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# New data on the scale insect pests of *Ephedra foeminea* in Greece

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**Summary** The dioecious gymnosperm self-sown ornamental plant *Ephedra foeminea* Forssk (Ephedraceae: Gnetales) has a special interest due to its nutraceutical and pharmaceutical properties. The present study contributes with data about the phenology of the plant in Attica, Greece, and the complex of its scale insect pests (Hemiptera: Coccomorpha), *Stotzia ephedrae* (Newstead) (Coccidae) (morphology, phenology, biology, natural enemies), *Dynaspidiotus ephedrarum* (Lindinger) (morphology, natural enemies) and *Leucaspis riccae* Targioni Tozzetti (Diaspididae) (natural enemies), based on observations made from April 2021 to June 2023. *Stotzia ephedrae* which was the scale species found in higher numbers on *E. foeminea* is a univoltine, oviparous and biparental species recorded as settled 1st instar nymph on the shoots of *E. foeminea* from May to January. The fecundity of the scale fluctuated between 370 and 598 eggs per female. The results on the phenology of *S. ephedrae* contribute to the knowledge of the critical period for the pest control, i.e. from May to January during which the scale remains in the sensitive first instar. In addition, the records of parasitoids and predators in the colonies of the scale insects infesting *E. foeminea* provide information on the available natural enemies for potential use in biological control schemes of these pests.

*Additional keywords*: ecology, parasitism, phenology, predators, scale insects

## Introduction

*Ephedra foeminea* Forssk (Ephedraceae: Gnetales) is a gymnosperm dioecious endemic plant species to the Mediterranean region. It is a plant of special interest. Among the ten species of genus *Ephedra, E. foeminea* and *E. aphylla* Forssk. are the two insect pollinated species by Diptera and Lepidoptera, many of them being nocturnal able to pollinate the plants during the full moon of July (Bolinder *et al.,*2015a; 2015b; 2016). A correlation is referred between its pollination period and the lunar cycle (Bolinder *et al.,* 2015b; Rydin and Bolinder, 2015). Additionally, the bibliography supports evidence for its medicinal properties, composing a promising source of new selective antioxidant and anti-cancer drugs (Rydin and Bolinder, 2015; Mpingirika *et al.,* 2020; Al-Saraireh *et al.,* 2021), as well as for its nutraceutical value especially of the fruits (Hegazy *et al.,* 2019; Khalil *et al.,* 2020).

Twenty-five (25) scale insect species (Hemiptera: Coccomorpha) have been reported in genus *Ephedra*: four of the family Coccidae, 15 of Diaspididae, five of Eriococcidae and one of Pseudococcidae. Records of scale insects in genus *Ephedra* in Greece include: the diaspidids (Hemiptera: Diaspididae) *Leucaspis (ephedrae) riccae* Targioni Tozzetti on *E. (vulgaris) distachya* in Athens and on *E. (campylopoda) foeminea* in Athens (on Lecabettus hill) and in Ano Lechonia (Magnessia) (Koroneos, 1934); *Dynaspidiotus (Aspidiotus) ephedrarum* (Lindinger) on *E. (campylopoda) foeminea* in Athens (on Lecabettus hill) (Koroneos, 1934); *Dynaspidiotus ephedrarum* on *Ephedra distachya* in East Attica (near Athens - Kato Sounio) (Szita, *et al.,* 2017); the coccid (Hemoptera: Coccidae) *Stotzia ephedrae* (Newstead) on *E. foeminea* in Athens (on Lecabettus hill) (Stathas *et al.,* 2023). Reports of other scale insects on *E. foeminea* in other countries include *Diaspidiotus cecconii* (Leonardi) in Palestine and

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*Diaspidiotus distinctus* (Leonardi) in Turkey (García Morales *et al*., 2016)

In April 2021, plants of *E. foeminea* were found infested by a complex of three species of scale insects: *S. ephedrae* (Stathas *et al.,* 2023), *D. ephedrarum* and *L. riccae*.

