

## Sites and Time of Oviposition of *Euphyllura phillyreae* Foerster on Olive Trees and on *Phillyrea latifolia*<sup>1</sup>

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### ABSTRACT

The distribution of eggs of *Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae) was recorded on twigs of olive trees and of *Phillyrea latifolia* L. in various locations of the Thessaloniki and Halkidiki prefectures of coastal northern Greece. On olive, the first eggs were observed in the last ten days of March and oviposition was intensified and became abundant only when the apical bud and the axillary leaf and flower buds started to swell. Eggs were laid mostly on swollen buds and on developing inflorescences. Oviposition preference was related to the stage of development of a bud and not to its location on the olive twig. On swollen leaf buds, most eggs were laid on the inner (upper) surface of the middle (second) pair of developing leaves, and fewer on the inner surface of the outer (first) pair and on the innermost (third) pair of leaves. On *Phillyrea*, eggs were deposited much earlier than on olive, on developing inflorescences. In choice and no-choice laboratory experiments, more eggs were laid on olive twigs bearing developing inflorescences, fewer eggs on twigs bearing only swollen terminal or axillary buds, and no eggs at all on twigs with only fully developed leaves of the previous year.

### Introduction

*Euphyllura phillyreae* Foerster (Homoptera: Aphalaridae) is the main, if not the only, psyllid species infesting olive trees (*Olea europea* L.) in northern Greece, and the most frequent species of *Euphyllura* infesting olive in central and southern continental Greece (Lauterer et al., 1986). Its geographic distribution is known to cover the Mediterranean, the Black Sea coast, and the Caucasus; it is an oligophagous insect feeding only on plants of the genera *Olea* (wild and cultivated), *Phillyrea* and *Osmanthus*, all Oleaceae.

In northern Greece *E. phillyreae* completes only one generation per year on olive and overwinters as a reproductively immature adult on the trees. Its reproduction is limited to a brief period in spring. On olive trees the eggs are laid mainly in April, on the young apical leaves, on swollen leaf and flower buds and on developing inflorescences (Prophetou & Tzanakakis, 1977; Stavradi, 1980). Lauterer et al., (1986) concluded that the species studied by Prophetou and Tzanakakis (1977) in N. Greece and found to have a single generation annually, was *E. phillyreae* although misidentified as *E. Olivina*.

In N. Greece *E. phillyreae* was also found on *Phillyrea latifolia* L. (Lauterer et al., 1986), an evergreen bushy perennial plant which blooms from March to May (Athanasias, 1986). The present paper presents the specific sites eggs are laid on various parts of olive and *Phillyrea*, and

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differences in the time of oviposition on these two host plants.

### Materials and methods

In the spring of 1981, 1982 and 1983, twigs were collected from olive trees of low-elevation locations of the prefectures of Thessaloniki and Halkidiki of northern Greece (Fig. 1). The twigs were collected at random from a height of 1-1.8 m. Depending on the location, up to three local varieties were sampled: Koroneiki, Megaritiki and Hondrolia (Halkidiki). Samples from two or more varieties of a given locality were pooled together.

Unpublished observations by the senior author had shown that no eggs are deposited basal of the first ten knots from the tip of an olive twig. Thus, only the distal 12 to 21 cm part of each twig, bearing ten pairs of last year's leaves, terminal bud, and axillary leaf or flower buds, was examined under a binocular microscope to record eggs. On the first date of sampling, March 20 of all three years, buds had not started to swell. On the following three sampling dates, March 30 and later, at least some twigs had swollen buds. In 1981 and 1982, only twigs having at least some swollen leaf or flower buds (Fig. 2A) were collected, whereas in 1983 twigs with only unswollen buds, as well as twigs with at least some swollen buds. In 1983 twigs were collected also from *Phillyrea lati-*

