ENTOMOLOGIA HELLENICA

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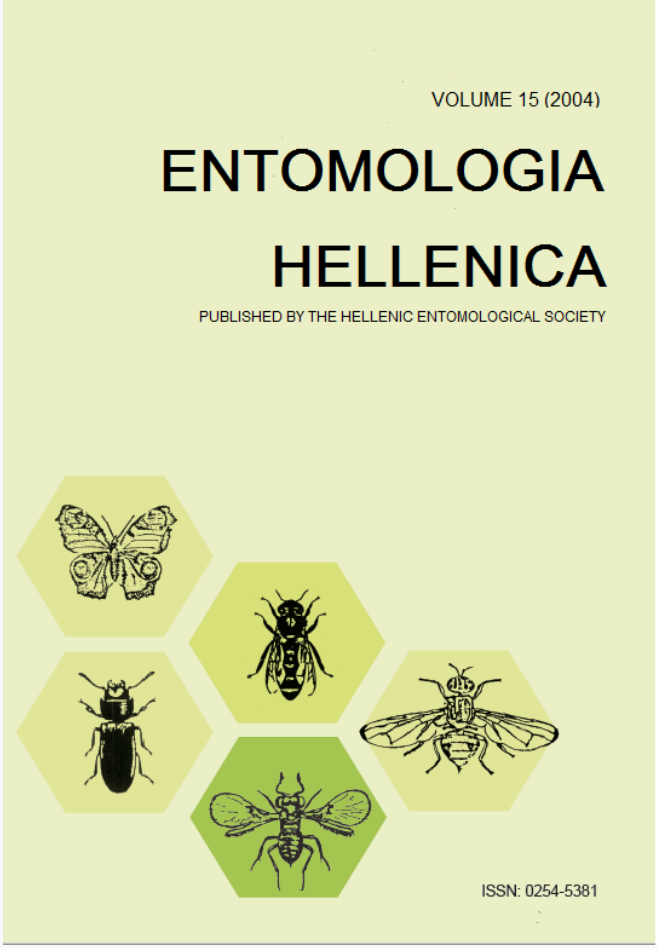

Εποχική εξέλιξη και συμπεριφορά ωοτοκίας του Marchalina hellenica (Hemiptera: Margarodidae)

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ENTOMOLOGIA HELLENICA **15** (2003-2004)

Seasonal development and ovipositing behavior of *Marchalina hellenica* (Hemiptera: Margarodidae)

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ABSTRACT

Marchalina hellenica, Gennadius (Hemiptera: Margarodidae) is the main honeydew producing insect of pine trees in Greece. Its behavior during oviposition, the reproductive capacity, oviposition period, pre-hatching period, and life span of the oviposited female and other aspects of its reproductive behavior in the field and in the laboratory, have been recorded in a three-year research. *M. hellenica* completes one generation per year. It is mainly parthenogenetic. The adult female appears on the trees, looking for an oviposition site from late March to late April. Its oviposition period in the field lasts about 20 days, while the hatching period lasts about 30 days. Live females can be found on the trees until the end of May, so there is a coexistence of three stages of *M. hellenica*, adult, eggs and 1st stage nymphs, in the field for about one month. *M. hellenica* is able to oviposit in the laboratory in room conditions. Its ovipositing period is as long as 16 days, while the total number of eggs laid is on average 262. Body weight was significantly correlated with the total number of eggs laid.

Introduction

Marchalina hellenica Gennadius (Homoptera: Margarodidae) is endemic in the Mediterranean countries, with large populations occurring in Greece and Turkey. In Greece, it mainly occurs on *Pinus halepensis* (allepo pine), and *P. brutia* (calabrian pine), but it is also found on *P. sylvestris* (scots pine), *P. pinea* (stone pine), *P. maritima* (maritime pine) and on *P. nigra* (black pine) in small populations (Kailidis, 1965; Avtzis, 1985). In Italy, it is also reported on *Pinus leucodermis* (bosnian pine) and *P. maritima* (maritime pine) (Fimiani & Solino,1994). In Greece and Turkey, *M. hellenica* is considered beneficial for beekeepers because it provides food for honey bees (*Apis mellifera*) (Kailidis, 1965; Avtzis, 1985; Cürkan, 1989). However, in Italy it is considered as a dangerous pest of pine trees.

