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The Reproductive System and Ovarian Development of the Adult Olive Psylla *Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae)

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ABSTRACT

The gross morphology of the reproductive system of adult females and males of the olive psylla *Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae) is given. Five stages of ovarian development are distinguished and described, based on the size and shape of the ovaries and ovarioles, on the separation of the vitellarium from the germarium, and on the appearance and shape of oocytes.

Introduction

Euphyllura phillyreae is the main psyllid species infesting olives in northern Greece, and the most frequently occurring species of *Euphyllura* in central and southern continental Greece (Lauterer et al. 1986). Its area of distribution is known to cover the Mediterranean, the Black Sea coast, and the Caucasus. At least in the northern part of its range, the species is oligophagous, feeding only on plants of the genera *Olea, Phillyrea,* and *Osmanthus,* all Oleaceae. Due to a misidentification the species was erroneously reported as *E. olivina* (Costa) by Prophetou and Tzanakakis (1977) who studied its seasonal development in N. Greece.

In northern Greece, *E. phillyreae* completes one generation per year. In olive groves, the adults are formed in the second half of May. The females remain reproductively immature through the summer, autumn and part of winter. After overwintering, the females were found with mature oocytes from late March to early May, and eggs were observed on the buds of olive in April and on inflorescences in May (Prophetou and Tzanakakis 1977, Prophetou-Athanasiadou 1993).

In N. Greece *E. phillyreae* was also found on *Phillyrea latifolia* L. (*P. media* L.) (Lauterer et al. 1986), an evergreen bushy perennial plant which blooms from March to May (Athanasiades 1986).

During a study of the termination of diapause of this species the reproductive system of the adults was studied and is described in the present paper.

Materials and Methods

During the triennium 1981-1983 and during 1988-1989, adults were collected from olive trees and *P. latifolia* by beating twigs over an insect net. They were killed with chloroform fumes, immersed in a Ringer solution and dissected under a stereoscopic microscope.

The descriptions of the reproductive systems of *Cacopsylla mali, C. pyri* and *Trioza erytreae,* by Grassé (1951), Bonnemaison and Missonnier (1955)

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and Blowers and Moran (1967) respectively, were used as a guide. Recorded were the presence of fat body, the shape and developmental stage of ovarioles, the presence of spermatophores in females, the shape and general structure of the spermatheca before and after mating, and the dimensions of the different parts of the male and female reproductive system. The presence of sperm in the testes and seminal vesicles was also recorded upon eclosion of the adults. Dimensions were measured with an accuracy of 13 μ. To determine the stage of ovarian development, microscopic slides were prepared in a Ringer solution, containing either the whole reproductive system or parts of it.

Results and Discussion

Female system

Its general structure is seen diagrammatically in Fig. 1. It consists of a pair of ovaries, a pair of lateral oviducts, a common oviduct, a paired accessory gland, a spermatheca, a pouch (bursa) of still unknown function, and a colleterial gland.

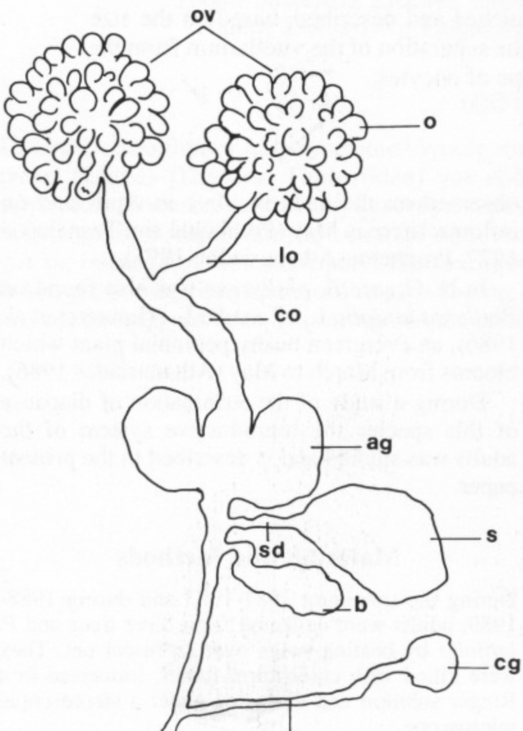

FIG. 1. Main parts of the reproductive system of the adult female of *Euphyllura phillyreae* shortly after ovarian development has started (stage II) (diagrammatically, X130). ag: accessory gland, b: bursa, cg: colleterial gland, cgd: colleterial gland duct, co: common oviduct, lo: lateral oviduct, o: ovariole, ov: ovary, s: spermatheca, sd: spermathecal duct.

