


Original Article

Development, survival and description of the life stages of *Zatrephina lineata* (Coleoptera: Chrysomelidae) fed on *Ipomoea pes-caprae* leaves

Desenvolvimento, sobrevivência e descrição das fases de vida de *Zatrephina lineata* (Coleoptera: Chrysomelidae) alimentada com folhas de *Ipomoea pes-caprae*

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Abstract

Zatrephina lineata (Coleoptera: Chrysomelidae) is a phytophagous insect, mainly of plants of the genera *Ipomoea* and *Mikania*. The objective was to study the development, survival and to describe the life stages of *Z. lineata* fed on leaves of *Ipomoea pes-caprae*. Biological observations were made daily with the aid of a stereoscopic microscope and the instars of this insect identified by the exuvia left between one moulting and the next. The duration of development and survival of the egg, larva and pupa stages and the first, second, third, fourth and fifth instars and of the nymph stage of *Z. lineata* differed, but not between sexes of this insect. The duration of development of *Z. lineata* was longer in the larval stage and in the fifth instar, and its survival greater in the egg and pupa stages and in the first and fifth instars. *Zatrephina lineata* eggs, cream-colored, are ellipsoid and deposited in groups on the adaxial surface of older *I. pes-caprae* leaves. The larvae of this insect go through five instars, with the first three being gregarious with chemo-behavioral defenses. The exarated pupae of *Z. lineata*, light yellow in color and with an oval shape flattened dorsoventrally, attach to the abaxial surface of the *I. pes-caprae* leaves. The shape of adults of this insect is oval, straw yellow in color with lighter longitudinal stripes and females are slightly larger than males.

Keywords: beach morning glory, bioerosion, biological aspects, Cassidinae, developmental stages, tortoise beetles.

Resumo

Zatrephina lineata (Fabricius, 1787) (Coleoptera: Chrysomelidae) é um inseto fitófago, principalmente de plantas dos gêneros *Ipomoea* e *Mikania*. O objetivo foi estudar o desenvolvimento, a sobrevivência e descrever as fases de vida de *Z. lineata* alimentada com folhas de *Ipomoea pes-caprae*. Observações biológicas foram feitas diariamente com auxílio de microscópio estereoscópico e os ínstars desse inseto identificados pela exúvia deixada entre uma muda e outra. A duração do desenvolvimento e sobrevivência dos estágios de ovo, larva e pupa e dos primeiros, segundo, terceiro, quarto e quinto ínstars e do período ninfal de *Z. lineata* diferiu, mas não entre os sexos deste inseto. A duração do desenvolvimento de *Z. lineata* foi maior na fase larval e no quinto ínstar, e sua sobrevivência maior nas fases de ovo e pupa e no primeiro e quinto ínstars. Os ovos de *Z. lineata*, de cor creme, são elipsoides e depositados em grupos na superfície adaxial das folhas mais velhas de *I. pes-caprae*. As larvas desse inseto passam por cinco ínstars, sendo os três primeiros gregários com defesas químico-comportamentais. As pupas exaradas de *Z. lineata*, de cor amarelo claro e formato oval achatado dorsoventralmente, fixam-se na superfície abaxial das folhas de *I. pes-caprae*. O formato dos adultos deste inseto é oval, de cor amarelo palha com listras longitudinais mais claras e as fêmeas são ligeiramente maiores que os machos.

Palavras-chave: glória da manhã na praia, bioerosão, aspectos biológicos, Cassidinae, estágios de desenvolvimento, besouros tartaruga.

1. Introduction

Chrysomelidae is one of the largest Coleoptera families with about 36,000 species described in the world (Bouchard et al., 2017). In South America, approximately 9,140 species have been recorded, 4,362 in 356 genera in

Brazil, representing 35% of species and 64% of genera from the Neotropical region (Borowiec and Świętojańska, 2019).

