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ECOSYSTEMS

New record of *Eupteryx decemnotata* (Hemiptera: Cicadellidae) in Argentina. Biological traits and description of the female and its immature stages

MORA PEREYRA, SUSANA L. PARADELL & ERICA LUFT-ALBARRACIN

Abstract: During a survey of leafhoppers (Hemiptera: Cicadellidae) associated with aromatic crops, the Ligurian leafhopper, *Eupteryx decemnotata* Rey was identified for the first time in the Platense Horticultural Belt in Buenos Aires province, Argentina. This study provides a detailed description of the female genitalia and immature stages, along with biological traits, including developmental time from egg to adult, sex ratio, oviposition sites preference and host plants. This leafhopper was reported on oregano, thyme, rosemary and mint plants. Significant damage to host plants was observed due to feeding activity, with females showing a marked preference for ovipositing in secondary veins of leaves (31.15%), followed by stems (21.31%) and main veins (19.67%). The total developmental time from egg to adult averaged 29.14 days under controlled conditions, with the fifth instar being the longest. The sex ratio was male-biased (59.49% males, 40.51% females). This study expands the known distribution of *E. decemnotata* and provides essential biological and morphological data that contribute to its taxonomic identification and pest management strategies.

Key words: aromatic crops, female genitalia, host plant, Ligurian leafhopper, nymphs, Typhlocybinae.

INTRODUCTION

The Ligurian leafhopper, *Eupteryx decemnotata* Rey (Hemiptera: Cicadellidae: Typhlocybinae), is a native species to the Mediterranean region, from where it quickly spread to several countries in central Europe through the international trade of its host plants (Maczey & Wilson 2004, Nickel & Holzinger 2006, Lubiarz & Musik 2015). In the Americas, this leafhopper was detected in California, North Carolina and Pennsylvania (Rung et al. 2009, Ciafré & Barringer 2017, Kittleberger 2018), later reported in Hawaii (Dietrich & Perreira 2019), and recently in Argentina, specifically in the province of Mendoza, where it was associated with oregano crops and the male of the species was redescribed (De Borbón et al. 2022). It is an oligophagous species that feeds on the mesophyll cells of plants belonging to the Lamiaceae family, such as lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), lesser calamint (*Calamintha nepeta* Savi), rosemary (*Rosmarinus officinalis* L.), hyssop (*Hyssopus officinalis* L.), peppermint (*Minthostachys mollis* Griseb.), basil (*Ocimum basilicum* L.), thyme (*Thymus vulgaris* L.) and common sage (*Salvia officinalis* L.) (Mazzoni & Conti 2006, Nickel & Holzinger 2006, Lubiarz & Musik 2015). Its feeding activity induces chlorotic spots on the leaves, called “stippling” (Pollard 1968), and can significantly degrade the quality of the final product (Lubiarz & Musik 2015). This species has been recorded causing significant

damage to aromatic ornamental plants in Tunisia (Chaieb et al. 2012) and oregano crops in Turkey and Greece (Arslan et al. 2012). At high population densities, the damage intensifies and can eventually result in the death of the host plant (Nickel & Holzinger 2006, Rung et al. 2009).

Traditionally, the taxonomy of Typhlocybinae has been mainly based on male morphological characters. However, in some groups, such as Agalliini (Megopthalminae) there is a long history of using the female sternum VII in identification (Oman 1933). Moreover, several studies on other Cicadellidae species have demonstrated that diagnostic features of the female genitalia are also valuable for taxonomic purposes (Carvalho & Mejdalani 2014). In recent years, the importance of ovipositor morphology and its associated structures, particularly the abdominal sternite VII, has increased, with several studies identifying numerous features of taxonomic interest. Even so, the morphological diversity of these structures remains relatively unknown compared to the current knowledge of male characteristics (Defea & Paradell 2017). Additionally, the nymph instars of *Eupteryx* Curtis species have received few taxonomic studies (Stewart 1986, Leonard & Barber 1923). Regarding the biological characteristics, studies were conducted on the immature stages of other species of the genus *Eupteryx* (Leonard & Barber 1923, Pollard 1968). However, there is limited information available regarding the biology of *E. decemnotata,* its immature stages and the effects of their activity on *Salvia officinalis* L. (Mazzoni & Conti 2006). Like other leafhoppers, *E. decemnotata* develops through five nymphal instars before reaching adulthood, with each instar exhibiting progressive morphological changes such as wing pad development and pigmentation.