Host plants of *S. ephedrae* belong to six plant genera of five families: *Bupleurum* (Apiaceae), *Asparagus* (Asparagaceae), *Ephedra* (Ephedraceae), *Coronila* and *Genista* (Fabaceae) and *Tamarix* (Tamaricaceae) (García Morales *et al.* 2016). *Dynaspidiotus ephedrarum* has been recorded worldwide to infest three plant genera of three families: Asparagaceae (*Asparagus* sp.), Ephedraceae (*Ephedra alata*, *E. altissima*, *E. distachya*, *E. foeminea*, *E. foliate*, *E. major*, *E. procera,* and *E. tilhoana*) and Pinaceae (*Pinus sylvestris*). *Leucaspis riccae* has been found on 6 plant genera of 6 families: Apocynaceae (*Nerium oleander*), Ephedraceae (*Ephedra alata*, *E. distachya*, *E. vulgaris*, *E. foeminea, E. campylopoda*, and *E. fragilis*), Ericaceae (*Erica arborea, E. manipuliflora*, Euphorbiaceae (*E. dendroides*), Moraceae (*F. carica*) and Oleaceae (*Olea europaea*) (García Morales *et al.,* 2016).

Morphological description of female adults of *S. ephedrae* is available by Ezzat and Hussein (1969) and by Hodgson (1994) but there is lack of information on biology and phenology of the scale in bibliography. Also concerning its natural enemies, the only records are the endoparasitoids *Metaphycus stephaniae* sp. nov. Guerrieri (Hymenoptera: Encyrtidae) in Israel (Guerrieri, 2006) and *Metaphycas hodzhevanishvilii* Yasnosh (Hymenoptera: Encyrtidae) in Iran (Fallahzadeh and Japoshvili, 2017).

In the present study, data on morphology of the developmental stages and on biology, phenology and natural enemies primarily of *S. ephedrae,* and to an extent of *D. ephedrarum,* are provided. *Dynaspidiotus ephedrarum* and *L. riccae* were found in low populations, therefore it was not possible to have clear information on their phenology, but their records were considered important for completing the complex of the existing scale insects entomofauna of *E. foeminea* in the area of the study. Furthermore, the records of active natural enemies against *D. ephedrarum* and *L. riccae,* contribute to the knowledge of their ecology and could possibly partly explain their weakness to develop high populations.

## Materials and Methods

The study was conducted with scales recorded on *E. foeminea* in Athens (Lecabettus Hill: 37º58΄ N, 23º44΄ E, altitude: 270m) from 20 April 2021 to 5 June 2023. The phenological stages of the host plant *E. foeminea* were recorded weekly, including the dates of appearance of the new vegetation and the blossom of *E. foeminea* bushes.

To study the morphology of *S. ephedrae*, measurements (length, width) were made in the laboratory under a stereoscopic microscope on 20 individuals of each developmental stage, except for crawlers: eggs, settled 1st instar nymphs, 2nd instar nymphs, the scale cover of the male adult nymphs, preovipositing female adults ovipositing female adults (female body with ovisac included). For *D. sephedrarum*, the same dimensions were measured in 12 individuals from each of the following stages, 2nd instar nymphs (19/2/2022), female adults and scale covers of males (15/10/2022). The mean number of the scales' length and width and Standard Error (SE) were calculated. Permanent slides of the scale insect species were made in the Laboratory of Entomology and Agricultural Zoology of the Department of Agriculture of the University of the Peloponnese, where identified. The confirmation of the scale species was made by Professor Giuseppina Pellizzari (Dipartimento di Agronomia, Animali, Alimenti), University of Padua, Italy. Vouchers of permanent slides of the scale insect species are kept in the Laboratory of Agricultural Entomology, Department of Agriculture, University of the Peloponnese.

To study the phenology of *S. ephedrae*, samples of 20–30 infested shoots of approx. 15 cm length, to ensure adequate populations of more than 300 individuals of *S. ephedrae* per sampling, were collected weekly.

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The samples were transferred to the Laboratory of Entomology (Department of Agriculture, University of the Peloponnese) and were examined under the stereoscopic microscope. The developmental stage and the numbers of all the alive *S. ephedrae* individuals were recorded and were expressed as percentage (%) of the total alive scales.

The biological parameters of *S. ephedrae* studied were the fecundity and sex ratio. The fecundity was measured by counting the total number of eggs in 30 ovipositing female adults on 23 April 2022. The sex ratio was estimated on 26 March 2022, on 375 individuals of the scale, by counting the female adults and the total number of males (alive and dead male nymphs + empty male scale covers).