M. hellenica feeds on the sap of pines and excretes the excess sugars as droplets of honeydew, which is attractive to honey bees. Honeybees collect, enrich and store this honeydew, and produce the characteristic pine honey. The only countries in the world where pine honey is produced are Greece and Turkey. The annual pine honey production in Greece is estimated between 60-65% of the total annual honey production. The economic importance of the presence of this insect for Greek beekeeping is high; however, information about its ecology, biology and reproduc-

tion is surprisingly scarce. Such data on oviposition, number of eggs, duration of the 1st stage nymphs to hatch and behavior of the adult during oviposition, are given by Nikolopoulos (1965), Kailidis (1965), Santas (1979) and Bikos (2000). However, this data does not agree on the date on which each stage appears; on oviposition period, number of eggs laid, and lifespan of the adult female.

Beekeeping in Greece means wandering. For honeybees to be able to take advantage of honeydew, it is necessary to be in large uniform forests at particular time and place. The amount of insect honeydew honey harvested each year varies, as does the exact timing of the maximum honeydew flow. The ability to forecast the availability of honeydew would be very valuable in minimizing the cost of honey produced. This has been studied by Pechhacker (1988) who counted the number of 2nd stage nymphs of *P. hemicryphus*, the main honeydew producing insect of fir tree, *Abies cephalonica*, in winter on particular trees and then compared this with the rate of infestation by young adults, both of which were found to provide good data as to where to place the bee hives. So the knowledge of the biology of *M. hellenica* and the parameters that influence its seasonal abundance will provide a firm basis for a forecast model of honeydew flow. In the present study, we provide data of three-year observations on the biology and reproduction behavior of *M. hellenica*.

Materials and methods

This study was carried out from March to June of 2001 to 2003 in the pinewood of Seih Sou, in Thessaloniki, northern Greece. An area of 100m^2 was delineated. The trees were 15 years old and their height reached 6-8 m. In this area five trees, at a distance of 2-3 meters each to another in a square, were selected based on the population density of *M. hellenica*. From these trees samples of pine, branches of 30 cm long and different diameter were collected. The samples were always cut from the lower parts of the western side of the trees. Previous observations (Gounari *et al.*, unpublished data) have shown that the density of *M. hellenica* colonies was similar among different trees and occupied the lower parts of the western side of the trees.

The first samples were taken in April of each year and were then collected weekly between April and the end of June in each of the three years (2001, 2002, 2003). In the laboratory, pine branches were cut into smaller pieces of 10-15cm to facilitate observations under a dissecting Olympus microscope. The observations were recorded with a digital Olympus camera, using the software Camedia C2000Z. Data relating to reproductive behavior of *M. hellenica* were collected from observations made in the field and also from pine cuttings transferred and kept in the laboratory.

Behavior during oviposition

Observations on the behavior of *M. hellenica* were made before and during oviposition *in vivo* on the samples of branches just as they were transferred to the laboratory, and also on braches with insects kept in the laboratory. In April and May 2002, four branches of pine of the weekly collections, 10 cm long, with a big number of *M. hellenica* attached, were placed in four wooden cages having at least one side made of glass. At this time, the insects were in 3nd-stage nymph. Daily observations were made and, when the insects had reached maturity, the number of insects dispersing to look for an oviposition site and the number of those that did not disperse were counted.

Observations on field collected branches

The data were collected from the samples of pine branches transferred to laboratory every week from April to the end of June during a three year period (2001, 2002, 2003). The time of oviposition, the number of eggs per ovisac (in each of four samples

from 50 ovisacs), the number of ovisacs containing females, eggs and 1st stage nymphs (crawlers), the duration of oviposition period, the life span of ovipositing female, the prehatching period and the time taken by 1st-stage nymphs to disperse from the ovisac were considered.