In this contribution, *E. decemnotata* is recorded for the first time in Buenos Aires province, Argentina, associated with aromatic crops. We provide a comprehensive description of the female genitalia and immature stages, along with biological data, including oviposition site preferences, developmental time and sex ratio.

MATERIALS AND METHODS

Collection of specimens

Samplings were conducted biweekly from 2021 to 2022 at La Anunciación orchard in Abasto, La Plata, Buenos Aires province, Argentina (34°56'37.6'' S – 58°07'49.8'' W, elevation 18 m). Specimens were collected using an entomological net and manual aspirator from aromatic crops [oregano, thyme, rosemary and mint (*Mentha spicata* L.)]. Collected specimens were preserved in 70% ethanol and the taxonomic identification was carried out based on the studies of Dietrich & Perreira (2019) and De Borbón et al. (2022). All measurements were expressed in mm.

Leafhoppers rearing

Laboratory studies were conducted at the Laboratorio de la División Entomología del Museo, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Buenos Aires province, Argentina. A laboratory colony of *E. decemnotata* was initiated with 10 adult specimens collected from rosemary crops at La Anunciacion orchard. The insects were reared into entomological cages (30 x 30 x 60 cm) covered with voile fabric under controlled laboratory conditions (25 ± 2°C, 50-60 % RH and L12:D12 photoperiod). Mint plants were used as the host plant.

Biological traits

To assess oviposition site preferences in the different structures of the plant, the following sites were evaluated: stem, petiole, lamina, primary main and secondary vein. Five females and three males were confined inside cylindrical PET (polyethylene-terephthalate) cages (20 cm length) covered on one side with voile fabric and closed on the other side with a cotton plug. Each cage contained a mint branch approximately 15 cm in length with five young and five mature leaves (Figure S1 - Supplementary Material). After 72 hours, the insects were removed, and the branches were left for an additional 72 hours to allow for egg development before dissection. Eggs were observed and counted under a Leica ICC50W stereomicroscope, using 35x magnification. This procedure was repeated with five branches of the mint plant. The stem was kept in Petri dishes with moistened plaster until nymph emergence. The dissected eggs were measured (n=19).

To obtain nymphs, the assays were performed using 15 females and 3 males, randomly collected from the colony previously described. Each group of 15 females were placed inside cylindrical PET cages 20.5 cm long, as mentioned above. Females were allowed to oviposit on mint leaves for 24h. At the end of this period, adults were removed, and plants were maintained inside the cages until nymphs emerged. The time of hatching was registered. Each nymph was individually placed inside glass test tubes (7.5 cm long x 1 cm diameter) covered with a moistened cotton plug at the upper end. The curved end of each glass tube was pierced and covered with voile fabric to avoid water condensation. The nymphs were fed daily with fresh leaf pieces of mint and the cotton was moistened daily. Individual insects were checked daily to register ecdysis and survivorship. Fifty nymphs were monitored to determine their development time. The assay was carried out in a rearing chamber under controlled conditions of temperature and photoperiod (25 ± 2° C, 50-60 % RH and L12:D12 photoperiod).

Nymphs from each instar were taken for description and biological data (developmental time of each stage and instar) until the adult stage, at point the sex ratio was determined.

Description of immature stages and female genitalia

Morphological characteristics, including chaetotaxy, measurements and color pattern, of the last (5th) instar were analyzed in detail and only the observed changes were added to the descriptions of earlier instars. Coloration was described based on assessment of anesthetized living specimens. Observations were conducted on 19 eggs and the nymphs from each instar. Morphological terminology follows Wilson (1978) and Stewart (1986).