The records of natural enemies included observations for the presence of predators in the field during the sampling of the aforementioned infested shoots and in the laboratory during the examination of the samples under the stereoscopic microscope. The parasitized scales that were found during the examination of the above samples were kept in cylindrical glass vials (diameter: 2.5 cm, height: 5cm covered by organtine) which were kept in incubators under controlled conditions (65-70% humidity and photoperiod 16:8 hours light per day) until the emergence of parasitoids. The parasitism was expressed as percentage (%) of parasitized scales out of the total number of the alive scales. The identification of the parasitoid of genus *Metaphycus* was made by Professor George Japoshvili (Entomology and Biocontrol Research Centre Agrarian), University of Tbilisi, Georgia.




**Figure 1.** Pollination drops on male (a) and female (b) blossoms of *Ephedra foeminea* in Athens (Lecabettus Hill), Greece.

## Results

### Morphology, phenology and biology traits of the scales

As regards the phenological stages of *E. foeminea*, sprouts of the new vegetation were grown on 10/4/2021, 17/4/2022, and 26/3/2023. From the end of August to the middle of October 2021 the bushes were significantly dehydrated but recovered their vitality until the next period. Blooming occurred during 10-17/7/2021, 1-23/7/2022 and 21/9/2022 – 17/10/2022. The secretion period of pollination drops on the female and male blossoms in the year 2022 were recorded during 9-16/7/2022 and 2-13/10/2022 (Fig. 1). The full moon of July and October 2022 occurred on 13/7/2022 and 10/10/2022, respectively.

Regarding the morphology of *S. ephedrae,* the settled 1st instar has an elliptic and thin flat shape with a light brownish color (Fig. 2a). The 2nd instar nymph of female has a darker brownish color, elliptic shape, with a longitudinal carina that arises on median site of the dorsum (Fig. 2b). The male 2nd instar nymph develops a white thin waxy cover (Fig. 2c). The pre-ovipositing female adult get a dark olive-green color secreting honeydew from dorsum (Fig. 3a). The color of

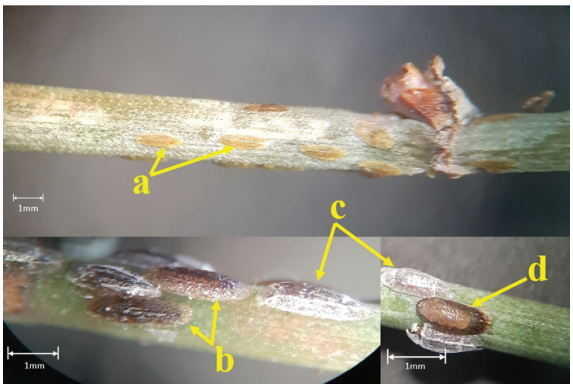



**Figure 2.** Nymphs of *Stotzia ephedrae* on *Ephedra foeminea*: (a) settled 1st instar nymphs, (b) 2nd instar nymphs of females, (c) 2nd instar nymphs of males, (d) larva of parasitoid in 2nd instar nymph.

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the body of the ovipositing female is purple brownish (Fig. 4a). The ovipositing female adult creates a white convex waxy ovisac that covers the eggs and the whole body of the female (Fig. 4b). The male adult is similar with the female in color and has two long waxy filaments in abdomen (Fig. 3b). Fig. 3 shows the empty male scale cover (3c) and male adult under the male scale cover before its emergence (3d). The eggs are oval ellipsoid light purple-reddish, laid under the ovisac to the posterior part of the body of the female (Fig. 4a). The crawler is elliptic, rather flattened with a slightly lighter tint than the eggs (Fig. 4b). The dimensions of the developmental stages of the scale are presented in Table 1.