Observations on branches kept in the laboratory

In 2001, 2002 and 2003 females of *M. hellenica* (Tabl. 1) were placed individually in Petri dishes and kept in the laboratory. On these females the following observations were made: Percentage of *M. hellenica* laying eggs *in vitro*; time between collection of females and start of oviposition (preoviposition period); ovipositing rate; total number of eggs laid per female; duration of oviposition; lifespan of ovipositing females and correlation between body weight and total number of eggs laid per female.

Each female collected during 2001 and 2002 (Tab. 1) was weighed before placed in Petri dish. During 2003, the nymphs were also placed in Petri dishes, but they were weighed just as they had completed their last ecdysis. In each case, the number of days until the begining of oviposition, the number of eggs laid and the ovipositing period were measured.

The lifespan of the ovipositing females was recorded during 2001 and 2003, whereas in 2002, the daily pattern of egg production of 50 females (Tab. 1) was studied using the following procedure: In group A (20 females), all eggs produced were counted and removed every day. In group B (30 females), the females were left to oviposit undisturbed until the time that females of group A have stopped ovipositing for 2 consecutive days. The total number of eggs was counted at this point then.

To check the viability of the eggs, a random number of eggs was placed into seven Petri dishes, which were kept at room temperature (18°-19°C) in a dark place until the 1st stage nymphs hatched.

Results

General observations on behavior during oviposition

Generally from about the end of March and during the first 10 days of April, *M. hellenica* is found on pine branches as 3rd stage nymphs, feeding on the sap of the pine and producing large droplets of honeydew. Honey bees collect this honeydew mostly from the end of February onwards, depending on the weather conditions. After the 25th March and up to about one month later, on 20th of April, the 3rd-stage nymphs reduce feeding and undergo their last ecdysis, emerging as adult females.

Twenty four hours before the beginning of ecdysis, the 3rd-stage nymphs stay motionless, discards any excess of honeydew, and the outer layer of the integument begins to slough off. The ecdysis (Fig.1) can last from 3 to 48 hours, depending on whether the insect is disturbed or not. The female is a non-feeding stage, as during ecdysis, its mouthparts are shed with the outer integument (Fig. 2) and they are never replaced. No honeydew is therefore produced by the females. Immediately after ecdysis, the body of the female is smooth and shiny, with no sign of wax secretions. At this time, about 60 to 90% (Table 2) of the females disperse from where they moulted towards the lower parts of the tree, searching for an oviposition site. This explains why the old cast exuvia are found in the previously attached places. The remaining 10-40% settled close to where they moulted.

The dispersing females can travel large distances on the branches or trunk of the host tree. Since they settle most frequently on the base of the trunk, they can be found almost anywhere nearby where there are sites which offer suitable protection during oviposition. These females, just before the beginning of

oviposition, show great ability to disperse, but are also strongly negatively phototaxic, so they prefer small dark niches. Since their body is very elastic at this stage, they can get into tiny cracks or crevices in the bark - indeed, anywhere away from light. After its settlement the female begin to secret long waxy filaments by ventral wax glands, formed a white ovisac, which encloses the whole body of the insect. Within this ovisac the female laid its eggs in a series, stuck together (Fig. 3).

Observations on field collected branches

The date when the adult females dispersed to lay their eggs was not the same in the three years (Fig. 4). It is considered that this was probably due to the environmental conditions but this needs further investigation. In 2001 and 2002, the dispersing females appeared on the trees after the 25th March. This dispersal phase lasted 10 days (until 5th April), during which all females had found sites for oviposition. This is more or less in agreement with the observations of other researchers (Nikolopoulos, 1965; Santas, 1979). However, in 2003, the dispersing females had not appeared until the 20th of April, although the period of dispersing lasted 10 days. By the 30th of April, all the insects had settled in the ovipositing site (Fig. 4, c). In all three years, all females had died by the 30th of May, irrespective of the date of their appearance. Thus, the females were present on the trees for about two months in 2001 and 2002 (from 25th March to 30th May), but for about one month in 2003 (from 20th April to 30th May). Oviposition began on early April in 2001 and 2002. However, in 2003, the ovisacs containing eggs appeared after 26th April, almost a month later.