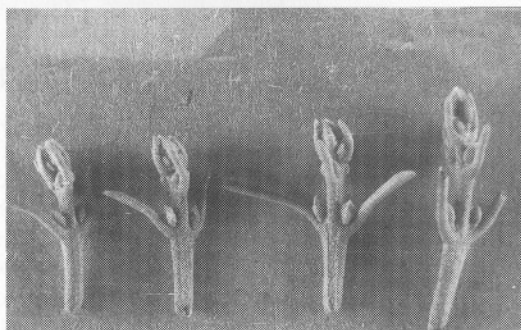
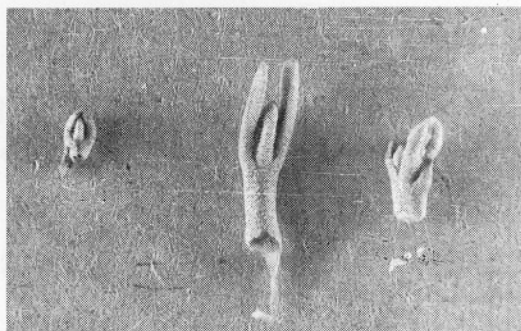


FIG. 2. A: Axillary buds of the Hondrolia olive variety as sampled on 8. IV. 1983 (x 2.64): sf: swollen (developing) flower bud; sl: swollen (developing) leaf bud; u: unswollen bud. B: Developing inflorescences as sampled on 20. IV. 1983 and used in the choice and no-choice oviposition experiments. (x 3.25).

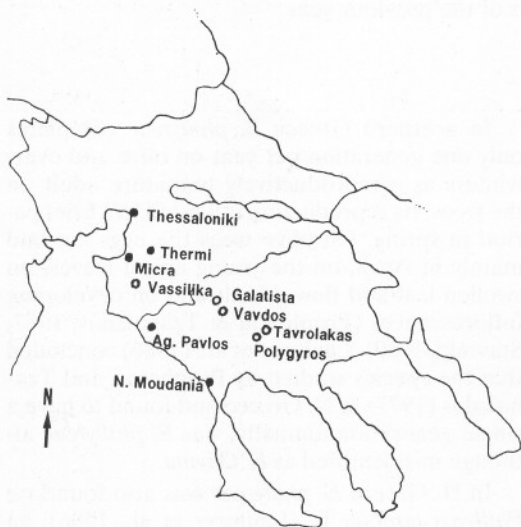


FIG. 1. Locations sampled in the prefectures of Thessaloniki and Halkidiki. Olive trees (●); *Phillyrea* (○).

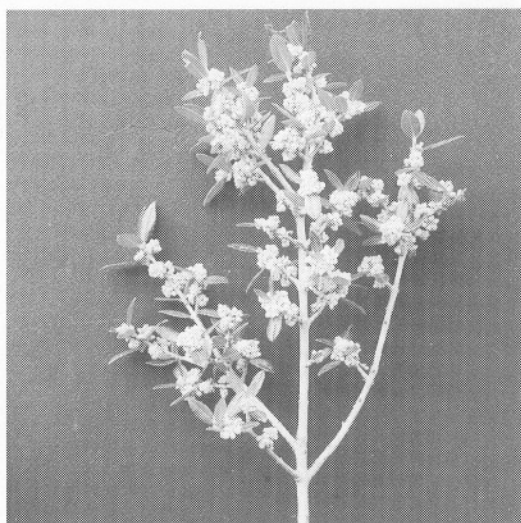


FIG. 3. Branch with twigs of *Phillyrea latifolia* bearing inflorescences as sampled on 12. VI. 1983 (x 0.34).

*folia*, (Fig. 3) in low-elevation and hilly locations of the same general area (Fig. 1).

For the laboratory choice and no-choice oviposition experiments, reproductively mature adults were collected from olive trees by beating twigs into a net. They were brought to the laboratory and maintained as described by Prophetou and Tzanakakis (1986). The adults were examined to make certain that they all belonged to the species *phillyreae*, which is the only species of *Euphyllura* we have encountered in the study area before and after our 1981-1983 survey (Lauterer et al., 1986). The laboratory experiments were carried out at 24+2°C, 65+5% RH, and natural daylight coming from a northeastern window. The photoperiod did not differ substantially from the one outdoors. The cages used in the choice experiment were wooden ones, 30 x 30 x 30 cm, with wire-screen top and sides and a glass front side facing the window. Six olive twigs, one of each treatment, as described below, and 11 pairs of adult insects were introduced in each cage. Each twig, of the variety Hondrolia, was positioned with its base in a vial with water, its free part above the vial being 6-8 cm. The eggs deposited were counted once at the end of the fourth day. In the no-choice experiment, one twig and one pair of adult insects were housed in a cylindrical 6 x 7.5 cm wide-mouth jar of transparent hard plastic, with its top side of white nylon organdie and its bottom perforated by the water vial holding the twig.