The Cassidinae subfamily, the second largest after Galerucinae, includes approximately 16% of Chrysomelidae

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species (Chaboo, 2007; Borowiec and Świątojańska, 2019). The body outline of Cassidinae beetles, known as “tortoise beetles” in North America, is oval or rounded, usually convex dorsally and flattened ventrally, with the margins of the elytra and pronotum flattened and dilated (Chaboo, 2007). Cassidinae species are phytophagous insects highly specialized in their hosts (Jolivet and Verma, 2005; Cuozzo et al., 2017), particularly plants with liana habit of the genera *Ipomoea* (Convolvulaceae) and *Mikania* sp. (Asteraceae) (Borowiec and Świątojańska, 2019). Larvae of most Cassidinae species feed in groups and their adults quickly disperse, feeding on their host plants, mainly females during the pre-oviposition period (Mphephu et al., 2017).

Chrysomelidae are predominantly pests of cultivated plants (Chaboo, 2007), but some of its species are used in the biological control of exotic weeds (Mphephu et al., 2017). *Zatrephina lineata* (Fabricius, 1787) (Coleoptera: Chrysomelidae), registered in Brazil, Colombia, French Guiana, Paraguay and Venezuela, feeds preferentially on plants of the Convolvulaceae family (Chaboo et al., 2014; Paleari, 2013; Gámez and Acconcia, 2019b) in localized areas of the sweet potato, *Ipomoea batatas* (L.) (Solanales: Convolvulaceae) crops and other species of this genus such as *I. asarifolia* (Paleari, 2021), *I. cairica* (L.) (Maia and Buzzi, 2005), *I. pes-caprae* (Bondar, 1953; Paleari, 2021) and *Ipomoea* sp. (Solanales: Convolvulaceae) (Buzzi, 1976; Gámez and Acconcia, 2019b).

Ipomoea pes-caprae, with adventitious roots forming true tangles, is a pioneer plant along tropical coasts reducing erosion in sand dunes (Castellani and Santos, 2005; Miryeganeh et al., 2014). The importance of sexual reproduction in maintaining local populations of this plant has been questioned (Castellani and Santos, 2005), suggesting that vegetative propagation explains its dominance in the floristic environment of coastal frontal dunes (Wilson, 1977; Bach, 1998; Tong and Lin, 2016). Defoliation by *Z. lineata* compromises the vegetative propagation of *I. pes-caprae* and induces the establishment and development of erosion processes. This bioerosion directly caused by organisms on a small scale is also important (Davis Junior and Fitzgerald, 2005) reinforcing the need of obtaining information on the bioecology of phytophagous insects associated to *I. pes-caprae*.

The aim of this work was to study the development, survival and to describe the stages of *Z. lineata* fed on leaves of *I. pes-caprae*.

2. Material and Methods

2.1. Study location

The research was carried out at the Entomology laboratory of Embrapa Algodão (7° 13'32"S latitude and 35° 54'19" W longitude) in Campina Grande, Paraíba state, Brazil, in a BOD-type climate chamber at 25 ± 1 °C, 60 ± 10% relative humidity and 12 hours of photophase.

2.1.1. Insect and plant material collection

Specimens of *Z. lineata*, obtained in the experimental field of Embrapa Algodão on *I. pes-caprae* leaves, were identified by Dr. Marianna Vieira dos Passos Simões, specialist in systematics

of the subfamily Cassidinae (Coleoptera: Chrysomelidae). *Zatrephina lineata* was reared in the laboratory for two generations fed on short leafy branches of stolons of *I. pes-caprae* collected in the experimental field of Embrapa Algodão.