An indication of the number of eggs a female might lay in field conditions was gained by counting the eggs in the egg-sacks of pine cuttings of four consecutive samples. However, since the females died as a result of this disturbance, egg production probably stopped prematurely. However, from this observation we estimated that each female could lay about 262 eggs on average (Table 3).

The pre-hatching period of the eggs was about 30 days (from 4th Apr.-7 th May 2002, and 26 th Apr.-9 th June 2003) (Fig. 4, b,c). Egg laying had already begun before the beginning of observations in 2001. The 1st-stage nymphs had dispersed by 9th June, in 2001; by 29th May,in 2002, and by 20th June, in 2003.

Observations on branches kept in the laboratory

In 2001 and 2002, dispersing females were taken to the laboratory, while in 2003 females and 3nd stage nymphs in pre-adult stage. Almost 100% of the adults oviposited successfully. The time between collection and beginning of egg-laying was in all cases very short. More specifically in 2001 and 2002, 80% and 60% of the females respectively started laying eggs in the third day. It also took three days for all the females to begin oviposition in 2003. The body weight of the female (39,46 mg in average in 2002) is in correlation with the time needed to start oviposition (Fig. 5). The rate of oviposition of 20 females (2002) – group A- under laboratory conditions is given in Fig. 6. A percentage of 67.7 of the total number of eggs was laid in the first five days. Some females laid eggs for 16 days and the total number of egg laid ranged between 133-394 (mean 262). The daily counting and removing of the eggs seems not to affect the total number of eggs laid. Thus, after 17 days the total number of laid eggs by insects of the two groups did not differ significantly at $P<0,05$ using the Duncan test. The number of eggs that the individuals of group B were oviposited ranged between 68-408 in a 17 days period. A positive correlation was found between the weight of the female (20mg: 2001, 39,4 mg: 2002, 37,3 mg: 2003, in average) and the total number of eggs laid in all the tree years of observations (Figs7 a, b, c).

TABLE 1: Procedure for the in vitro study of the oviposition behavior of *M. hellenica*

year	date	Number of insects	Stage of development when collected
2001	9 April	15	Dispersing adults
2002	4 April	50	Dispersing adults
2003	22 April	10	Pre-adult stage

TABLE 2: Proportion of *M. hellenica*, to move looking for a new site of oviposition

Date	Number of insects		
	Total	Dispersal	Not dispersal
11/04/2002	18	16 (89%)	2 (11%)
25/04/2002	50	31 (62%)	19 (38%)
18/05/2002	50	46 (92%)	4 (8%)
21/05/2002	50	39 (78%)	11 (22%)

TABLE 3. Number of eggs / ovisac in collected pine braches in four consecutive samples

Date	Number of eggs/ovisac (n=50)	
	Min-Max	Mean±se
10/04/01	60 - 351	198 ± 46,9
17/04/01	23 - 271	123 ± 77,4
24/04/01	92 - 313	222 ± 41,4
02/05/01	78 - 302	192 ± 37,5

