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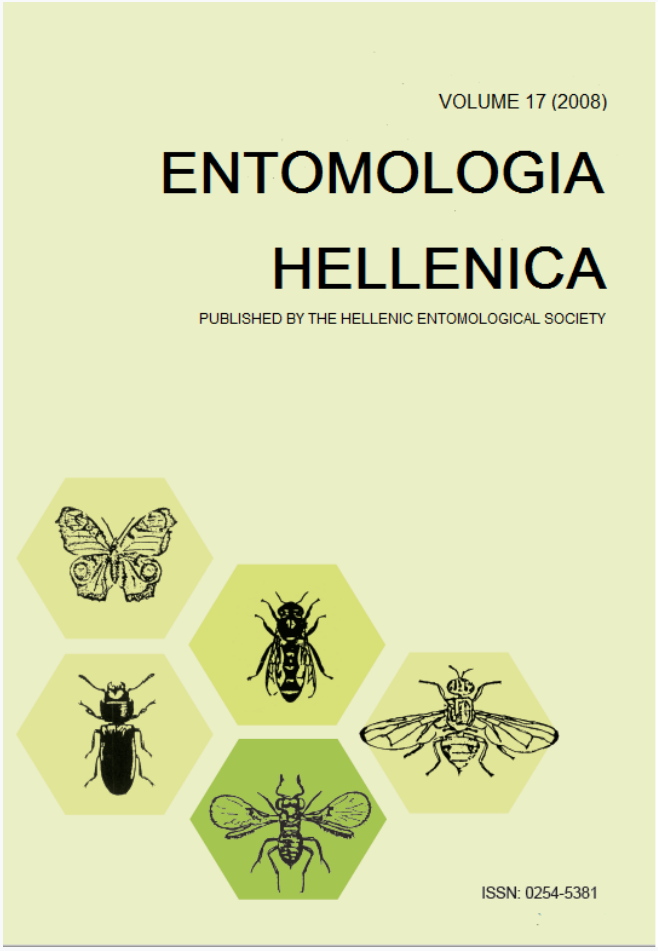

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Contribution to the ecological management of the seed chalcid wasp *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae) in pistachio orchards

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ABSTRACT

Aspects related to the management of the pistachio seed wasp *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae), were investigated. *E. plotnikovi* has internal feeding habits. However, the external appearance of the fruits could be used to distinguish between infested and uninfested fruits, since fruits partly blackish or brownish and shriveled had higher infestation levels than those without any discolouration. The adult emergence from the fruits was completed within a short period of 12 days, from the middle until the end of May. This pest overwinters as larva inside the mummified fruits. The application of sanitation measures for the control of this pest was experimentally investigated. The results showed that no adults emerged from fruits that were buried in the soil but also from those that remained on the soil surface during winter. Thus, it seems that there is no need for burying them by tillage under the soil as it had been proposed. This result might be essential in the wider adoption of this method in the management of this pest.

Introduction

The seed wasp *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae) is included in the most important insect pests of pistachios in Greece and several areas of Middle East (Nikol'skaya 1934, Davatschi 1956, Mourikis et al. 1998). This wasp may cause damage as high as 90% (Mourikis et al. 1998). It is a univoltine species that completes its development inside the pistachio fruit. It hibernates at the stage of fully developed larva in diapause inside the infested mummified fruits (Tsourgianni 1989). Both temperature and photoperiod were proved to be involved for the introduction of larval diapause (Tzanakakis et al. 1992). In mid-spring the larva pupates inside the fruit and the adult emerges through a round hole that it bores on the fruit shell.

E. plotnikovi has internal feeding habits and thus, inflicts damage to the interior of the fruit. However, it would be worthwhile to examine whether the external fruit coloration patterns tend to relate to larval infestation. This information could contribute to the evaluation of damage levels in the field and application of industrial techniques to separate the healthy from the infested fruits. In the case of another pest, the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) the association between the external appearance of pistachio fruits after harvest and the infestation levels has been proved a suitable tool to separate the healthy from

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the damaged fruits in industrial scale (Doster and Michailides 1999).

The control of *E. plotnikovi* is almost exclusively based on the application of insecticide sprayings (Mourikis et al. 1998). However, the use of chemicals increases the cost of the production and disturbs the management programs against other important insect pests such as psyllids, because it reduces substantially the population levels of their natural enemies (Souliotis et al. 2002).