2.2. Survival, development and description of the stages *Z. lineata*

Fifty *Z. lineata* eggs, collected from the rearing colony of this insect, were placed in a Petri dish (6.0 cm in diameter × 1.5 cm in height) on a leaf disc of *I. pes-caprae* with the same diameter as this dish. After emergence, 40 neonate larvae of *Z. lineata* were distributed in groups of ten to four Petri dishes on leaf discs of this plant. The branches and leaves offered to the caterpillars were washed with a solution of water and 1% sodium hypochlorite, and left to dry for about 2 hours in the laboratory. Two short branches and their leaves of *I. pes-caprae* were arranged in a 25 ml glass flask filled with water to delay their moisture losses, and this arrangement of leaves was maintained in cages made with PVC tube (14 cm in diameter × 21 cm tall). The upper part of the tube was sealed with voile fabric fixed with elastic tape and the base with Styrofoam disc. Leafy branches were changed after consumed by the larvae until pupa formation. Ten pupae of *Z. lineata*, after formed, were transferred to new PVC cages for adult emergence and sexing. *Zatrephina lineata* adults were sexed by separating couples in copulation, considering as males the smaller individuals mounted on females and with expanded elytral margin (Gámez and Acconcia, 2019a, b). The cages were kept in an acclimatized chamber under the same conditions of temperature, relative humidity and photophase as mentioned, until the emergence of the adults.

Biological observations were made daily, at 8:00 A.M. and 4:00 P.M., with the aid of an EL224 stereomicroscope (BEL Engenharia, Monza, Milan, Italy) with a 20x magnification until the emergence of *Z. lineata* adults. The survival and duration of the immature stages of individuals that originated males or females were determined, and the developmental stages of this insect described. The instars of *Z. lineata* were identified by observing the exuvia left by the insect between one molt and the next, and the larvae were measured after removing the excrements and exuvia (Buzzi, 1976).

2.3. Data analysis

Data were submitted to the Liliefors normality test and transformed, when necessary, to meet the variance analysis prerequisites. Survival and development data from egg to adult of males and females *Z. meticulous* were submitted to analysis of variance and the means compared by Fisher's LSD test at 5% probability. The data, with normality, were analyzed using the System of Statistical and Genetic Analysis (SAEG) of the Federal University of Viçosa.

3. Results

3.1. Survival and development of *Z. lineata*

Survival of egg ($F = 0.00$, $df = 1.6$, $P > 0.05$), larva ($F_{1,6} = 0.10$, $df = 1.6$, $P > 0.05$) and pupal ($F = 0.00$, $df = 1.6$, $P > 0.05$)

(Table 1) and the first ($F = 0.00$, $df = 1.6$, $P > 0.05$), second ($F = 0.86$, $df = 1.6$, $P > 0.05$), third ($F = 3.43$, $df = 1.6$, $P > 0.05$), fourth ($F = 0.20$, $df = 1.6$, $P > 0.05$) and fifth ($F = 0.00$, $df = 1.6$, $P > 0.05$) instars was similar between sexes (Table 2), but differed between stages ($F = 115.11$, $df = 2.9$, $P < 0.01$) and instars ($F = 17.39$, $df = 4.15$, $P < 0.01$) of *Z. lineata*. Survival per stage was higher in the egg and pupal stages, lower in the larval stage, with a higher value for fifth and first instars, and lower for the third instar.

The development periods of the egg ($F = 0.00$, $df = 1.11$, $P > 0.05$), larva ($F = 2.04$, $df = 1.11$, $P > 0.05$) and pupa ($F = 1.04$, $df = 1.11$, $P > 0.05$) stages (Table 1) and the first ($F = 4.41$, $df = 1.11$, $P > 0.05$), second ($F = 0.25$, $df = 1.11$, $P > 0.05$), third ($F = 0.74$, $df = 1.11$, $P > 0.05$), fourth ($F = 0.05$, $df = 1.11$, $P > 0.05$) and fifth ($F = 2.280$, $df = 1.11$, $P > 0.05$) instars were similar between the sexes

(Table 2), but differed between stages ($F = 2507.76$, $df = 2.63$, $P < 0.01$) and instars ($F = 126.28$, $df = 4.96$, $P < 0.01$) of *Z. lineata*. The period of development per stage was longer in the larval stage and shorter in the pupal stage of *Z. lineata*. The period of larval development was longer for fifth instars and shorter for the second instar.