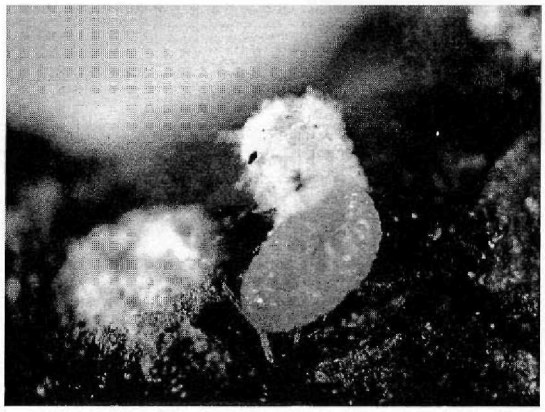


FIG. 1. The last ecdysis of *M. hellenica* and the emerge of the adult

FIG. 2. Exuvia with the mouthparts of the last ecdysis of *M. hellenica*

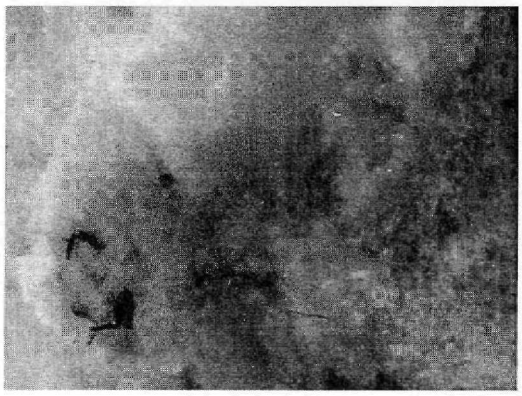


FIG. 3. The ovisac of the adult *M. hellenica* with eggs

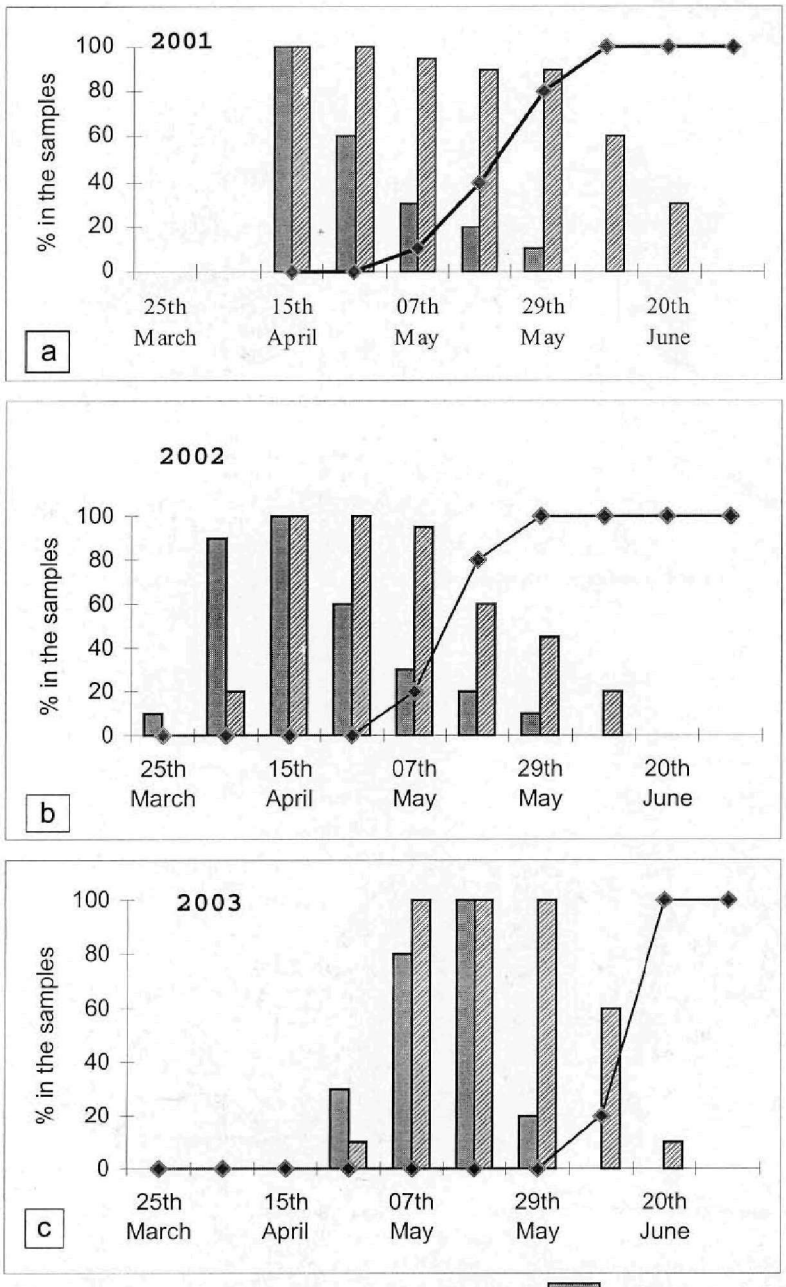


FIG. 4. Proportion of alive adults, moving or ovipositing ovisacs containing eggs and ovisacs with dispersing nymphs of the samples during the three years of observations