Female genitalia were examined in specimens collected from the field. The abdomen was rinsed in 10% KOH at room temperature for 24 h, then washed in water and afterward mounted for microscopic observation using a Leica ICC50W stereoscopic microscope with an attached digital camera. Illustrations were produced by initially photographing the female genitalia (sternite VII, pygofer and valvulae), followed by digital tracing and drawing using CorelDRAW X.7. The female genitalia were described based on the terminology of Hill (1970), Dietrich (2005) and Catalano & Dietrich (2017). Average is expressed as mean ± SE. Material examined was deposited in the entomological collection of the Museo de La Plata, Argentina (MLP).

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey´s test was used to compare the duration of the different instars and to assess significant

differences in the length of the nymphal stage between female and male individuals. The Kruskal-Wallis test was performed to compare the number of eggs laid on different plant sites.

RESULTS

Biological information

In this study, *E. decemnotata* is reported on oregano, thyme, rosemary and mint plants. Considerable leaf damage was observed on plants used in the laboratory for rearing and bioassays, caused by the Ligurian leafhopper (Figure 1).

Oviposition sites. Eggs were individually laid on various plants structures. Females exhibited a preference for ovipositing in secondary veins of leaves, which accounted 31.15% of the total eggs observed. Other oviposition sites included by the stem (21.31%), main vein of leaves (19.67%), petioles (18.03%), and leaf lamina (9.83%). Despite these trends, no significant differences were found among oviposition site preferences (χ^2=3.185, df= 4, p>0.05).

Developmental time. The average egg hatching time was 12.27 ± 1.53 days. The mean developmental times (16.87 ± 1.54 days) for the first to fifth instars were 2.79 ± 0.86, 2.82 ± 0.62, 2.63 ± 0.62, 3.30 ± 0.76 and 4.95 ± 0.99 days, respectively (Table I). Significant differences were observed between the durations of the fourth and fifth instars compared to the earlier instars (F= 61.27, df=4, p≤0.05), with the fifth instar being the longest. On average, the total duration of the immature stage of *E. decemnotata* was 29.14 days under controlled laboratory conditions.

Out of 59 eggs deposited, 37 individuals successfully reached the adult stage, resulting in a sex ratio of 59.49% males and 40.51% females. The average nymphal developmental time was 16.93 ± 1.62 days for females (n:15) and 16.73 ± 1.52 days for males (n:22), there were no statistically significant differences between sexes (F= 0.33; *p* = 0.58).

Description of the immature stages

Eggs (Figures 2a-b)

Dimensions: length: 0.6 ± 0.1 mm; width: 0.1 ± 0.01 mm. The endophytic eggs are ellipsoidal, with the anterior pole pointed and the posterior one rounded. The ventral surface is slightly concave and the dorsal side convex. At oviposition, the *E. decemnotata* eggs are translucent (Fig. 2a); three days later, they turn to whitish and the eye spots