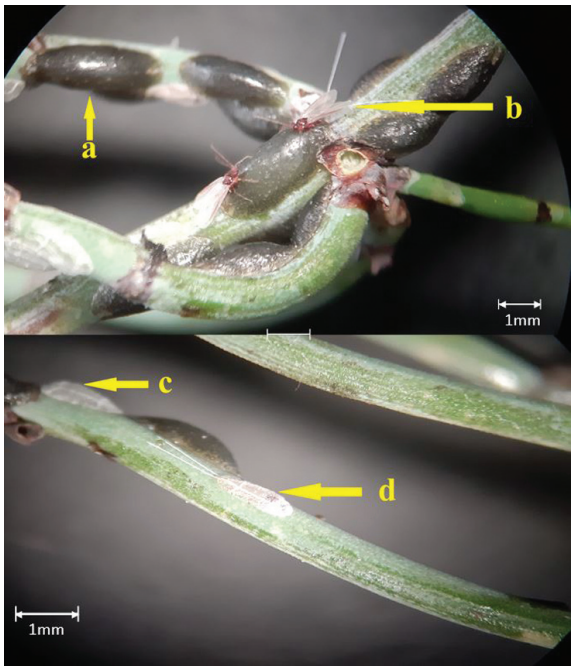



**Figure 3.** *Stotzia ephedrae* adults on *Ephedra foeminea:* (a) preovipositing female, (b) male adult, (c) empty male scale cover and (d) male adult under the scale cover before its emergence.

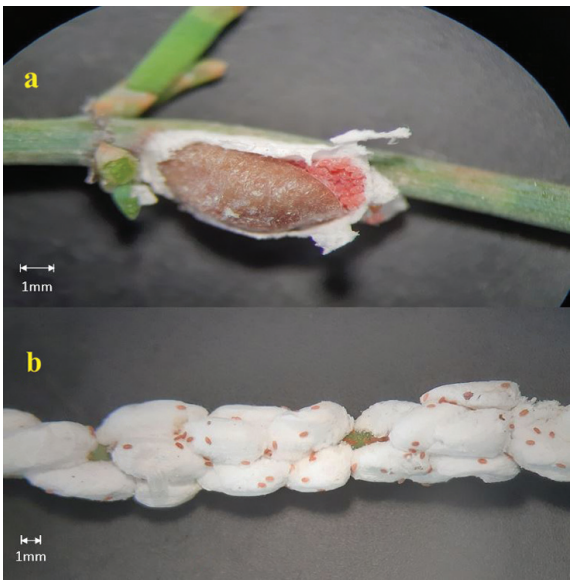



**Figure 4.** Ovipositing female adults of *Stotzia ephedrae* on *Ephedra foeminea*: (a) the body of ovipositing female, (b) female adults covered by their scale covers, and hatched crawlers.

**Table 1.** Dimensions (mm) of the developmental stages of *Stotzia ephedrae* (n=20).

| Developmental stage | Length<br>Mean ± SE<br>(min – max) | Width<br>Mean ± SE<br>(min-max) |
|---|---|---|
| Egg | 0.22 ± 0.001<br>(0.21-0.22) | 0.14 ± 0.003<br>(0.12-0.15) |
| Settled 1st instar nymph | 1.48 ± 0.03<br>(1.21-1.87) | 0.63 ± 0.02<br>(0.43-0.76) |
| 2nd instar nymph | 2.08 ± 0.09<br>(1.62-3.10) | 0.86 ± 0.03<br>(0.60-1.06) |
| Preovipositing female adult | 3.46 ± 0.09<br>(3.10-4.58) | 1.39 ± 0.02<br>(1.20-1.52) |
| Ovipositing female<br>(including ovisac) | 5.80 ± 0.09<br>(5.24-6.41) | 3.15 ± 0.09<br>(2.43-3.75) |
| Male adult | 1.27 ± 0.01<br>(1.10-1.35) | 0.44 ± 0.02<br>(0.32-0.56) |