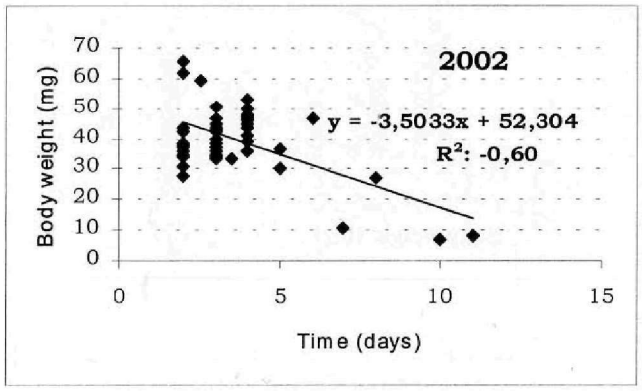


FIG. 5. Relation of the body weight of the adult *M. hellenica* to the time needed to begin oviposition, under laboratory conditions (2002).

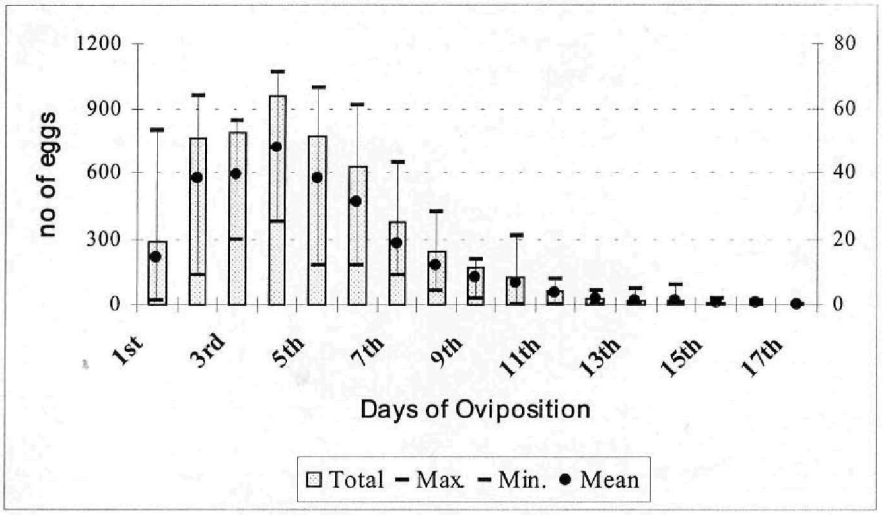
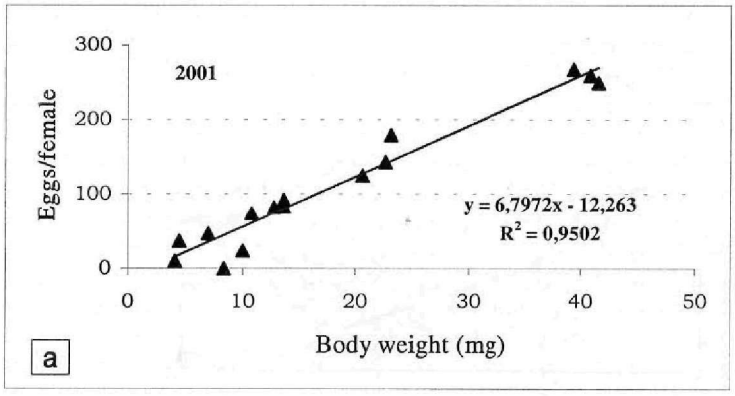
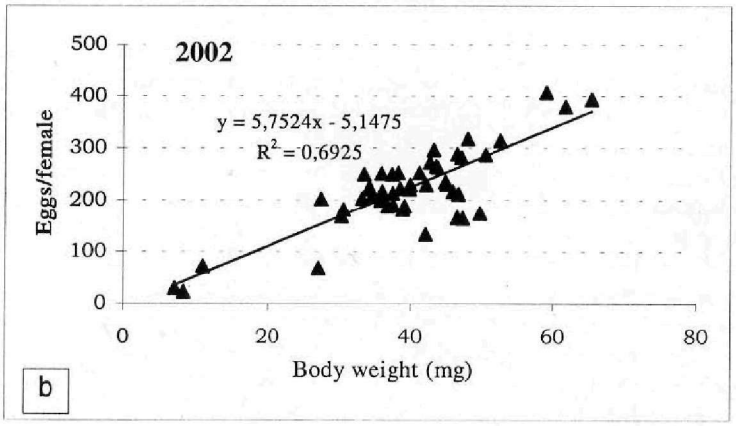