Ovaries. Each ovary contained 29-51 ovarioles (mean 40 ± 3.8, n=201). Fig. 2 shows the frequency distribution of ovarioles in the 201 females that were dissected. The ovarioles are of the meroistic telotrophic type. During ovulation, the oocytes descend to the vitellarium, while the trophocytes remain in the germarium and provide the necessary nutrients to the developing oocytes through a trophic cord.

The ovarioles of a reproductively immature female (Figs. 3 I, II; 4 I, II) are subspherical and only the germarium is distinguishable, having an intense white colour (milky) and consisting of oocytes, trophocytes, and epithelial tissue. The ovarioles of a reproductively maturing or mature female (Figs. 3 III, IV, V; 4 III, IV, V), are oblong and consist of the terminal filament, the germarium, the vitellarium, and the pedicel. We were able to distinguish the terminal filament only in ovaries that were in stages IV and V of development (see below).

Stages of ovarian development

Upon emergence from the last instar's exuvium, sometime in May, the adult female's ovaries are transparent and not easily distinguishable. A few days later they turn opaque white and are easily seen. In females living on olive trees in coastal northern Greece, the ovaries remain in this undeveloped condition for approximately two to three months. In late August a small percentage starts developing to the next (second) stage, and by late December more than 60% has reached that stage. Ovarian development continues in the field throughout winter, so that by the end of March virtually all females have ovaries fully developed, with oocytes ready to be laid (Prophetou-Athanasiadou and Tzanakakis 1986).

The ovarioles of an ovary do not all develop simultaneously, but in groups of 4 or 5 ovarioles. Thus, while some ovarioles are at a given stage of development, others are at a less or at a more advanced stage, as seen in Fig. 5 III, IV, V.

During ovarian development we have never observed more than two oocytes in any ovariole. The second (younger) oocyte starts developing when the first one has reached its final shape and size and is ready to move to the lateral oviduct. While still in the ovariole, the mature oocyte (Figs. 3 V; 4 V; 6) does not differ in shape and size from the egg as deposited on the host plant.

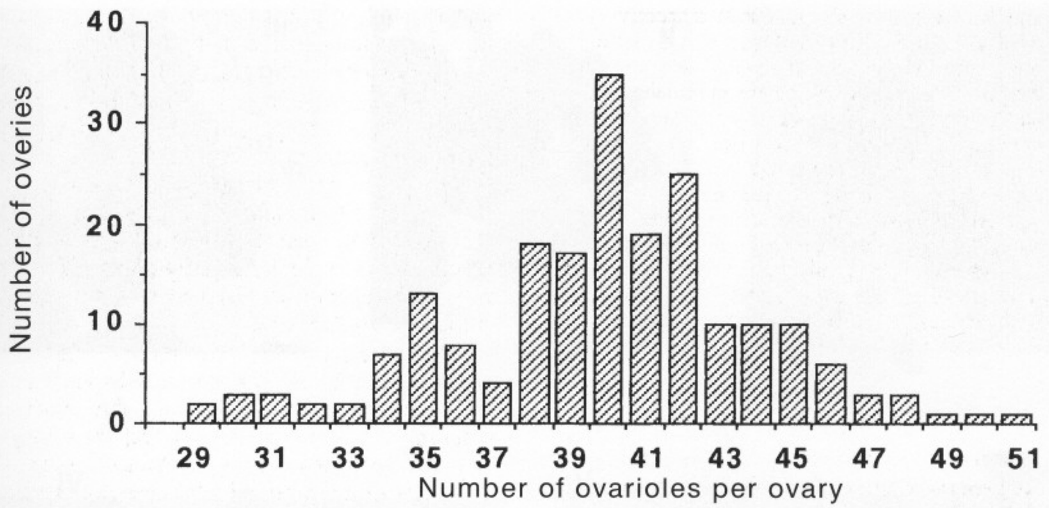

FIG. 2. Frequency distribution of the number of ovarioles in the ovaries of *Euphyllura phillyreae*. Number counted in one ovary of each of 201 females.