The eggs deposited were also counted once at the end of the fourth day. To count eggs, buds or inflorescences were gently removed from each experimental twig using forceps. The treatments consisted of twigs with leaves of the previous year, terminal bud and developing inflorescences (A), leaves and developing inflorescences (B), leaves, terminal bud and axillary leaf buds (C), leaves and terminal bud (D), leaves and axillary leaf buds (E) and leaves only (G). The buds, were swollen and the inflorescences in the stage of developing, as seen in Fig 2B. There were 6 replicates per treatment. Analysis of variance (ANOVA) was used to test the effect of the growth stage of the twig on the oviposition. Then the Duncan-test was used to test the differences between the mean numbers. Further details are given in the Results section.

## Results

### Field sampling, olive trees

The distribution of eggs on twigs taken at random in March and April from trees of four varieties, pooled together, is given in Table 1. In all three years, the first eggs were found in the March 30 samples. In 1983, from March 30 onwards, twigs with as well as twigs without swollen buds were sampled, while in 1981 and 1982 only

TABLE 1. Distribution of *E. phillyreae* eggs on randomly collected olive twigs of three varieties in low-elevation locations of Thessaloniki and Halkidiki, 1981-1983

Date of collection	Location	No. of twigs	Percent twigs bearing eggs	Total eggs	Mean no. eggs per twig that was	
					Collected	Oviposited
20.III. 81	Thermi	25	0.0	0	0.0	0.0
30.III. 81		25*	8.0	8	0.3	4.0
8.IV. 81		83*	0.0	0	0.0	0.0
18.IV. 81	Thermi	109*	68.8	208	1.9	2.8
20.III. 82		30	0.0	0	0.0	0.0
30.III. 82		30*	3.3	12	0.4	12.0
8.IV. 82		77*	2.6	15	0.2	7.5
18.IV. 82		40*	75.0	680	17.0	22.7
20.III. 83		30	0.0	0	0.0	0.0
30.III. 83	Thermi	34*	8.8	10	0.3	3.3
		52	0.0	0	0.0	0.0
8.IV. 83		30*	53.3	457	15.2	28.6
	Micra	42	4.8	4	0.1	2.0
8.IV. 83		26*	46.2	43	1.7	3.6
	Ag. Pavlos	30	0.0	0	0.0	0.0
8.IV. 83		25*	36.4	174	7.0	19.3
	N. Moudania	40	5.0	3	0.1	1.5
8.IV. 83		55*	38.2	327	5.9	15.6
	Thermi	40	5.0	4	0.1	2.0
18.IV. 83		38*	76.3	867	22.8	29.9
		51	5.8	7	0.1	2.3

\* These twigs had each at least some swollen leaf or flower buds. The rest, without an asterisk, had only unswollen buds.

twigs bearing at least some swollen buds. The 1983 data show that on any given sampling date, the twigs with swollen buds received many more eggs than the twigs with unswollen buds. This shows that females had a definite preference to oviposit on swollen buds. The developmental stages of buds and inflorescences on which eggs were found are seen in Fig. 2 and the location of eggs on swollen (developing) buds and developing inflorescences in Fig. 4.