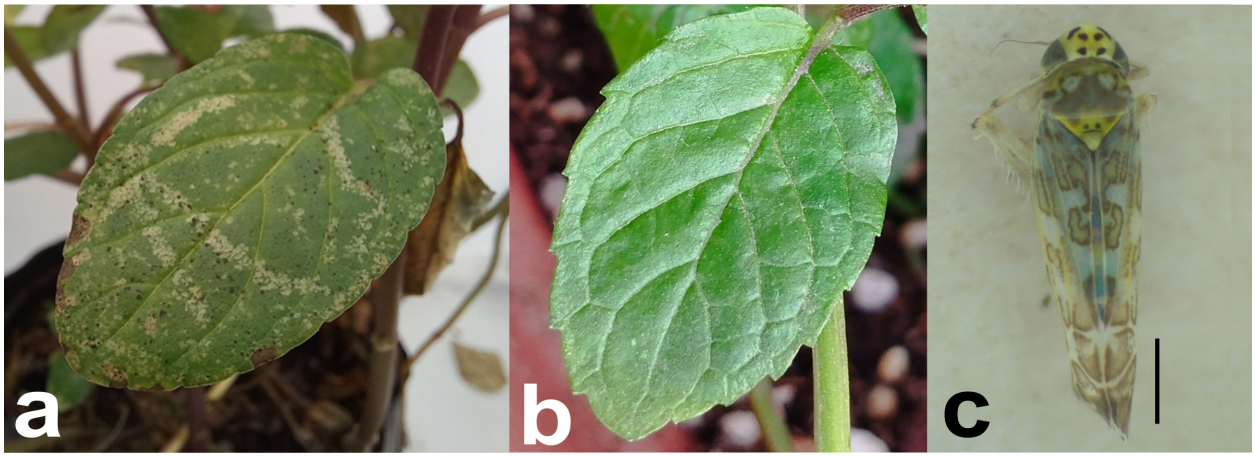

Figure 1. a) Damages caused by *Eupteryx decemnotata* on leaves of mint plant; b) Healthy mint leaf; c) female *Eupteryx decemnotata* Rey. Scale bars: 0.5 mm (c).

Table I. Developmental time of each immature stage of *Eupteryx decemnotata* (mean ± SD) under laboratory conditions (25 ± 2°C, 50-60 % RH and L12:D12 photoperiod) reared on mint plants. Different letters between instars denote significant differences ($p < 0.05$).

	Egg	First Instar	Second instar	Third instar	Fourth instar	Fifth instar	Total Nymphal Development
Developmental time (days)	12.27±1.53	2.79±0.86a	2.82±0.62a	2.63±0.62a	3.30±0.76b	4.95±0.99c	16.87±1.54
Range	10-15	1-4	1-4	1-4	2-6	2-7	14-22
n	59	47	44	41	40	40	40