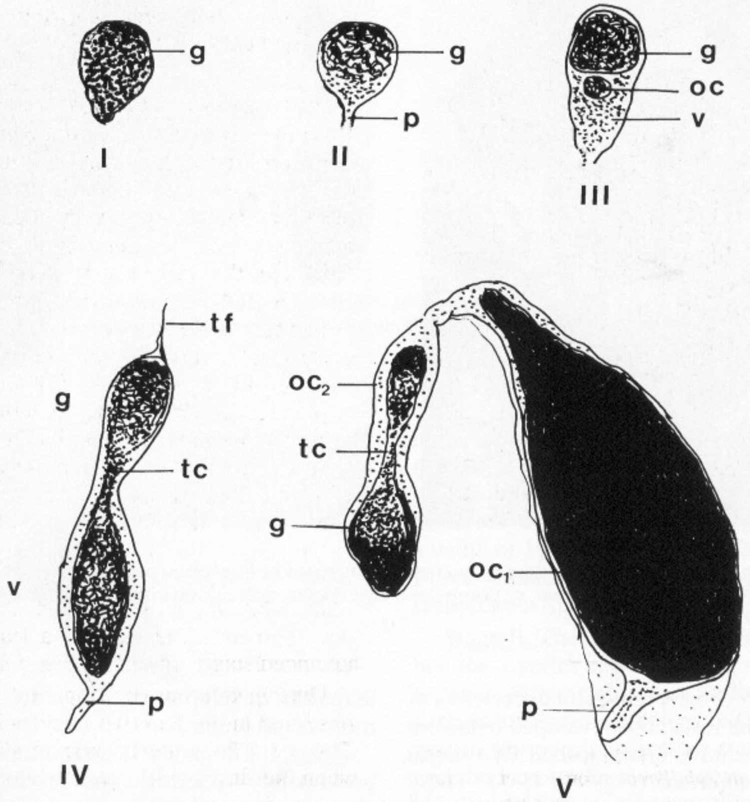

FIG. 3. Ovarioles during each of the five stages of ovarian development in *Euphyllura phillyreae* (diagrammatically, X 130). g: germarium, oc: oocyte, oc1: first oocyte, oc2: second oocyte, p: pedicel (stalk), tc: trophic cord, tf: terminal filament, v: vitellarium.