In 1983, the number of eggs in each bud was also recorded. In the case there were two buds per node, the mean number of eggs per bud was calculated and presented in Fig. 5. For this reason the total number of eggs per sampling date seems to be smaller in Fig. 5 than in Table 1. It is

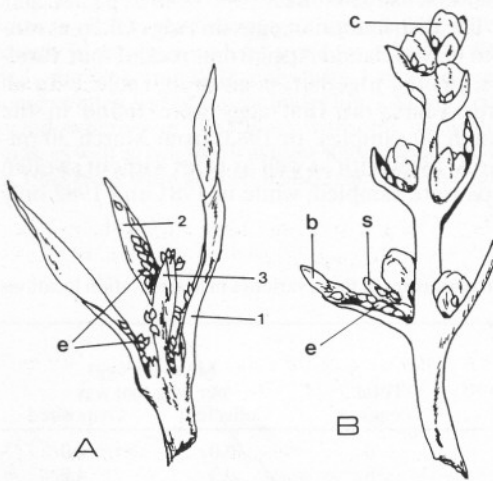


FIG. 4. Oviposition sites on swollen (developing) bud (A) and on developing inflorescences (B) of the Hondrolia olive variety, as recorded in April 1983 (x 9). 1: leaf of the first (outer) pair; 2: leaf of the second pair; 3: leaf of the third (inner) pair. b: bract; c: corolla; e: eggs; s: sepal. One leaf of the second pair of the terminal bud was removed to show the eggs on the inner pair of leaves.

seen (Fig. 5) that on the first two dates the eggs were found only or mostly on the terminal bud (T) and on the buds of axillae 1, 2 and 3, while on April 18 they were more or less evenly distribut-

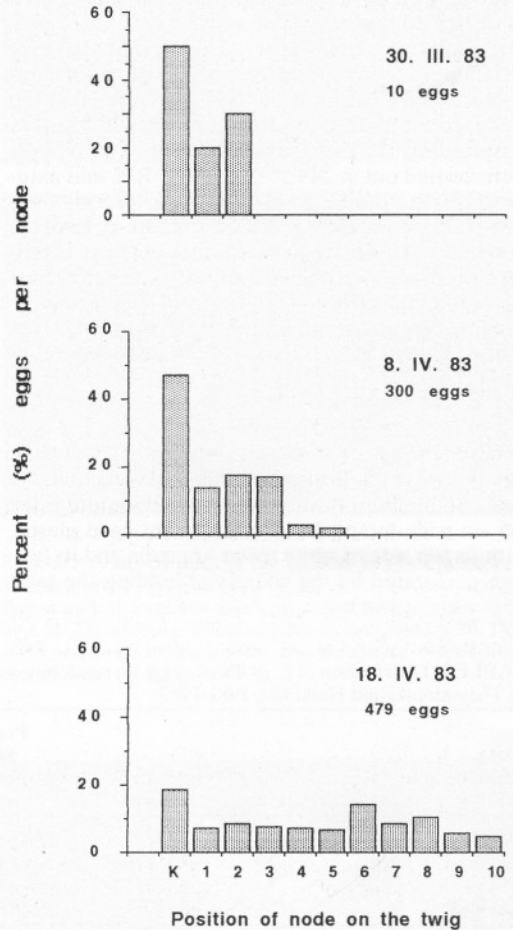


FIG. 5. Egg distribution on olive twigs of the varieties Megaritikiki and Hondrolia in the spring of 1983 in Thermi. K: terminal bud; 1: next-to-the-terminal pair of axillary buds, and so on.

TABLE 2. Distribution of eggs of *E. phillyreae* in various parts of developing terminal and axillary buds, collected at random from olive trees of the variety Hondrolia in Thermi, Thessaloniki

Date	Buds with eggs	Total eggs	Percent eggs per pair of bud leaves		
			Outer pair	Middle pair	Inner pair
30. III. 83	2	10	30.0	70.0	0.0
8. IV. 83	44	353	7.9	78.5	13.6
18. IV. 83	93	519	1.2	82.4	16.4

\* See Fig. 5.

ed along the whole twig. On this last sampling date, swollen leaf and flower buds were found on all the axillas, from 1 to 10. This was not the case on the previous two sampling dates. Of the buds bearing eggs on April 18, 12 were terminal, 29 pairs were axillary leaf buds, and 6 pairs were axillary flower buds.