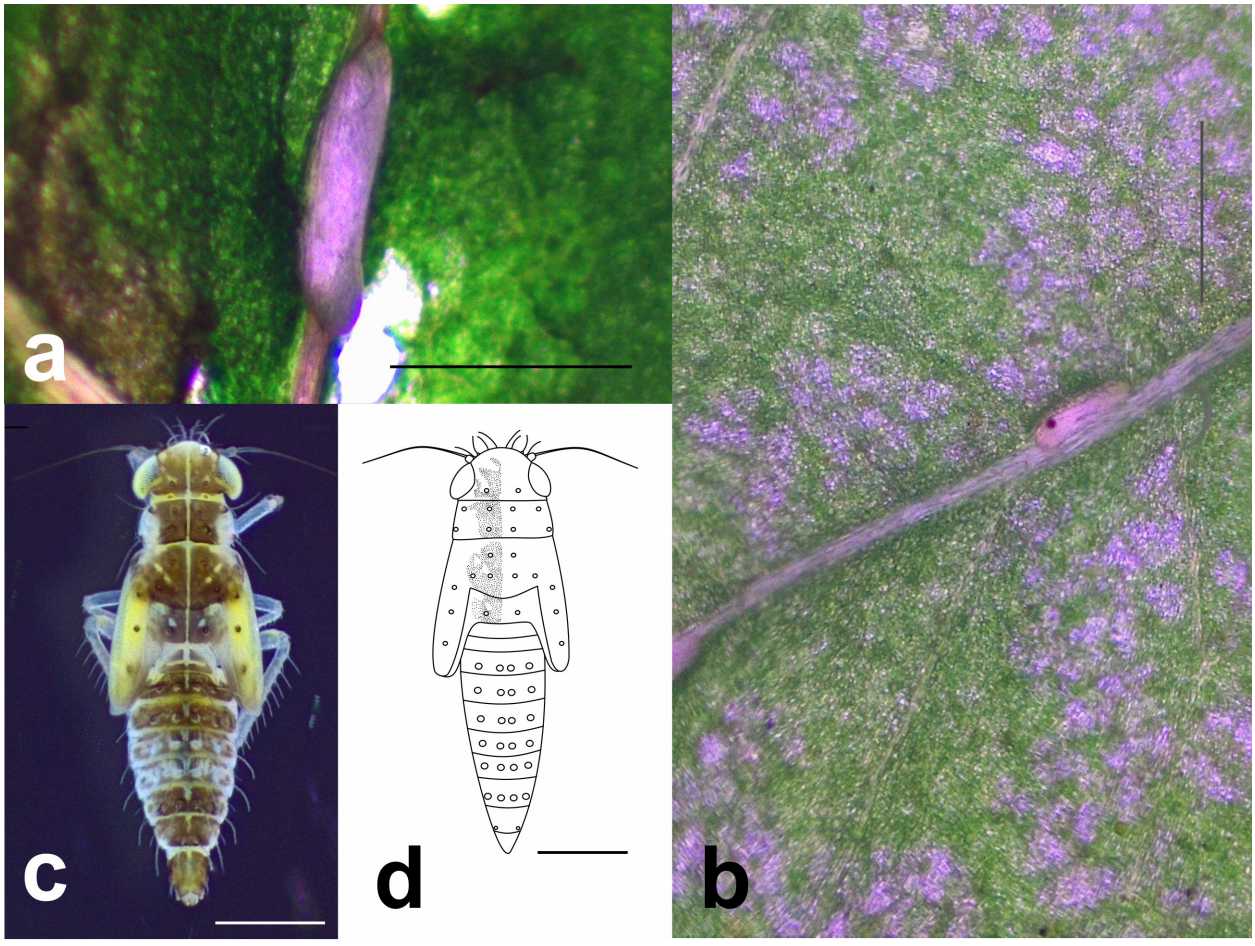

Figure 2. *Eupteryx decemnotata* Rey. a) Early egg laid in a secondary vein on mint plant; b) a roughly 10-day-old egg, showing visible eye spots, deposited on a secondary vein of a mint leaf; c) fifth instar coloration; d) chaetotaxy instars. Scale bars: 0.5 mm (a, c, d); 1 mm (b).

become visible 8 to 10 days after oviposition (Fig. 2b).

Early instars

After the eggs hatch, this leafhopper passes through five instars. Instar I is pale yellow, with setae mainly on the head and no visible wing pads. Instar II is pale yellow with light thoracic spots, and wing pads become visible from this instar. Instar III has spots on the head, thorax and first abdominal segments. Instar IV presents spots on the head, thorax and abdomen. The

chaetotaxy of this instar is the same as that of the instar V. A progressive development of wing pads (from instars II-V) can be observed, along with an increase in body size and the gradual appearance of darker and larger brown spots across various body parts.

Fifth instar (Figures 2c-d)

Overall dorsal coloration is pale yellow with dark areas extending from head to abdomen, contrasting with a clear medial line along the entire dorsal surface, dividing the body into two equal halves (Fig. 2c). *Head*: rounded vertex with two pairs of spines, posterior margin of crown with one pair of spines and two pairs of spines on upper face whose tips are visible from above. *Prothorax*: two pairs of spines on the anterior and posterior margin. *Mesothorax*: one medial pair of spines on the anterior margin and two pairs on posterior margin, and three pairs on the wing pads. *Metathorax*: one pair of medial spines. *Abdomen*: segments III-VII with one medial pair and one lateral pair of spines, segment VIII with one medial pair of spines, segment IX with one medial pair of microspines, and segments I and II without spines (Fig. 2d).

Description of the female (Figures 1b, 3a-f)

Measures: total length 2.48 mm; crown 0.21 mm, interocular width 0.26 mm; pronotum length 0.26 mm; pronotum width 0.53 mm General coloration similar to male, characterized by the presence of five pairs of black spots on the head: two pairs of spots on the crow, one pair of oval spots on the margin and two pairs of spots on the face, located below the latter.

Female genitalia. Sternite VII subquadrangular in ventral view, with a medial notch in the sclerotized anterior margin and an angular slightly projected posteriorly in posterior margin (Fig. 3a). Pygofer with strong macrosetae arranged in a row along the ventral margin and numerous microsetae in all surface, concave apex in lateral view (Fig. 3b). Valvula I thin, dorsally curved, tapering apex; apical third with dorsal margin obliquely striated and ventral margin striated (Fig. 3c). Valvula II asymmetrical; large valve, in lateral view, strongly curved to apex, dorsal margin apical third with countless pointed denticles arranged to apex, ventral margin with 7 teeths on apex (Fig. 3d); small valve, in lateral view, with 8-11 small rounded teeth in apical third distributed on dorsal margin (Fig. 3e); both valvula with conspicuous ducts, pointed apex. Valvula III, in lateral view, narrow anterior portion longer than posterior portion, which is markedly expanded, margin of apical third with numerous spines extending towards apex of dorsal margin, blunt apex (Fig. 3f).