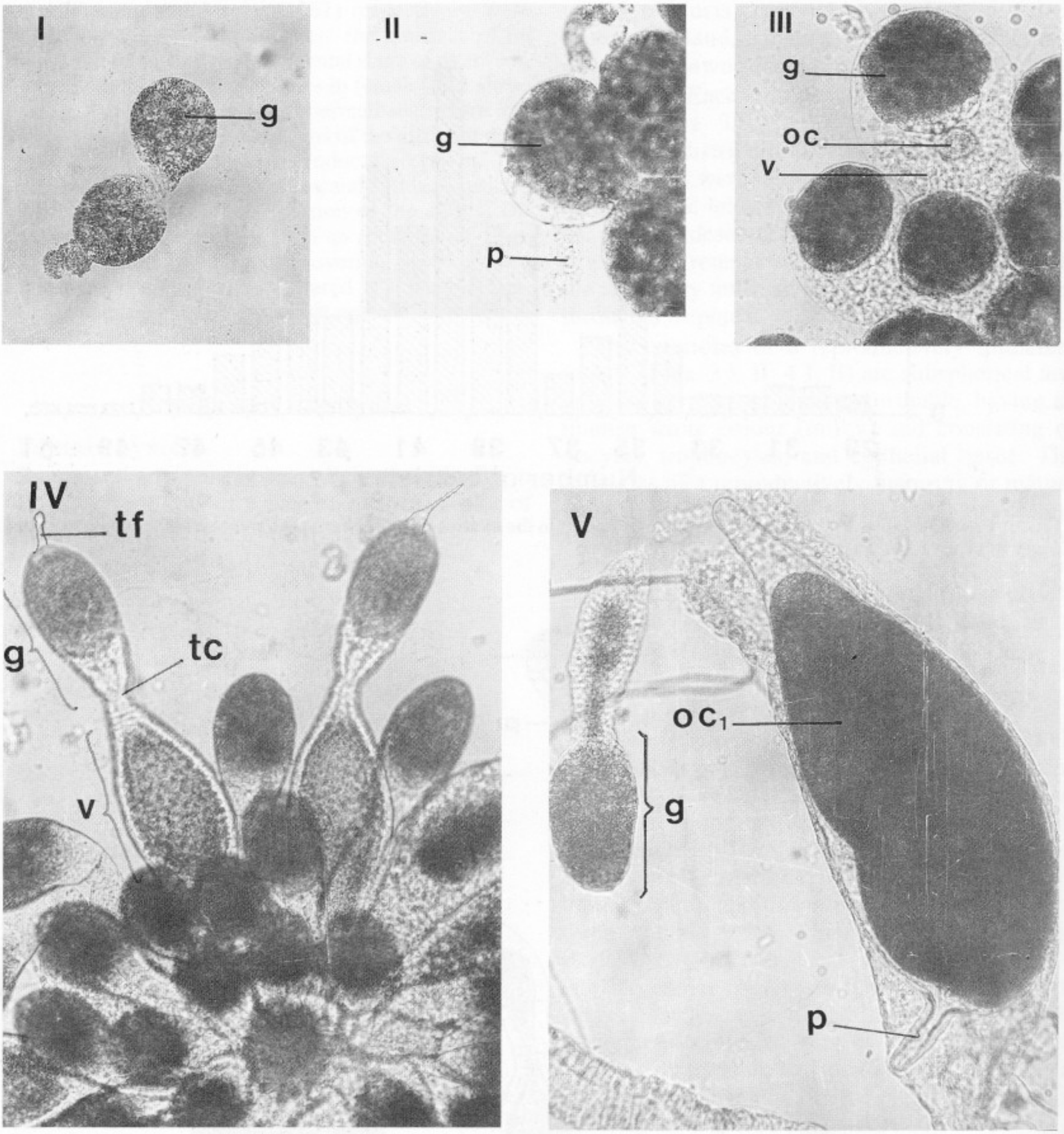

FIG. 4. Ovarioles during each of the five stages of ovarian development of *Euphyllura phillyreae*. I (x 289), II (x 395), III (x 523), IV (x 241), V (x 259). g: germarium, oc oocyte, oc1: first oocyte, oc2: second oocyte, p: pedicel, tc: trophic cord, tf: terminal filament, v: vitellarium.

Based on distinct morphological differences of the ovaries and the few most developed ovarioles of each ovary, we have distinguished five stages of ovarian development (Figs 3; 4; 5). These stages have been briefly described by Prophetou-Athanasiadou and Tzanakakis (1986) and used to distinguish females of *E. phillyreae* which were in diapause from those in various stages of post-diapause development. They are described in more detail below.

Stage I. The ovary is undeveloped, white, approximately spherical, and resembles a blackberry. It is surrounded by abundant green fat body. At this stage, the fat body surrounds also other parts of the female reproductive system, such as the oviducts, the accessory glands, and the sper-