Fig. 4 shows where the eggs are deposited on a developing leaf bud and on a developing inflorescence. Of the 882 eggs found on leaf buds of variety Hondrolia (Table 2), whether terminal or axillary, most were laid on the inner (upper) surface of the middle (second) pair of developing leaves. Fewer eggs were laid on the inner surface of the outer (first) pair of leaves and on either side of the inner (third) pair of leaves. Of the leaves of the middle pair, 37.2% had 1-3 eggs each, 39.4% had 4-6, 13.9% had 7-9, 7.3% had 10-12, and 2.2% had 13-15 eggs each, the mean being  $5.1 \pm 3.03$  eggs per leaf.

#### Field sampling, *Phillyrea*

Oviposition on *Phillyrea latifolia* was earlier than on olive, not only in the low-elevation locations of Vasilika but also in the hilly and cooler ones of Galatista, Tavrolakas, Polygyros and Vavdos. In late March 1983, while olive flower buds had not yet started to swell and no oviposition taken place on olive, *Phillyrea* on Seih Sou (Thessaloniki) were already in full bloom, egg

hatch had been completed and immatures were in the third and fourth instar. Two weeks later, samplings in other locations, mostly of a higher elevation, gave the data seen in Table 3. It is worth noting, that on that date all females had ovaries at stages IV and beyond. It is seen that on plants which had no developing inflorescences, no eggs were laid, despite the fact that most females collected from those plants had oocytes in the last stage (V) of development, ready to be laid. On the contrary, in locations where the plants had developing inflorescences, eggs were recorded, and where the plants were in full bloom egg hatch had been completed and the larvae were in various instars, including the last one.

A total of 300 eggs were found on 79 oviposited still closed flowers. The eggs were found on the sepals and the outer surface of the still closed corolla, as well as on the inner surface of bracts. Of all flowers on which oviposition occurred, 51.9% had 1-3 eggs each, 39.2% had 4-6, 7.6% had 7-9 and 1.3% had 10-12 eggs each, the mean number being  $3.9 \pm 1.82$  eggs per flower.

#### Oviposition preference in the laboratory

Both choice and no-choice experiments (Table 4) show that significantly more eggs were laid

TABLE 3. Adult female ovarian condition and immature stages of *E. phillyreae* recorded on *Phillyrea* in various locations of Halkidiki on 12.IV. 1983. Twenty four adult females and 4 infested *Phillyrea* twigs per location.

Location	Altitude in m	Exposure of site	Neighbouring perennial vegetation	Developmental stage of <i>Phillyrea</i>	Percent females with ovaries in stages		Presence (+) or absence (-) of immatures of the filial generation					
					IV	V*	Eggs	L1	L2	L3	L4	L5
Polygyros A	500	Northern	<i>Arbutus andrachne</i> <i>Erica verticillata</i> <i>Pistacia terebinthus</i>	Without developing inflorescences	33.3	66.7	-	-	-	-	-	-
Vavdos	938	Southern	<i>Quercus coccifera</i>	Without developing inflorescences	10.0	90.0	-	-	-	-	-	-
Tavrolakas	480	Northern	<i>Q. coccifera</i>	With developing inflorescences	0.0	100.0	+	-	-	-	-	-
Polygyros B	500	Southern	<i>Q. coccifera</i> <i>Q. pubescens</i>	With developing inflorescences	0.0	100.0	+	-	-	-	-	-
Polygyros C	500	Northern	<i>A. andrachne</i> <i>E. verticillata</i> <i>P. terebinthus</i>	In full bloom	0.0	100.0	+	+	+	+	+	-
Galatista	450	Southern	<i>Q. coccifera</i>	In full bloom	0.0	100.0	-	-	-	+	+	+
Vasilika	70	Southern	<i>Q. coccifera</i>	In full bloom	0.0	100.0	-	-	-	-	+	+

\* Last stage of oocyte maturation (fully formed oocytes).

TABLE 4. Eggs of *E. phillyreae* laid in April 1983 in choice and no-choice tests, on olive twigs of the variety Hondrolia, from which certain categories of buds were removed. (n=6).