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*Stotzia ephedrae* is an oviparous biparental species. The recorded sex ratio (females/males) was 52.8/47.2. The eggs laid by females fluctuated between 370 to 598 per female. The mean (± SE) was 493.13 ± 15.90 eggs per female. The percentages of the developmental stages of *S. ephedrae* in the sampled shoots are presented in Fig. 5. As it is shown in the Figure, the hatching of crawlers was recorded in May (2021, 2022 and 2023). The settled 1st instar nymphs were present until the end of January (2022, 2023), thus this instar covers the longest period of the year, comparatively with the other stages of the scale. The 2nd instar nymphs were recorded from the end of December 2022 to the end of February 2023 and from the end of December 2022 to the beginning of March 2023. The immature stages of males (nymphs under their cover) were recorded between the end of February 2022 to the end of March 2022 and by the middle of February 2023 to middle of March 2023. Pre-ovipositing females were recorded from the beginning of March 2022 to the beginning of April 2022 and from the end of February 2023 to the beginning of April 2023. Ovipositing females were recorded from the start of the study (20/4/2021) to the end of April 2021, from the end of March 2022 to the end of April 2022 and from the middle of March to the middle of April 2023. Until the crawler hatching the ovisacs remained settled on the host plant, containing the dehydrated body of the dead females under their frontal portion and alive eggs under the posterior portion (Fig. 5). The mean number of *S. ephedrae* eggs per female was 493.13 ± 15.90.

*Stotzia ephedrae* is settled only on the shoots of *E. foeminea* and the development of the scales is not affected by the development of the shoots. The infestation by *S. ephedrae* damages the plant by sucking the sap, dehydrating the shoots (Fig. 2) and by the secretion of honeydew by the preovipositing female adults, which encourages the growth of sooty molds and obstructs photosynthesis.

*Dynaspidiotus ephedrarum* is an oviparous biparental species. The dimensions of the 2nd instar nymphs, of the scale covers of male adults and of female adults, are presented in Table 2. The scale cover of female adult is convex white to grey - white, with a brownish subcentral exuvia (Fig. 6a). The scale cover of male has an oval shape and white color with brownish subcentral (Fig. 6b). The developmental stage of *E. foeminea* does not affect the development of *D. ephedrarum*, but its infestation damages the host plant by sucking the sap and drying the shoots (Fig. 6).

**Natural enemies of the scales**

Regarding the presence and action of natural enemies, larvae of an endoparasitoid *Metaphycus* sp. (Hymenoptera: Encyrtidae) (Fig. 2d) were found in 1st and 2nd instar nymphs of *S. ephedrae*. The percentage of parasitism in the total population of the scale is presented in the Figure 7. As it is shown in this Figure, the parasitism fluctuated from 1.1 to 14%. The majority of the parasitized nymphs (up to 80%) were mainly found near to the internal basal side of the leaves. Smal number of individuals of the coccophagus predators *Chilocorus bipustulatus* (L.) and *Exochomus quadripustulatus* (L.) (Coleoptera: Coccinellidae) were observed sporadically in the colonies of *S. ephedrae* during the period of the study: three adults and two larvae of *C. bipustulatus* (10/6/2021), 2 adults *C. bipustulatus* (16/7/2022), and 2 adults of *E. quadripustutatus* (20/9/2023).

In the case of *D. ephedrarum*, predated scales by three adults of the predator *Cybocephalus fodori* Endrödy-Younga (Coleoptera: Cybocephalidae) were recorded on 10/6/2022, as well as one larva and two adults on 27/3/2023. Also, parasitism of female adults was recorded by an unidentified endoparasitoid species. Encapsulated eggs (1-2 per female adult), and parasitoid exit holes (1-3 per female adult scale cover) were found (Fig. 6).

Regarding *L. riccae,* an unidentified ectoparasitoid was found to parasitize the female adults of the scale (Fig. 8).