FIG. 6. Fecundity rate of 20 *M. hellenica* per day (2002)





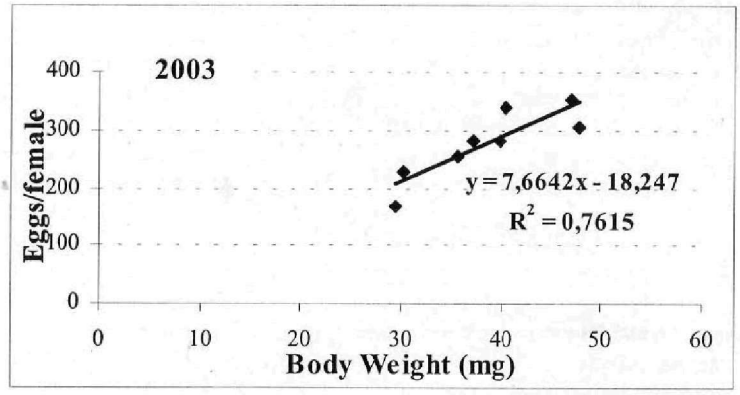


FIG. 7. Relationship between the adult body weight and the total number of eggs laid per female in 2001 (a), 2002(b) and 2003 (c).

The total number of eggs laid was on average 111, 222 and 258 in 2001, 2002 and 2003 respectively. The mean oviposition period was 30.6 days (range 20-37 days) in 2001 and 26.7 days (range 18-39 days) in 2003. This period is completely different from that determined by other researchers. More specifically, Nikolopoulos (1965) stated that *M. hellenica* females lay eggs for three days and then die, while Santas (1979) stated that the oviposition period is a few days.

A high percentage (60.2%-97.6%, mean 81.4%) of crawlers hatched succefully *in vitro* at 18°-19°C without being in an ovisac which, in the field, would have protected the eggs.

Discussion

This three-year study indicated some new data on biology of *M. hellenica* which enrich our knowledge and will help us to reach our goal, which is the ability to forecast the timing and quantity of honeydew flow. There are two main periods in which honey bees tap the honeydew secretions of *M. hellenica*, in autumn (September – October), and in spring (March-April). As the female is not able to feed, it cannot also produce honeydew. So the appearance of the female on the trees in spring indicates the end of the spring period of production of honeydew secretions. This time is not distinct for each year. In the three years of the study, it ranged from the 25th of March to the 25th of April. The biotic and non- biotic parameters influencing this must be investigated in order to be capable to determine every year the timing in which the spring honeydew flow will stop and the beekeepers will have to move their beehives from the forests.

It is interesting to point out that the delayed appearance of adult *M. hellenica* on the trees in 2003 appeared to shorten only the oviposition period of one month instead of two months and the life span of the female (from about 30 days in 2002 to 14-22 days in 2003), but no significant effects were noted on the reproductive capacity of the insect (total number of eggs/female in average in 2002, 222 and in 2003, 258), nor on the date of the settlement of the new generation, the crawlers, to their new feeding sites.