Twigs left with	Mean percent of eggs laid $\pm$ SD	
	No-choice (1102 eggs)	Choice (1951 eggs)
A Leaves*, terminal bud**, developing inflorescences	34.5 $\pm$ 3.8 (a)***	41.8 $\pm$ 14.1 (a)
B Leaves, developing inflorescences	40.5 $\pm$ 4.1 (a)	47.7 $\pm$ 18.8 (a)
C Leaves, terminal bud, axillary leaf buds	14.9 $\pm$ 6.5 (b)	6.1 $\pm$ 11.2 (b)
D Leaves, terminal bud	5.7 $\pm$ 3.2 (c)	2.9 $\pm$ 4.3 (b)
E Leaves, axillary leaf buds	4.4 $\pm$ 5.8 (c)	1.5 $\pm$ 2.6 (b)
G Leaves only	0.0 (d)	0.0 $\pm$ 0.0 (d)

\* Fully developed, of the previous year.

\*\* Most terminal and axillary leaf buds were swollen.

\*\*\* Values on the same column followed by the same letter not significantly different from one another at 1%, Duncan-test.

on olive twigs which had developing inflorescences than on twigs which had swollen leaf buds (no-choice test:  $F_{5,30}=69.638$ , ; choice test:  $F_{5,30}=24.153$ ,  $P=0.0001$ ). There were no significant differences between twigs having terminal (D) and twigs having axillary leaf buds (E). The additional presence of either terminal or axillary buds (C) gave significantly more eggs in the no-choice but not significantly so in the choice test. We have no explanation for this. No eggs were laid on twigs which had only leaves of the previous year. No eggs were found on unswollen buds.

A total of 1971 eggs were found on 138 oviposited still closed flowers. The eggs were found on the sepals and the outer surface of the still closed corolla, as well as on the inner surface of bracts (Fig. 5B). Of oviposited flowers, bracts included, 29.7% had 1-3 eggs each, 19.4% had 4-6, 17.4% had 7-9, 12.3% had 10-12, 8% had 13-15, 2.2% had 16-18, 2.9% had 19-21, and 8% had 22-30 eggs each.

## Discussion

In interpreting the results of field sampling, we should bear in mind that the counts in the figures and tables are in essence cumulative, each count including the eggs laid to that date. The field samplings showed that, in all three years, oviposition on olive trees started in the last ten days of March and became intense only when the apical bud and the axillary leaf or flower buds started to swell, sometime between late March and mid April. Once buds started to swell, eggs were laid only on swollen buds and not on still unswollen ones of the same twig. On leaf and flower buds,

oviposition preference was not related to a certain site (axilla) of the twig. For example, on March 30, the eggs were laid only on the distal axillae which then had swollen buds. Later, on April 18, when all the buds along the twig were swollen, the buds of all axillae were found oviposited, oviposition having been extended also to the basal buds of the twig. Therefore, when buds at a suitable developmental stage are available on all or most axillae along the twig, there seems to be no preference for distal versus basal axillary buds. This is one of the findings of the present work. The laboratory choice and no-choice experiments showed a preference for developing olive inflorescences as compared to swollen leaf buds.

In the area sampled, the reproductive diapause of *E. phillyreae* is known to be terminated for most females overwintering on olive trees after mid December, and for those overwintering on *Phillyrea* much earlier in mid November (Prophetou-Athanasiadou, 1993). Most females on olives are known to have mature oocytes, ready to be laid, in mid March or shortly thereafter (Prophetou and Tzanakakis, 1977; Prophetou-Athanasiadou and Tzanakakis, 1986; Prophetou-Athanasiadou, 1993). On the contrary, females on *Phillyrea* have mature oocytes much earlier, in mid February and, in certain locations on earlier-blooming shrubs, even in late December. Also, eggs on *Phillyrea* are laid much earlier than on olive, as observed by Prophetou-Athanasiadou (1993), who concluded that the time of oviposition differs between olive and *Phillyrea* and coincides with the proper stage of growth of the host plant on which the adults reside. Our data pro-

vide both field and laboratory experimental evidence in support of this conclusion. They also show that preference for oviposition is expressed for certain organs when at a given stage of development and that oviposition sites on a plant may change with time, depending on the suitability of each organ, as it develops. The fact that, on olive, on all three dates, most eggs were laid on the second pair of leaves of the developing leaf buds, whether terminal or axillary, also supports this conclusion.