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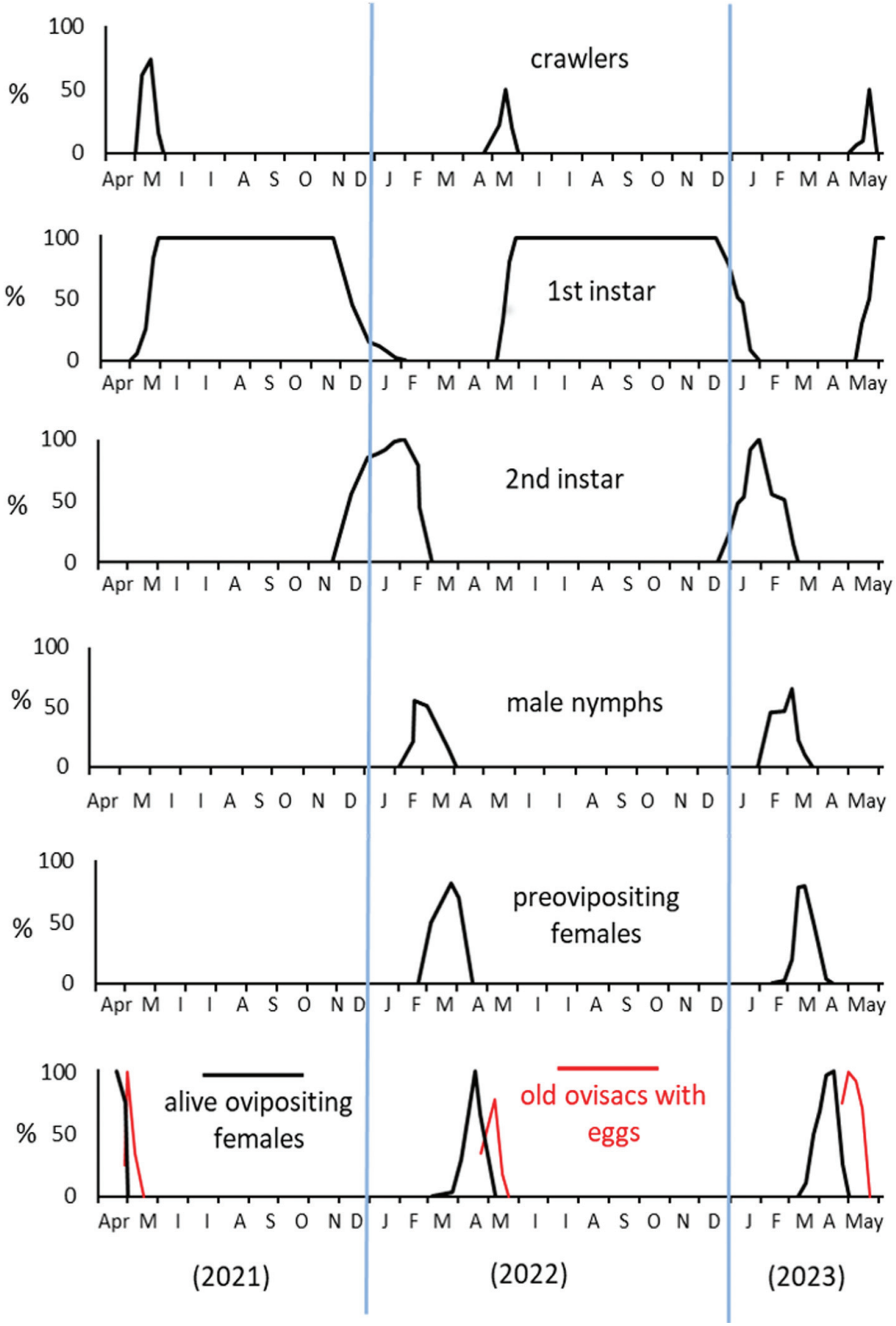



**Figure 5.** Percentage (%) of the developmental stages of *Stotzia ephedrae* on *Ephedra foeminea* from 20 April 2021 to 5 June 2023, Athens (Lecabettus Hill), Greece.

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**Table 2.** Dimensions (mm) of the developmental stages of *Dynaspidiotus ephedrarum* (n=12).

| Developmental stage | Length<br>Mean ± SE<br>(min-max) | Width<br>Mean ± SE<br>(min-max) |
|---|---|---|
| 2nd instar nymph | 1.14 ± 0.02<br>(1.05-1.22) | 0.83 ± 0.04<br>(0.62-0.97) |
| Ovipositing female<br>(with scale cover) | 1.88 ± 0.07<br>(1.56-2.30) | 1.18 ± 0.01<br>(1.13-1.21) |
| Scale cover of male adult | 1.31 ± 0.04<br>(1.14-1.49) | 0.62 ± 0.01<br>(0.60-0.66) |