DISCUSSION

In this study, we reported for the first time the presence of the Ligurian leafhopper, *E. decemnotata,* in Buenos Aires province, Argentina, associated with crops of oregano, thyme, rosemary and mint. Although this leafhopper had previously been recorded on various of these aromatic crops, our findings represent the first report of its association with mint plants, expanding its known range of host plants. The previous record of *E. decemnotata* in Mendoza province provides an important precedent, though there are notable differences in climate and altitude between the two regions, showing its high potential for adaptation. Mendoza is characterized by a semiarid climate, with low annual precipitation and marked diurnal temperature variation, in contrast to the humid temperate climate of Buenos Aires, which has more stable temperatures and higher humidity levels. Additionally, Mendoza is situated at a considerably higher altitude (approximately 900 m a.s.l.) than Buenos Aires (approximately 20

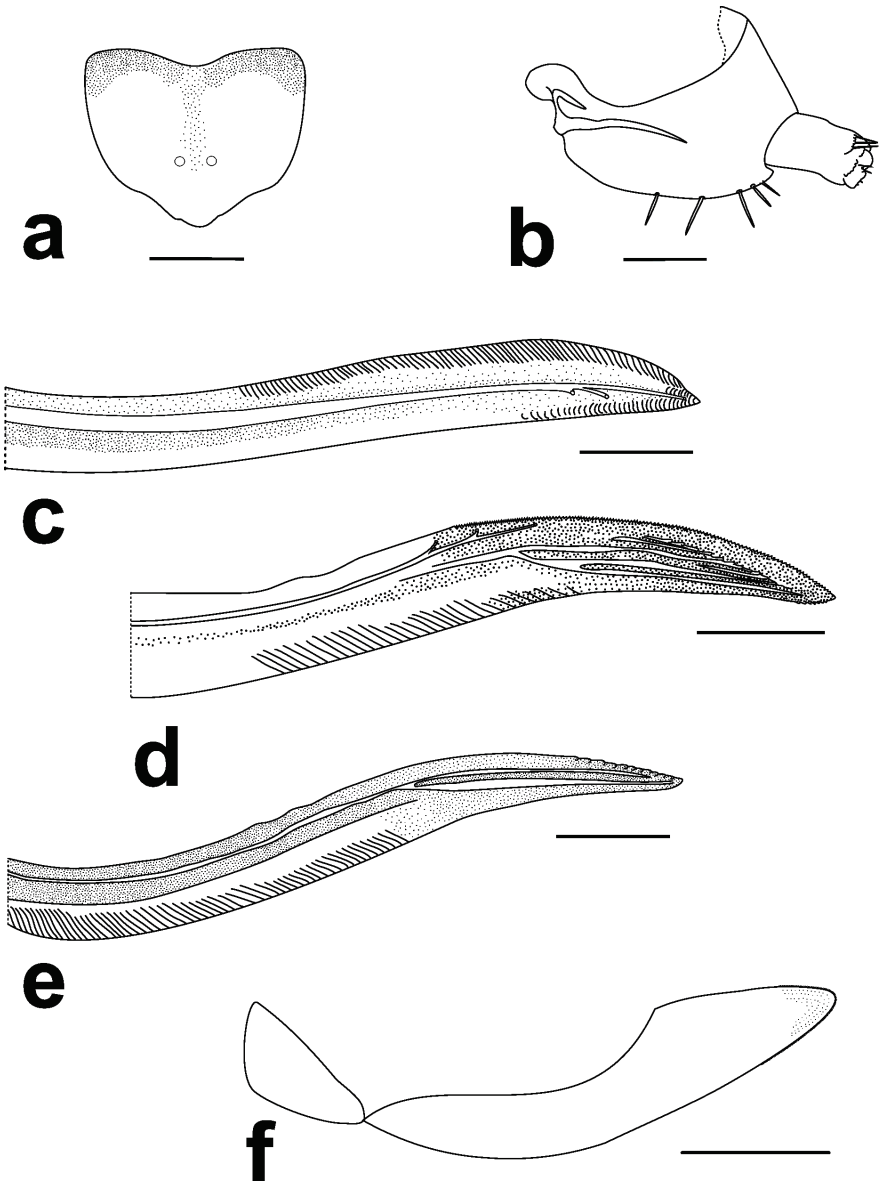

Figure 3. Morphological characteristics of the female genitalia of *Eupteryx decemnotata* Rey. a) Sternite VII; b) Pygofer; c) valvula I; d) large valvula II; e) small valvula II; f) valvula III. Scale bars: 0.2 mm (b, f); 0.05 mm (a, c, d, e).