The period we recorded eggs on olive trees falls within the known periods oviposition occurs in the same general area of coastal northern Greece, as reported by Prophetou and Tzanakakis (1977). Oviposition on *Phillyrea* needs further work to be elucidated.

The infestation of *Phillyrea* by the immatures of *E. phillyreae* we observed on the Seih Sou hill, near Thessaloniki, in 1982 has many similarities with that of olive as was described by Prophetou and Tzanakakis (1977). After hatching, the young nymphs formed colonies and produced a white waxy secretion and spherical honey-dew droplets. The waxy secretion was abundant mainly during the 5th (last) nymphal stadium.

Another species of the same genus long known to infest olives and *Phillyrea* is *E. olivina* Costa. It is multivoltine in such regions as the Sfax of Tunisia, completing two spring and normally one autumn generation there. There are many similarities with our species as to the olive organs it selects for oviposition as given by Silvestri (1934) and supplemented by Arambourg (1964). It is not known, however, which are the preferred oviposition sites on each particular organ and the pattern of egg distribution for females of each of the spring and autumn generations of *E. olivina*.

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**KEY WORDS:** Homoptera, Aphalaridae, *Euphyllura phillyreae*, *Euphyllura olivina*, *Phillyrea latifolia*, olive trees, oviposition, egg distribution

## Θέσεις και Εποχή ωτοκίας του *Euphyllura phillyreae* Foerster σε ελαιόδενδρα και σε *Phillyrea latifolia*

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### ΠΕΡΙΛΗΨΗ

Σε κλαδίσκους ελαιοδένδρων και *Phillyrea latifolia* L. διαφόρων τοποθεσιών των νομών Θεσσαλονίκης και Χαλκιδικής, έγινε καταγραφή της κατανομής των αυγών του *Euphyllura phillyreae* Foerster (Homoptera, Aphalaridae). Σε ελαιόδενδρα, τα πρώτα αυγά παρατηρήθηκαν το τελευταίο δεκαήμερο του Μαρτίου και η ωτοκία εντάθηκε μόνον όταν ο κορυφαίος και οι πλάγιοι φυλλοφόροι ή ανθοφόροι οφθαλμοί άρχισαν να φουσκώνουν. Μόνο σε φουσκωμένους οφθαλμούς και σε αναπτυσσόμενες ανθοταξίες διαπιστώθηκαν αυγά. Τα θηλυκά απέθεσαν λίγα μόνο αυγά σε μη φουσκωμένους οφθαλμούς, δηλαδή σε οφθαλμούς των οποίων δεν είχε αρχίσει η έκπτυξη. Η προτίμηση για ωτοκία είχε σχέση με το στάδιο ανάπτυξης του οφθαλμού και όχι με τη θέση του στον κλαδίσκο. Στους φουσκωμένους οφθαλμούς, τα πλείστα αυγά αποτέθηκαν στην εσωτερική (άνω) επιφάνεια του μεσαίου (δεύτερου) ζεύγους των αναπτυσσόμενων φύλλων και λιγότερα στην εσωτερική επιφάνεια του πρώτου (εξωτερικού) ζεύγους και στο εσωτερικό (τρίτο) ζεύγος. Στη *Phillyrea*, τα αυγά αποτέθηκαν πολύ ενωρίτερα από ότι στα ελαιόδενδρα, σε εκπτυσόμενες ανθοταξίες.

Σε εργαστηριακά πειράματα με επιλογή και χωρίς επιλογή, περισσότερα αυγά αποτέθηκαν σε κλαδίσκους ελιάς που είχαν αναπτυγμένα φύλλα του προηγούμενου έτους και αναπτυσσόμενες (εκπτυσόμενες) ανθοταξίες, λιγότερα σε κλαδίσκους που είχαν φύλλα και φουσκωμένους ακραίο ή πλάγιους οφθαλμούς και κανένα σε κλαδίσκους που είχαν μόνο αναπτυγμένα φύλλα.