogramma* wasps may offer effective method of control than control measures currently available. This could be achieved when the right adapted candidate *Trichogramma* would be used, taking in consideration the right time of release, the duration of activity of releasing wasps and emergence time of wasps from released cards. Hommay *et al.*(2002), in their work with mass releases of *T. evanescens* and *T. cacoeciae* against grapevine moth (*Lobesia botrana*), reported that, under optimal conditions, the release of these *Trichogramma* can reduce damage caused by grape moth and give results equivalent to insecticide treatments. Further impact of treatment by inundative release on population of the olive moth was observed. The population density of mature larvae and pupae of the olive moth/tree (data not shown) on the soil surfaces was higher by 1.4 times in CO site than TR one. Some hymenopterous and dipterous parasitoid were found attacking the mature larvae and pupae of olive moth on the soil surface (data not shown). In most cases, the percentage of total parasitism on larvae and pupae of *O. oleae* in TR site exceeded those recorded in CO site.

Figure (7) shows that the fruit yield/olive tree in TR site was higher than in CO one. The small plot studies had shown that the percentage of parasitism by *T. evanescens* was generally low, suggesting a question about the effectiveness and practical application of this wasp in olive farms. However, the economic impact of *T. evanescens* on the olive moth, *Prays oleae* in treated sites is shown in reduction of the pest population size (Figs. 3 and 4), decrease in number and weight of fallen fruits (Fig. 6) and increase in crop yield (Fig. 7). Although the use of *T. evanescens* may at first seem simple, effective *P. oleae* control is determined by many factors including: the species of *Trichogramma* used; the quality and fitness of the parasite product; the numbers released and the timing of the release; and the releasing method. In short, the outcome of inundative release was partially successful and could provide a model for control of *P. oleae* and other lepidopterous pests of olive tree, especially when the endemic species, e.g., *T. cordubensis*, will be used in the next trials.

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التطبيق الناجح للإطلاقات المكثفة لطفيل البيض *Trichogramma evanescens* West. لمكافحة فراشة الزيتون *Prays oleae* Bern.

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أ. زيتون [•] ، س. السيد [•] ، أ. الشاذلى [•] ، ع. المنشاوى [••] ، هـ. كرم [••] ، ن. خميس [•] ، س. القمنى [•]

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تم إطلاق ٩٠٠،٠٠٠ طفيل/هكتار/إطلاق من طفيل البيض *Trichogramma evanescens* West. ضد جيل الأزهار (٩ مارس – ٢٥ مارس ، ٤ أبريل، ٢٢ أبريل) وجيل الثمار (١٣ مايو، ٢٧ مايو، ١٠ يونيو، ٢٤ يونيو) لفراشة الزيتون *Prays oleae* Bern. تم إختيار مزرعة مقارنة تحوى نفس الأصناف (تفاحى وشامى) دون إطلاق لغرض المقارنة. كما تم متابعة أسبوعية لطيران ذكور فراشة الزيتون بالمصائد الفرمونية الجنسية، مع تسجيل أسبوعى لمعدلات الإصابة بالآفة على أوراق الزيتون والأزهار، والثمار والثمار غير الناضجة الساقطة وكذلك نسبة التطفل. كما تم جمع ووزن محصول الثمار بعناية فى المناطق المعاملة وغير المعاملة. من المثير ملاحظة إنخفاض فى تعداد ذكور الحشرة فى مصائد المناطق المعاملة إبتداء من الإطلاق الثالث إلى الإطلاق الأخير ضد الجيل الثانى لفراشة الزيتون. نتج عن إنخفاض ذكور الفراشة فى القطاعات المعاملة إنخفاضا فى عدد بيض الآفة على أفرع الزيتون فى المناطق المعاملة مقارنة بالمناطق غير المعاملة. سجلت أعلى نسبة للتطفل على بيض الآفة فى ٢٧ مايو. أظهرت نتائج فاعلية الإطلاقات على كمية الثمار غير الناضجة الساقطة ومحصول الثمار/شجرة (بالوزن والعدد) فى المناطق المعاملة وغير المعاملة نتائج مشجعة فيما يخص حماية محصول الزيتون بالإطلاقات المكثفة بطفيل الترايكوجراما.