Little research has been conducted on the development and evaluation of alternative control measures against *E. plotnikovi* under the concept of the Integrated Pest Management (IPM). Apart from chemical control, sanitation measures have been proposed to contribute to the control of this pest. Sanitation is aimed at the elimination of the number of larvae that survive in fruits during winter and thus minimizing the pest inoculums in the forthcoming growing season (Tzanakakis et al. 1992). Apparently, *E. plotnikovi* could potentially survive during winter either inside the fruits that remain on the trees after harvest and/or in those that have fallen on the ground. The proposed sanitation method is accomplished by dropping the fruits from the trees on the ground and plowing them (Mourikis et al. 1998). Sanitation is used against another pistachio pest the seed wasp *Megastimus pistaciae* Walker (Hymenoptera: Torymidae) (Mart et al. 1995) and recommended for the almond seed wasp *Eurytoma amygdali* Enderlein (Katsoyannos et al. 1992). This method is important in the control of *A. transitella*. In this case, its effectiveness was largely dependent on the depth that the pistachios were buried in the soil (Siegel et al. 2004).

The sanitation measures are environmentally friendly and compatible with the IPM practices which can be a fundamental tool for the control of *E. plotnikovi* in ecological farming. However, their effectiveness has not been proved experimentally. In addition, modifications in the application of these measures that could probably reduce their cost and make them more widely accepted by the farmers have not been assessed. This kind of research is well justified since the effectiveness of sanitation is strongly related to the extent of its application in the crop area. Under this concept it would be worthwhile to investigate the emergence rates of the pest from fruits that remain on the trees after harvest as well as from fruits kept on the ground surface or are buried in the soil.

Towards the more effective management of this pest under IPM practices, an essential measure is the optimal timing of the application of chemicals against females in spring. This technique is largely appreciated and widely used (Mourikis et al. 1998). However, it has not been investigated whether its predictability could be improved by investigating a possible relationship of adult emergence rate to temperature fluctuations.

Following the above considerations the current study aimed to: 1) search whether the external appearance of the fruits could be used to distinguish between infested and uninfested fruits, 2) evaluate the effectiveness of different sanitation measures on the elimination of *E. plotnikovi* numbers and 3) record adult emergence rate and investigate its possible associations with temperature fluctuations.

Materials and Methods

Experimental orchard

The experiments were conducted in a pistachio orchard of 3.5 ha located in the farm of biological cultivation that belongs to the Athens Organization 1999, located

in the Liossion municipality, 7 km north of Athens centre. The pistachio trees were 20 year old. They were irrigated by microsprinklers. The soil of the orchard consisted of clay (31-46%) and sand (31-45%). The orchard was cultivated by applying the standard cultivation practices in the area.

Fruit colouration and infestation by *E. plotnikovi*

Fruit samplings were conducted to investigate whether the external fruit colouration patterns could be related to the presence of *E. plotnikovi* larva inside them. A preliminary sampling showed that based on their external appearance, the fruits could be classified into ones that were: almost entirely black (type A), partly blackish (type B), brownish and shriveled (type C) and also ones without any change of color (type D). Fruits were sampled on August 13, September 22, and October 5 and 12. These dates were selected in order to associate external fruit coloration with damage levels in the period close to harvest. On each sampling date 10 fruits were collected at random from each of 20 randomly selected trees. The fruits were brought to the laboratory, where they were examined under the stereomicroscope. The fruits were separated in the previously mentioned coloration types and then, according to their content the fruits were distinguished in three categories: seedless, normally developed and infested by *E. plotnikovi*.

Sanitation measures evaluation

Fruits were buried in the soil at different depths and the following spring the percentage of adult emergence was compared with that of fruits kept on the soil surface or maintained on the trees.

Prior to the experiments, the fruit damage level was estimated. For this purpose, about 15.000 fruits were randomly collected from trees in the entire orchard in the period from 15 to 22 September 2000. The level of infestation was then recorded in a subsample of 1.000 fruits. Fruits from this initial stock were also used in the experiments.

In mid-October, under the canopy of 7 randomly selected trees of the orchard a surface area of about 90x90cm was delineated. In this area, two pits of about 90x40cm each were opened, separated 10cm of undisturbed soil. The depth of the pit closer to the trunk was 20cm and of the other 10cm. Each pit was divided into two parts of 40x40cm each. The bottom surface of each part was covered with a plastic net on which 200 fruits from the stock were placed. The soil that was dug out from each pit was used to refill it up to the soil surface together with additional nearby soil if necessary. This soil was carefully examined to exclude any fruits. The pit sites were marked and they were left undisturbed for the rest of the experimental period. In order to facilitate records of adult emergence, the plants developed on or close to the tips were cleared.