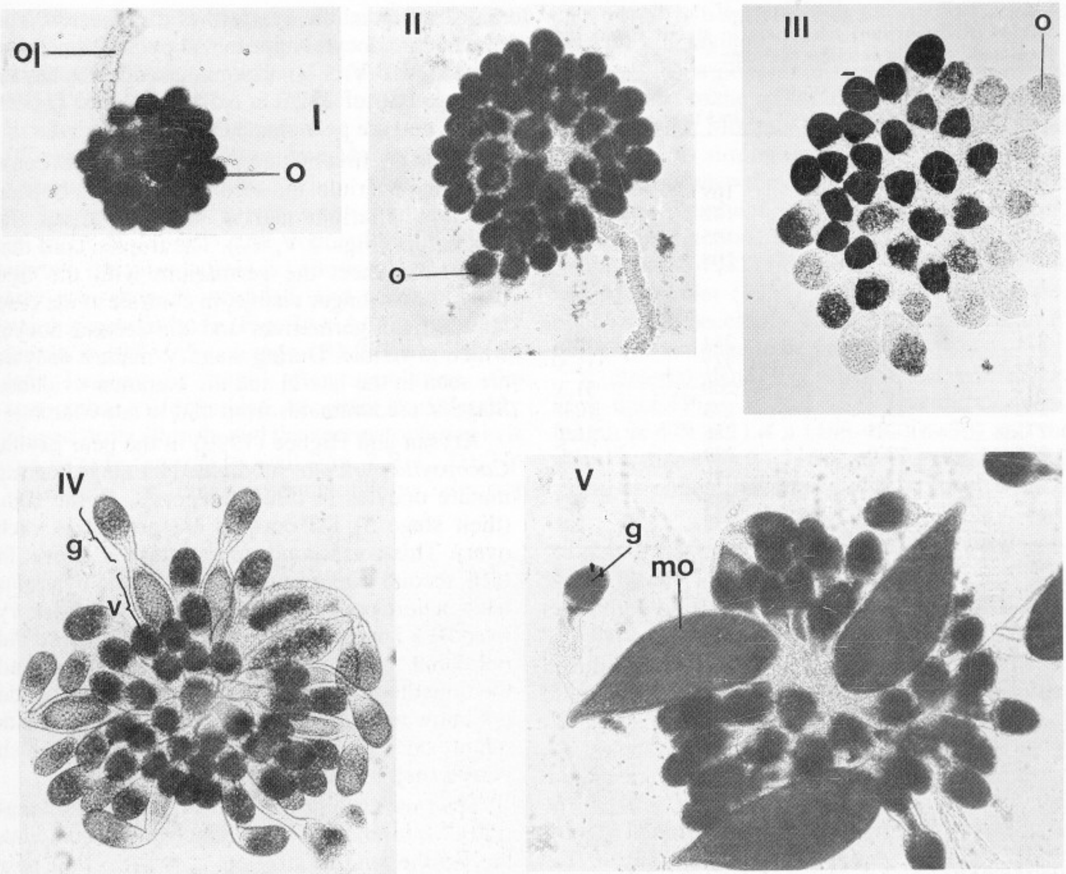

FIG. 5. Stages of ovarian development (I-V) of *Euphyllura phillyreae*. Stage I (x80), stage II, III and V (x116), Stage IV (x100). g: germarium, lo: lateral oviduct, mo: mature oocyte, o: ovariole, v: vitellarium. (From Prophetou-Athanasiadou and Tzanakakis, 1986).

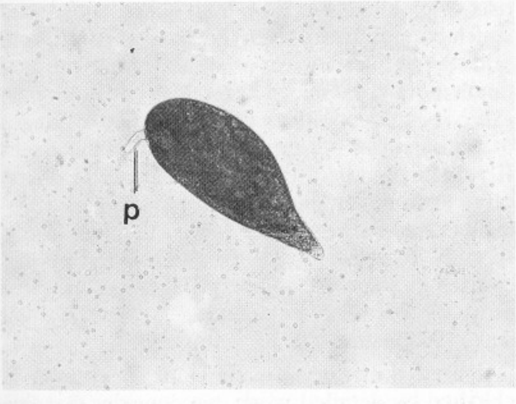

FIG. 6. Mature oocyte of *Euphyllura phillyreae* as seen in the ovarioles shortly before being laid. p: pedicel.

matheca. The ovarioles are close to one another so that their number cannot be counted. Only the germarium is distinguishable. The ovary has a mean length of 190 ± 9.8 μ (range 169-208 μ) and a width of 196.8 ± 10.3 μ (169-208) (n=80). Stage I predominates until sometime in mid December (Prophetou-Athanasiadou and Tzanakakis, 1986).

Stage II. There is still abundant green fat body, but the ovaries have started to loosen and the number of ovarioles can be counted. Only the germarium is still distinguishable, but the basal part of the ovariole is more transparent than the rest, and more development is detectable in the basal part of the germarium (Figs. 3 II; 4 II; 5 II). Ovarian length is 247 ± 21.2 μ (221-329) and width 271 ± 27.8 μ (234-338) (n=80). The frequency distribution of ovarian size at stages I

TABLE 1. Frequency distribution of the dimensions of ovaries of *Euphyllura phillyreae* in stages I and II of development. Eighty ovaries per stage.

Length class (μ)	Percent	Width Class (μ)	Percent
	Stage I		
169	7.5	169	2.5
182	32.5	182	17.5
195	51.3	195	43.7
208	8.7	208	36.3
	Stage II		
221	18.8	234	12.5
234	30.0	247	12.5
247	12.5	260	31.3
260	22.5	273	10.0
273	7.5	286	11.2
286	5.0	299	7.5
299	3.7	312	6.3
		325	6.2
		338	2.5

and II is given in Table 1. Stage II predominates from mid December to late January.

Stage III. The green fat body has been reduced considerably. The ovarioles are much more separated from one another than in stage II. Ovarian dimensions can no longer be measured, certain ovarioles being longer than others and the ovary taking an irregular shape (Figs. 4 III; 5 III). In the ovarioles the vitellarium has started to form and the first oocytes appear in it (Figs. 3 III; 4 III). At first the vitellarium appears as a continuation of the germarium. Later at this stage, the first oocyte appears in the vitellarium. The trophic cord is not yet distinguishable. At the time stage III appeared in females living in olive groves, the first matings were observed. In the olive grove, stage III predominates from late January to mid February.