During this study, no males of *M. hellenica* were observed, matching the description given by Nikolopoulos (1964). Yet, a number of insects with male morphological appearance collected from the branches of the samplings were sent to the National Museum of Wales in Cardiff, UK for identification. So, it is to be investigated whether *M. hellenica* is exclusively parthenogenetic or not. The great ability of the female to disperse to long distances, far away from the previously attached site, on another part of the tree or to another tree, and also the form of the ovisac which can easily be taken away with the air seems to be the two the main ways of expansion in the trees of a forest. The appearance of *M. hellenica* on neighboring trees of other species, such as cypress (*Cupressus* sp.) seems to be coincidental. Female *M. hellenica* use *Cupressus* sp. as an alternative oviposition site, like other sites on the ground, on benches and walls.

Acknowledgements

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KEYWORDS: *Marchalina hellenica*, pine tree, honeydew, oviposition, Greek pine honey, soft scale insects, Margarodidae

Εποχική εξέλιξη και συμπεριφορά ωοτοκίας του *Marchalina hellenica* (Hemiptera: Margarodidae)

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ΠΕΡΙΛΗΨΗ

Το *Marchalina hellenica*, Gennadius (Hemiptera: Margarodidae) είναι το κύριο μελιτογόνο έντομο του πεύκου στην Ελλάδα. Στην παρούσα εργασία παρουσιάζονται τα αποτελέσματα έρευνας τριών ετών σχετικά με τη συμπεριφορά του εντόμου κατά την ωοτοκία, την περίοδο εκκόλαψης των ερπουσών, τη διάρκεια ζωής του ωοτοκούντος θήλεος, καθώς και άλλα στοιχεία σχετικά με την συμπεριφορά του κατά την αναπαραγωγή , στο ύπαιθρο και στο εργαστήριο. Το *M. hellenica* έχει μία γενεά το έτος. Η υπόθεση ότι είναι κατά κανόνα παρθενογενετικό διερευνάται. Τα ώριμα προς ωοτοκία θήλεα παρουσιάζονται στα δέντρα, κινούμενα προς ανεύρεση θέσεως ωοτοκίας κατά τις 25 Μαρτίου ή και ένα μήνα αργότερα κατά τις 25 Απριλίου. Η περίοδος ωοτοκίας στο ύπαιθρο είναι περίπου 20 ημέρες, ενώ η περίοδος εκκόλαψης διαρκεί 25-30 ημέρες. Πριν την ωοτοκία το ακμαίο παράγει νήματα κηρώδους σύστασης με τα οποία καλύπτει ολόκληρο το σώμα του. Η ύφανση αυτών είναι πιο συμπαγής στο πίσω μέρος του σώματός του, δημιουργώντας εικόνα «σάκου», μέσα στον οποίο αποθέτει τα ωά κολλημένα κατά σειρές. Ζώντα ωοτοκούντα θήλεα μπορούν να βρεθούν στα δέντρα έως και το τέλος Μαΐου. Έτσι παρουσιάζεται συνύπαρξη τριών σταδίων του εντόμου, ακμαίου, ωών και ερπουσών για μία περίοδο περίπου ενός μήνα. Το *M. hellenica* μπορεί να ωοτοκήσει και σε συνθήκες εργαστηρίου, χωρίς ιδιαίτερες απαιτήσεις. Η περίοδος ωοτοκίας στο εργαστήριο ήταν κατά το 2002 16 ημέρες, ενώ ο συνολικός αριθμός των ωών που αποτέθηκαν ήταν κατά μέσο όρο 262. Κατά τα τρία έτη παρατηρήσεων παρουσιάστηκε συσχέτιση μεταξύ του βάρους του σώματος του θήλεος εντόμου και του χρόνου προ-ωοτοκίας, όπως και του συνολικού αριθμού ωών, που μπορεί να αποθέσει. Η διάρκεια ζωής του ωοτοκούντος θήλεος κυμαίνεται μεταξύ 26-30 ημέρες, χωρίς να συσχετίζεται με το βάρος του σώματός του. Κατά τη διάρκεια ζωής του το θήλυ δεν τρέφεται καθώς δεν διαθέτει στοματικά μόρια.