Adult emergence from the buried fruits was recorded by placing a wooden box of 40x40cm covered with fine mouslin, on each of the two parts of each pit, on April 24. The mouslin at the sides of the boxes was long enough and was buried in the soil to a depth of 5cm, so as to prevent adult escapes through the sides. Plants that developed on or close to the bags were cleared. The boxes were inspected 2 day intervals from 1 to 31 May to monitor adult emergence.

On the soil surface, beside each of the 7 sites of the pits, two envelope-shaped bags of 30x30cm, made from plastic net bearing holes of 0.5cm diameter were placed. In each bag 200 fruits were enclosed. Care was taken to place the fruits on the soil surface in places that did not keep water. Plants that developed on or

close to the bags were cleared. To record emergence on April 24, a wooden box of 40x40cm covered with fine mouslin was placed above each bag. The bags were inspected at 2 day intervals from 1 to 31 May 2001 to record adult numbers.

Adult emergence from fruits that were maintained during winter on the trees was also monitored. This experiment was conducted on 6 randomly selected trees of the orchard. Fruits were collected in October and after being counted they were enclosed in bags made by plastic net and placed on the canopy of the same tree on which they had been collected. In mid-April each bag was covered with fine mouslin to prevent escape of the adults emerged in each bag. The bags were inspected at 2 day intervals from 1 to 31 May to record adult emergence.

The temperature data of the period of adult emergence were collected by the laboratory of General and Agricultural Meteorology of the Agricultural University of Athens in the Agricultural University Campus.

Statistical analysis

The number of fruits was analysed using a two-way ANOVA with factors the type of external appearance (A, B, C and D) and the condition of the fruits (seedless, normal and infested). Means were separated using the Tukey-Kramer HSD test ($P< 0.05$).

A logistic regression equation was used to describe the emergence rate of the adults:

$$Nt = \frac{K}{1+\exp(a-rt)}$$

where Nt is the number of exits in the sampling day and t is the time in days that have passed from the start of the exit period. The analyses were performed by using the statistical package JMP (SAS Inc. 2007).

Results

Fruit colouration and infestation by *E. plotnikovi*

The number of normal fruits was significantly higher than that of seedless and infested fruits ($F_{2,36}=4.92$, $P < 0.012$) (Fig. 1). The fruits of the type D were more frequently recorded in the samples than those of the other types ($F_{3,36}=3.31$, $P < 0.031$). The number of normal, seedless and infested varied among the four fruit colour types, but not significantly ($F_{6,36}=2.04$, $P > 0.08$). However, the fruits of the Type B had the highest percentage of damage (total average 25%) followed by that of the type C (average 17%) whereas, the damage was kept at much lower level in fruit types A and D (3% and 5%, respectively).

Sanitation measures evaluation

The damage level of the fruit sample that was taken from the stock fruits that were to be subsequently used in the field experiments, to investigate the emergence rate of the adults, was estimated by dissection to be 10.3%.

No adult emergence was recorded from the fruits buried in the soil either at 20 or 10cm depth, neither from the fruits that were maintained on the soil surface. However, adults emerged from 10.6% of the fruits that were kept in the net bags on the trees. These adults belonged to *E. plotnikovi*.

The adult emergence from the fruits enclosed in the net bags on the trees, started on May 13 and lasted until May 23. Adult emergence peaked on May 19 reaching 35 adults/ 2d. The temperature fluctuations during the period of adult emergence are shown in Fig. 2. In the first 2 records the adult exit rates were kept at low levels. However, afterwards the emergence of the adults was much