Stage IV. The fat body has been reduced even more than in stage III, and the ovary appears as in Fig. 5 IV. In the ovarioles the vitellarium is already developed and separated from the germarium by a narrow intermediate zone. The vitellarium is often bigger than the germarium. (Figs. 3 IV; 4 IV; 5 IV). The first oocyte which since late stage III has been in the vitellarium, is surrounded by the epithelial cells. The trophic cord is formed and is distinguishable. Vitellogenesis starts, and as a result of it there is a fast increase of the size of oocytes and of the vitellarium. Stage IV predominates in early March.

Stage V. It is the most advanced stage that we observed during the present study. The fat body that surrounded the ovaries has disappeared. The first mature oocytes appear, and oviposition starts. (Figs. 3 V; 4 V; 5 V). The mature oocytes have a mean length of 382.3 ± 6.54 μ (range 377-390, n=22), and are pear-shaped (Fig. 6).

While the first mature oocyte has not yet come out of the ovariole, the second oocyte can be seen in the vitellarium and is separated from the germarium (Figs. 3 V; 4V). The trophic cord that used to connect the germarium with the first oocyte is no longer visible, in contrast to the cord between the germarium and the second oocyte which is visible. During stage V mature oocytes are seen in the lateral and the common oviducts. Stage V predominates from mid to late March.

Krysan and Higbee (1990) in the pear psylla, *Cacopsylla pyricola,* subdivided the stage bearing mature oocytes in three categories. In the first, (their stage 5) 1-3 oocytes are present in each ovary. This corresponds to our stage V above. In their second category up to 50% of the ovarioles have mature oocytes, while in their third category over 50% have such oocytes. In our work we did not count the number of mature oocytes beyond the time the first ones appeared. Therefore, we do not know whether more than a few ovarioles with mature oocytes are observed per ovary and which period such a condition may cover.

The time each of the above five stages of ovarian development lasts in nature may vary with the weather and be affected also by the time new growth and flower buds develop on the host plant. It may also differ considerably between adults living and feeding on different species of host plant, as found between olive and *Phillyrea latifolia.* Thus in mid June all females had ovaries in stage I, irrespective of species of host plant. The first ovaries in stage II on both host plant species and locations were observed in mid July. From mid September on, ovarian development was much more pronounced in females developing on *Phillyrea* than on olive. This concerned all stages of ovarian development. In mid September stages III and beyond had started on *Phillyrea,* while on olive such development started on the 20th December and exceeded 75% only in mid February. In mid November, most insects on *Phillyrea* had ovaries at stage III and beyond, while on olive only 4% of the population had ovaries at that stage (Prophetou-Athanasiadou 1993).

Paired accessory gland. Until the matter is clarified by detailed work, we consider that this pair of pouches (Figs. 1 and 7B) represents an accessory gland. We reach this tentative conclusion because similar paired pouches located in the same part of the common oviduct are reported as accessory glands by Weber (1930) (from Grassé, 1951) for *Cacopsylla mali* (Schmidberger), by Bonnemaison and Missonnier (1955) for *C. pyri* (Foerster) and by Blowers and Moran (1967) for *Trioza erytreae* (Del Guercio).

Spermatheca. On olives, when the ovaries are at stage II (above) and copulations have not started, the spermatheca is yellowish, approximately pear-shaped, wrinkled, and surrounded by dense green fat body (Fig. 7A). At that period its length is 195 ± 15 μ (range 182-221, n=110). In late January or later, after copulation, the spermatheca is distended, becomes considerably bigger, turns whitish, and the spermatophores in it can be seen through its wall (Fig. 7 B, C, D). Up to several or many spermatophores are not uncommon in a spermatheca during the period of massive egg laying.

Bursa. This pouch empties into the common oviduct next to the spermatheca (Figs. 1 and 7B). Blowers and Moran (1967) consider as bursa copulatrix a similar pouch in the psyllid *T. erytreae*, because they found no visible contents in it, no glandular cells in its wall, and no glandular function. On the other hand, Weber (from Grassé 1951) in *C. mali* and Bonnemaison and Missonnier (1955) in *C. pyri* consider this pouch as an accessory gland. Its exact role in *E. phillyreae* remains to be proven.