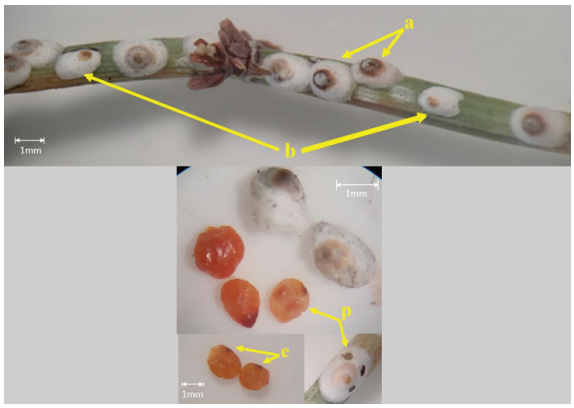



**Figure 6.** *Dynaspidiotus ephedrarum* infestation on *Ephedra foeminea*: (a) preovipositing female adults, (b) male nymphs, (e) encapsulated eggs by endoparasitoid in the body of female adults, (p) larvae (up) and exit holes (down) of endoparasitoid of the scale.

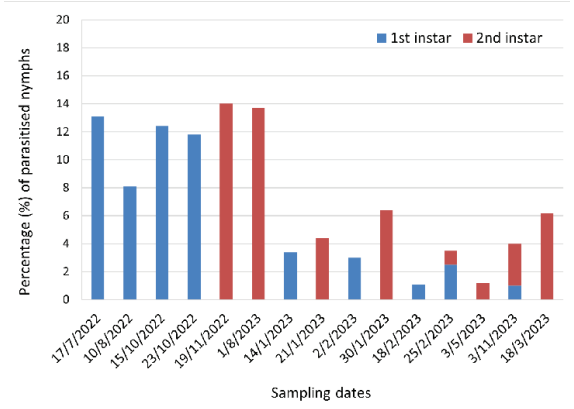



**Figure 7.** Percentage (%) of parasitism of 1st and 2nd instar nymphs of *S. ephedrae* by *Metaphycus* sp. during the period of the study.




**Figure 8.** *Leucaspis riccae* on *Ephedra foeminea*: (a) female nymph, (b) larva of ectoparasitoid under the scale cover of female scale nymph.

## Discussion

The average number of eggs laid by *S. ephedrae* females was 493.13 ± 15.90 eggs per female. This number could be considered as indicative of the fecundity of the scale and cannot represent its total fecundity, as more eggs could be laid by the examined females after the date of the sampling (23 April 2022). *Stotzia ephedrae* completes one annual generation in the area of the present study. From the beginning of May to the end of January

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was recorded as settled 1st instar nymph, although the weather conditions of this period are suitable for the development of scale insects to next instars in Greece. This could indicate that this instar enters diapause. Similar behavior with a long period of diapause of the 1st instar is also recorded to other scale insect species in Greece, e.g. *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccomorpha: Coccidae) infesting *Citrus sinensis* from the beginning of June to the beginning of the next April in Athens (Stathas and Karipidis, 2020), as well as *Kermes echinatus* Balachowsky (Hemiptera, Coccomorpha: Kermesidae) on *Quercus ilex* from the end of June to mid-April of the following year in Peloponnese (Stathas *et al.,* 2018). Some minor differences recorded on the presence of the several developmental stages of *S. ephedrae* could be related with differences of climatic conditions of each year during the period of the study.

The size of female (1.88 mm length, 1.18 mm width) and male covers (1.31 mm length, 0.62 mm width) of *D. ephedrarum* are similar to those found in other studies, e.g. according to Lindiger female scales are 1.5 mm in diameter (García Morales *et al.,* 2016) while according to Balachowsky the female scale has a diameter of 2 – 2.2 mm and the scale cover has a length 1.4 - 1.5 mm (García Morales *et al.,* 2016).

The *Metaphycus* endoparasitoid of *S. ephedrae* could not be identified in species level. Nevertheless, two *Metathycus* endoparasite species of *S. ephedrae* are known: *M. stephaniae* sp. nov. in Israel (Guerrieri, 2006) and *M. hodzhevanishvilii* in Iran (Fallahzadeh and Japoshvili, 2017). The coccinellid predators *C. bipustulatus* and *E. quadripustulatus* are common coccophagus species worldwide, e.g. *Chilocorus bipustulatus* has been recorded as a natural enemy of 76 scale insect species and *E. quadripustulatus* on 40 species (García Morales *et al.,* 2016).