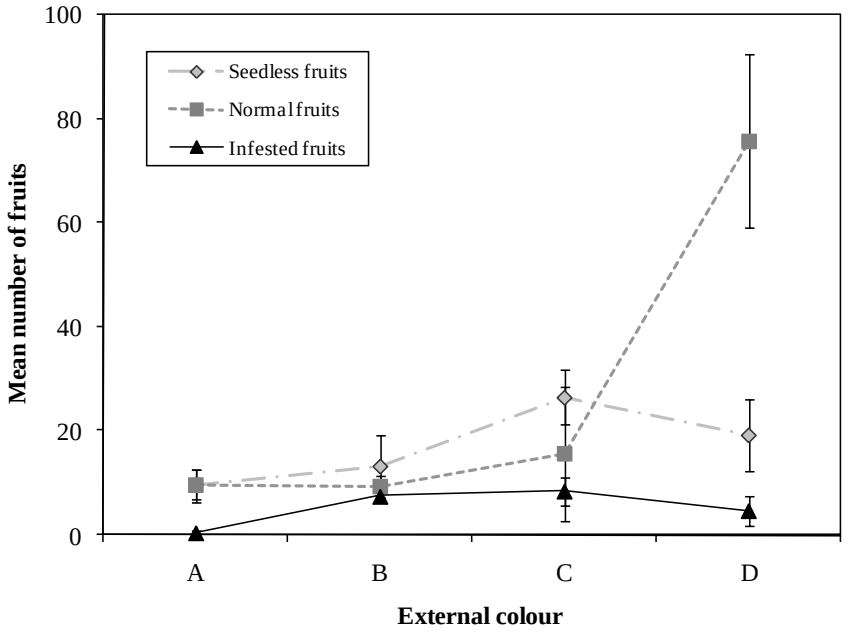

FIG 1. Mean (±SE) of seedless, normal and infested fruits by the wasp *Eurytoma plotnikovi* in fruits of different external appearance: type A almost entirely black, type B partly blackish, type C brownish with shriveled and type D without any discoloration.

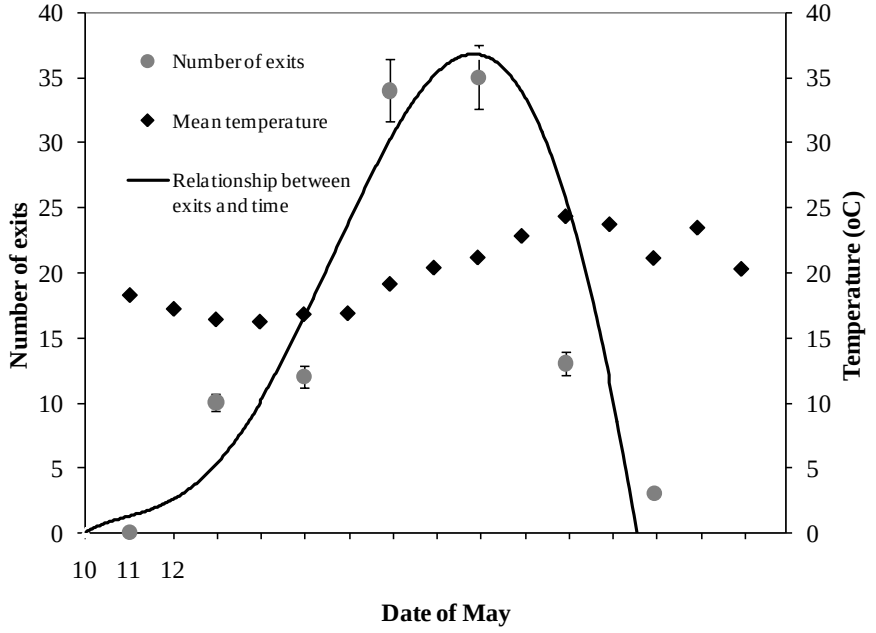

FIG 2. Adult emergence rate of the wasp *E. plotnikovi* from 107 pistachio fruits and the respective temperature fluctuation during May 2001.

accelerated. The fluctuation of exit rates was well described by a logistic regression (K = 39.10, a = 3.39, r = 0.77, R^2 = 0.95) (Fig. 2).

Discussion

The external appearance of pistachio fruits can be related with the absence of larva inside them. This is because fruits of types A (largely discoloured, i.e. almost entirely black) and D (without any discoloration) host significantly less larvae than the type B (partly blackish) and C (brownish and shriveled) (Fig. 1). Thus, our results support the expectation that fruits without discolouration are of highest quality. This knowledge could assist for the easier and timely estimation of the damage percentages at the harvest time. In addition, it could be also useful for processors to recognize the poor quality fruits (Doster and Michailides 1999). However, further research is required to sort more accurately these fruits according to their quality.

Records showed that adults emerged from the fruits belonged to *E. plotnikovi*. This result supports the statement of Mourikis et al. (1998), that although the closely related species *M. pistaciae* is known to occur in Greece, it is much less important than *E. plotnikovi*.

The adult emergence from the fruits was completed within a short period of 12 days, from the middle until the end of May (Fig. 2). This result is also supported by those of the study of Tsourgianni et al. (1989), where this period lasted from the end of May to early June.