Colleterial gland. It is subspherical, with a very long duct (Figs. 1 and 7E). The duct's mean length is 479 ± 11.4 μ (468-494)(n=30) and the gland's diameter 84.5±6.8 μ. We consider this gland as colleterial, because of its location near

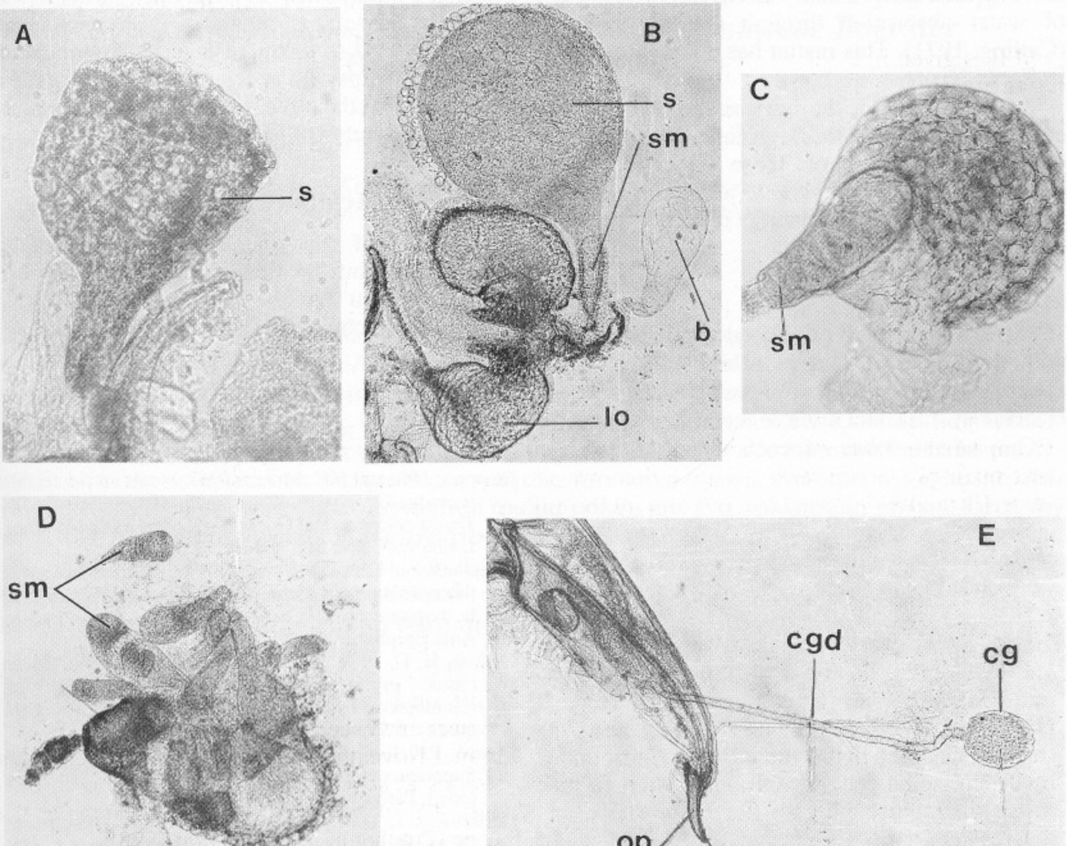

FIG. 7. Spermathecae, spermatophores and colleterial gland of *Euphyllura phillyreae*. A: before copulation (x267); B and C: after copulation (x140 and x194); D (x300); E (x140). ag: accessory gland, b: bursa, cg: colleterial gland, cgd: colleterial gland duct, op: ovipositor, s: spermatheca, sm: spermatophore.

the end of the common oviduct and of the fact that a similar organ with also a very long duct was described as such in *C. pyri* by Bonnemaison et Missonnier (1955), in *C. mali* by Weber (1930) and in *T. erytreae* by Blowers and Moran (1967).

Eggs. The mature oocytes, as seen in the ovariole shortly before being laid, are pear-shaped and white. Each egg or mature oocyte is approximately 65 μ long and possesses a stalk near its wider end (Figs. 5 and 6A). This egg stalk, which occurs in the eggs of all psyllids, and is inserted in the plant tissue, functions not only as a point of attachment, but also of water uptake from the plant (Bonnemaison and Missonier, 1955; Blowers and Moran, 1967; Catling, 1971; Hodkinson, 1974 and references therein; White, 1968). The progressive decline in egg hatch in *T. erytreae* with an increase in leaf size is possibly due to physiological changes in maturing citrus leaves which detrimentally affect this mechanism of water absorption through the egg pedicel (Catling, 1971). This matter has not been studied in *E. phillyreae*.