m a.s.l.), which may influence the biology of *E. decemnotata*, particularly its developmental time, survival rates, and reproductive behavior. Despite the latitudinal similarity between both provinces, these environmental differences could play a key role in shaping the distribution and adaptation of the species in each region.

We provided a detailed description of the female genitalia and immature stages, along with biological traits such as oviposition sites preference, developmental time and sex ratio. Our results indicated that females predominantly laid their eggs in secondary veins of leaves, although less than 50% of the times. This finding differs from previous studies on the related species such as *Eupteryx melissae* Curtis, which reported higher egg deposition in petioles of catnip leaves (*Nepeta cataria* L.) (Lamiaceae) (Leonard & Barber 1923).

The developmental time observed in our study showed that under laboratory conditions (25°C), the total duration of the immature stage was approximately 29 days. These results contrast with studies conducted at 20°C on

common sage plants which reported a total duration of nearly 41 days for the same species, highlighting temperature as a critical factor influencing developmental time (Mazzoni & Conti 2006).

Among the nymphal stages, the fifth instar was the longest, lasting around five days, result consistent with previous studies (Mazzoni & Conti 2006). Interestingly, our study found the fourth instar to be the second longest, differing from other studies (Mazzoni & Conti 2006) which indicated the first instar as the second longest. Our results revealed that the average egg hatching period lasted nearly two weeks, showing a more gradual pattern, with hatching dispersed over the first five days. In contrast, Mazzoni & Conti (2006) reported an egg hatching period of three weeks, with 82% of the eggs hatching within the first three days. These variances may be attributed to differences in experimental temperatures and host plant species used.

Regarding nymphal chaetotaxy, *E. decemnotata* exhibits the same chaetotaxy pattern described by Stewart (1986). Nonetheless, in addition to this basic arrangement, it presents two pairs of medial spines (anterior and posterior) on the pronotum, a lateral pair of spines on abdominal segments III and IV, and segment IX with one medial pair of microspines. These characters combined with coloration patterns shown in the Figure 2d can be useful for accurate identification.

This study contributes valuable information to the taxonomic and biological knowledge of *E. decemnotata*, including developmental times, oviposition preferences and sex ratios under controlled conditions. These data are essential for designing monitoring protocols and developing effective strategies within integrated pest management (IPM) programs for aromatic crop systems in Argentina.

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SUPPLEMENTARY MATERIAL

Figure S1.

How to cite

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MORA PEREYRA[1]
https://orcid.org/0009-0000-6838-4635

SUSANA L. PARADELL[1]
https://orcid.org/0000-0002-4680-8035

ERICA LUFT-ALBARRACIN[2,3]
https://orcid.org/0000-0002-4081-8953

[1]Universidad Nacional de La Plata, División Entomología, Edificio Anexo Laboratorios del Museo, Facultad de Ciencias Naturales y Museo, Av. 122 y 60, s/n, Paseo del Bosque, CP 1900, La Plata, Buenos Aires, Argentina

[2]PROIMI (Biotecnología (CONICET)), División Control Biológico, Av. Belgrano y Pje Caseros, CP 4000, San Miguel de Tucumán, Tucumán, Argentina

[3]Fundación Miguel Lillo, Instituto de Entomología, Miguel Lillo 251, CP 4000, San Miguel de Tucumán, Tucumán, Argentina

Correspondence to: **Erica Luft-Albarracin**
E-mail: erluft@hotmail.com

Author contributions

MP: conceptualization, investigation, methodology and writing – original draft. SLP and ELA: conceptualization, investigation, review of the article and funding acquisition. All authors reviewed and approved the final version of the manuscript.