Evaluation of sanitation measures and possible modifications showed that the burying of the fruits to a depth of 10 or 20cm prevented adult emergence. However, adults emerged from fruits that remained on the trees.

Most interestingly, no emergence was observed from fruits kept on the ground surface. Very likely, the conditions on the soil surface such as humidity levels and subsequent infection by fungi were unfavorable for the survival of the pest. This is a very interesting finding because it shows that it is not necessary to bury or destroy the fruits, but instead, it is adequate to only remove the fruits from the trees after harvest and leave them on the ground. This simplifies the application of this method and evidently reduces its cost since there is no need for tilling the fruits into the soil which on the other hand, is not a necessary and proper technique this time of the year in the pistachio orchards. Following this modification, sanitation may become more attractive to the farmers. This could lead to higher success rates in the management of seed wasp because effectiveness of the sanitation is apparently increased if applied to a larger area.

The results obtained prove the effectiveness and largely simplify the application of the sanitation control method against the pistachio seed chalcid wasp. Thus, the incorporation of this method in the management practices against this pest can be more widely adopted. Its application would have also positive effects in the management of other insect pests by natural enemies occurring during that period (Souliotis et al. 2002), since the control of *E. plotnikovi* is otherwise mostly based on insecticidal sprayings, mainly with organophosphates, that negatively affect the population levels of natural enemies in the orchards. Therefore, the application of this method could favor the ecological stability in the pistachio agroecosystems with positive impacts in the production of pistachios under less intensive and environmentally friendly farming practices.

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KEYWORDS: Adult emergence, fruit discolouration, cultural control, seed wasp, sanitation.

Συμβολή στην οικολογική διαχείριση του ευρυτόμου της φιστικιάς *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae)

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ΠΕΡΙΛΗΨΗ

Η μελέτη αυτή αφορά στην εκτίμηση του ποσοστού προσβολής, της περιόδου εξόδου των ακμαίων και στην αντιμετώπιση του ευρυτόμου της φιστικιάς *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae). Η προνύμφη του *E. plotnikovi* αναπτύσσεται στο εσωτερικό του καρπού. Επομένως, η δυνατότητα διάκρισης των προσβεβλημένων από τους μη προσβεβλημένους καρπούς μπορεί να βοηθήσει στην έγκαιρη εκτίμηση του ποσοστού προσβολής. Οι καρποί διακρίθηκαν σε 4 κατηγορίες ανάλογα με την εξωτερική τους εμφάνιση και εξετάστηκαν τα επίπεδα προσβολής τους. Βρέθηκε ότι καρποί με χρώμα μερικώς μαύρο ή φαιό είχαν μεγαλύτερα ποσοστά προσβολής. Η έξοδος των ακμαίων βρέθηκε να ολοκληρώνεται σε χρονικό διάστημα 12 ημερών, από τα μέσα έως τα τέλη Μαΐου. Το ευρύτομο διαχειμάζει στο στάδιο της προνύμφης εντός των καρπών και ως σημαντικό μέτρο αντιμετώπισης προτείνεται η συλλογή και καταστροφή των καρπών που μένουν στο δένδρο ή πέφτουν στο έδαφος. Στα πλαίσια της παρούσας μελέτης εξετάστηκε εάν το έντομο μπορεί να επιβιώσει: 1) σε καρπούς που πέφτουν στο έδαφος και 2) σε καρπούς που τοποθετήθηκαν σε διαφορετικά βάθη (10 και 20cm) εντός του εδάφους. Ως μάρτυρας χρησιμοποιήθηκαν καρποί που παρέμειναν στα δένδρα κατά τη διάρκεια του χειμώνα. Τα αποτελέσματα έδειξαν ότι ακμαία εξήλθαν μόνο από τους καρπούς που παρέμειναν επί των δένδρων. Επομένως, φαίνεται ότι δεν χρειάζεται να γίνεται συλλογή των καρπών που πέφτουν στο έδαφος την περίοδο μετά τη συγκομιδή. Αυτό το αποτέλεσμα μπορεί να είναι σημαντικό για την εφαρμογή της μεθόδου αυτής σε ευρύτερη κλίμακα (λόγω μείωσης του κόστους εφαρμογής) και επομένως να συμβάλει στην προσπάθεια για αντιμετώπιση του ευρυτόμου με μέσα πιο φιλικά προς το περιβάλλον.