After being laid, the eggs according to their colour at 23Æ C could be grouped in three age classes: Up to 2 days old those being white, 3-4 days old those being yellow, and 5-6 days old those being light orange. Eggs ready to hatch (8 days old) were dark orange.

Male system

It consists of a pair of bilobed yellowish testes, with their ducts leading to a bilobed yellowish seminal vesicle, a pair of accessory glands, an ejaculatory duct, and a seminal pump (Fig. 8).

One of the lobes of each testes is usually

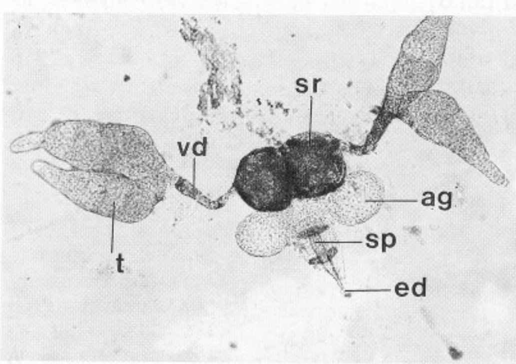

FIG. 8. Male reproductive system of *Euphyllura phillyreae* (x70). ag: accessory gland, ed: ejaculatory duct, sp: sperm pump, sr: seminal receptacle, t: testis, vd : vas deferens.

smaller and shorter than the other. The mean length of the larger lobe was 473 ± 42.9 μ (416-520) (n=10) and that of the smaller one 288.6 ± 28.6 μ (247-325) (n=10). The length of the seminal ducts was 208 ± 10.6 μ (195-221) (n=10), and of the seminal vesicle 222.3 ± 11.4 μ (208-234) (n=10).

The accessory glands are almost spherical. Their exact role in our species is not known. We expect that, same as in other insects, their secretion(s) takes part in the formation of spermatophores.

Located near the outlet of the ejaculatory duct is the seminal pump, similar to the one described by Bonnemaison et Missonier (1955) in *C. pyri.* Same as in *C. pyri,* it must push the spermatophores through the ejaculatory duct into the female reproductive system.

We have not studied in detail the genitalia of either sex in *E. phillyreae*. The only parts known are the interior side of the male's right parameres which are used to distinguish *E. phillyreae* from the other two psyllid species of the same genus that also infest the olive tree in the Mediterranean region (Lauterer et al. 1986).

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KEY WORDS: Homoptera, Psylloidea, Aphalaridae, *Euphyllura phillyreae,* olive psyllid, reproductive system.

Το Αναπαραγωγικό Σύστημα και η Ανάπτυξη των Ωοθηκών της Ενήλικης Ψύλλας της Ελιάς *Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae)

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ΠΕΡΙΛΗΨΗ

Από ελαιώνα της περιοχής Θεσσαλονίκης συλλέχθηκαν ενήλικα της ψύλλας της ελιάς *Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae) διάφορες εποχές του έτους και εξετάστηκε το αναπαραγωγικό τους σύστημα μετά από ανατομή. Το σύστημα του αρσενικού αποτελείται από δύο δίλοβους όρχεις με τους αγωγούς τους, ένα δίλοβο σπερματικό θύλακο, ένα ζευγάρι βοηθητικών αδένων, τον εκσπερματικό αγωγό και μία σπερματική αντλία. Το σύστημα του θηλυκού αποτελείται από δύο ωοθήκες, δύο πλευρικούς ωαγωγούς, ένα κοινό αγωγό, ένα διμερή βοηθητικό αδένα, μία σπερμοθήκη, ένα θύλακο άγνωστου ακόμα ρόλου και ένα κολλητήριο αδένα. Κατά την ανάπτυξη του αναπαραγωγικού συστήματος του θηλυκού, από τα τέλη του θέρους ως τα τέλη Απριλίου, διακρίθηκαν και περιγράφονται πέντε στάδια ανάπτυξης των ωοθηκών, με βάση σαφείς μορφολογικές διαφορές των ωοθηκών στο σύνολό τους και των πιό προχωρημένων σε ανάπτυξη ωοφόρων σωλήνων. Δίνονται διαγράμματα, εικόνες και διαστάσεις διαφόρων οργάνων.