In the present study, parasitism of female adults of *D. ephedrarum* and *L. riccae* was found by an unidentified endoparasitoid and ectoparasitoid, respectively. Reports for parasitoid species of *D. ephedrarum* include *Coccobius sybariticus* Pedata (Hymenoptera: Aphelinidae) (Pedata, 1999), while for *L. riccae,* the aphelinids *Aphytis libanicus* Traboulsi and *A. mytilaspidis* (Le Baron) (Hymenoptera: Aphelinidae) (Rosen and DeBach, 1979). The coccophagus predator *C. fodor,* found in low populations in the colonies of *D. ephedrarum*, could also be considered as a natural enemy of the scale. In fact, *C. fodori* is referred as predator of five scale insect species of the family Diaspididae in Turkey and Greece: *Comstockaspis perniciosa* (Comstock) in Antalya, Turkey (Erler and Tunç, 2001), *Diaspis echinocacti* (Bouché) in Kalamata, Greece (Stathas *et al.,* 2021), *Parlatoria pergandii* Comstock in Leonidion, Peloponnese, Greece (Stathas, 2001), *Parlatoria ziziphi* (Lucas) in Tyros, Peloponnese, Greece (Stathas *et al.,* 2007) and *Targionia vitis* (Signoret) in Kalamata, Greece (Stathas *et al.,* 2021).

The results on the phenology of *S. ephedrae* which was the scale species found in higher numbers on *E. foeminea* contribute to the knowledge of the critical period for the pest control, i.e. from May to January during which the scale remains in the sensitive instar of crawlers. In addition, the records of parasitoids and predators in the colonies of the scale insects infesting *E. foeminea* provide information on the available natural enemies for potential use in biological control schemes of these pests.

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# Νέα δεδομένα σχετικά με τα κοκκοειδή που προσβάλλουν το φυτό *Ephedra foeminea* στην Ελλάδα

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**Περίληψη** Το δίοικο γυμνόσπερμο φυτό *Ephedra foeminea* Forssk (Ephedraceae: Gnetales), είναι ένα αυτοφυές καλλωπιστικό φυτό που έχει ιδιαίτερο ενδιαφέρον. Στην παρούσα εργασία παρουσιάζονται στοιχεία για τη φαινολογία του φυτού στην Αττική, και για τα επιβλαβή για το φυτό κοκκοειδή έντομα (Hemiptera: Coccomorpha): *Stotzia ephedrae* (Newstead) (Coccidae) (μορφολογία, φαινολογία, βιολογία, φυσικοί εχθροί), *Dynaspidiotus ephedrarum* (Lindinger) (μορφολογία, φυσικοί εχθροί) and *Leucaspis riccae* Targioni Tozzetti (Diaspididae) (φυσικοί εχθροί), που εξασφαλίστηκαν από παρατηρήσεις που έγιναν από τον Απρίλιο του 2021 έως τον Ιούνιο του 2023. Το είδος *Stotzia ephedrae* σε σχέση με τα άλλα δύο είδη παρατηρήθηκε σε μεγαλύτερους πληθυσμούς επί του *E. foeminea*. Συμπληρώνει μία γενεά το έτος, είναι είδος ωοτόκο και αμφιγονικό και παραμένει εγκατεστημένο στο στάδιο της νύμφης 1ης ηλικίας στους βλαστούς του *E. foeminea*, από τον Μάιο έως τον Ιανουάριο. Η γονιμότητά του κυμάνθηκε από 370 έως 598 ωά ανά θηλυκό. Από τα αποτελέσματα της μελέτης της φαινολογίας του *S. ephedrae* φαίνεται ότι η καταλληλότερη περίοδος για την καταπολέμηση του εντόμου είναι κατά το διάστημα Μαΐου – Ιανουαρίου, καθώς το έντομο βρίσκεται στο ευαίσθητο στάδιο της νύμφης 1ης ηλικίας. Επίσης, οι καταγραφές της δράσης παρασιτοειδών και αρπακτικών εναντίον των κοκκοειδών που προσβάλλουν το *E. foeminea*, παρέχουν πληροφορίες για τους διαθέσιμους φυσικούς εχθρούς που δυνητικά θα μπορούσαν να χρησιμοποιηθούν ως παράγοντες βιολογικού ελέγχου αυτών των προσβολών.

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