3.2. Description of the immature and adult stages of *Z. lineata*

The laying of *Z. lineata* is a cluster of eggs imbricated in 3-5 rows juxtaposed on the adaxial surface of the oldest *I. pes-caprae* leaves. The color of eggs of this insect is cream with elongated ellipsoid shape measuring 1.96 ± 0.00 mm long by 0.12 ± 0.00 mm wide (Figure 1A). A rounded, translucent yellow substance, through which the neonate larvae hatch, covers the apical portion of the egg chorion of *Z. lineata*.

Table 1. Survival and duration (mean \pm standard error) of the egg, larval and pupal stages of *Zatrephina lineata* (Coleoptera: Chrysomelidae) at the temperature of 25 ± 1 °C, relative humidity of $60 \pm 10\%$ and a 12-hour photophase. Campina Grande, PB, 2023.

Stage	Female	n	Male	n	Female + Male	n
Survival (%)						
Egg	100.00 \pm 0.00	20	100.00 \pm 0.00 ^{n.s.}	20	100.00 \pm 0.00 a	40
Larva	31.58 \pm 1.75	06	32.50 \pm 1.50 ^{n.s.}	06	32.50 \pm 4.38 b	12
Pupa	100.00 \pm 0.00	06	100.00 \pm 0.00 ^{n.s.}	06	100.00 \pm 0.00 a	12
Duration (days)						
Egg	9.00 \pm 0.00	20	9.00 \pm 0.00 ^{n.s.}	20	9.00 \pm 0.00 b*	40
Larva	18.25 \pm 0.63	06	18.13 \pm 0.31 ^{n.s.}	06	18.54 \pm 0.31 a	12
Pupa	5.38 \pm 0.24	06	5.25 \pm 0.14 ^{n.s.}	06	5.35 \pm 0.09 c	12

^{n.s.}Means of survival and duration of each immature stage between genera do not differ by Fisher's LSD test. *Means followed by the same letter in the column per stage and parameter do not differ by Tukey's test at 5% probability. n = number of individuals. Survival means transformed into arc sin root ($x/100$) for statistical analysis.

Table 2. Survival and duration (mean \pm standard error) of the first, second, third, fourth and fifth instars of *Zatrephina lineata* (Coleoptera: Chrysomelidae) at the temperature of 25 ± 1 °C, relative humidity of $60 \pm 10\%$ and 12 hour photophase. Campina Grande, Paraíba State, Brazil. 2023.

Instar	Female	n	Male	n	Female + Male	n
Survival (%)						
First	95.00 \pm 2.00	19	90.00 \pm 2.00 ^{n.s.}	18	92.50 \pm 3.75 a*	37
Second	63.16 \pm 1.50	12	66.67 \pm 1.50 ^{n.s.}	12	61.67 \pm 5.83 bc	24
Third	66.67 \pm 3.00	08	58.33 \pm 3.00 ^{n.s.}	07	37.50 \pm 5.63 c	15
Fourth	87.50 \pm 3.00	07	85.71 \pm 3.00 ^{n.s.}	06	87.75 \pm 6.13 ab	13
Fifth	100.00 \pm 0.00	07	100.00 \pm 0.00 ^{n.s.}	06	100.00 \pm 0.00 a	13
Duration (days)						
First	3.00 \pm 0.00	19	3.25 \pm 0.25 ^{n.s.}	18	3.05 \pm 0.07 bc	37
Second	3.00 \pm 0.00	12	2.88 \pm 0.13 ^{n.s.}	12	2.76 \pm 0.09 c	24
Third	2.88 \pm 0.31	08	3.00 \pm 0.00 ^{n.s.}	07	3.00 \pm 0.14 bc	15
Fourth	3.63 \pm 0.24	07	3.00 \pm 0.20 ^{n.s.}	06	3.46 \pm 0.15 b	13
Fifth	5.75 \pm 0.32	07	6.00 \pm 0.20 ^{n.s.}	06	6.04 \pm 0.13 a	13

^{n.s.}Means of survival and duration per instar between sexes do not differ by Fisher's LSD test. *Means followed by the same letter in the column per stage and parameter do not differ by Tukey's test at 5% probability. n = number of individuals. Duration averages transformed into arc sin root ($x/100$) for statistical analysis.

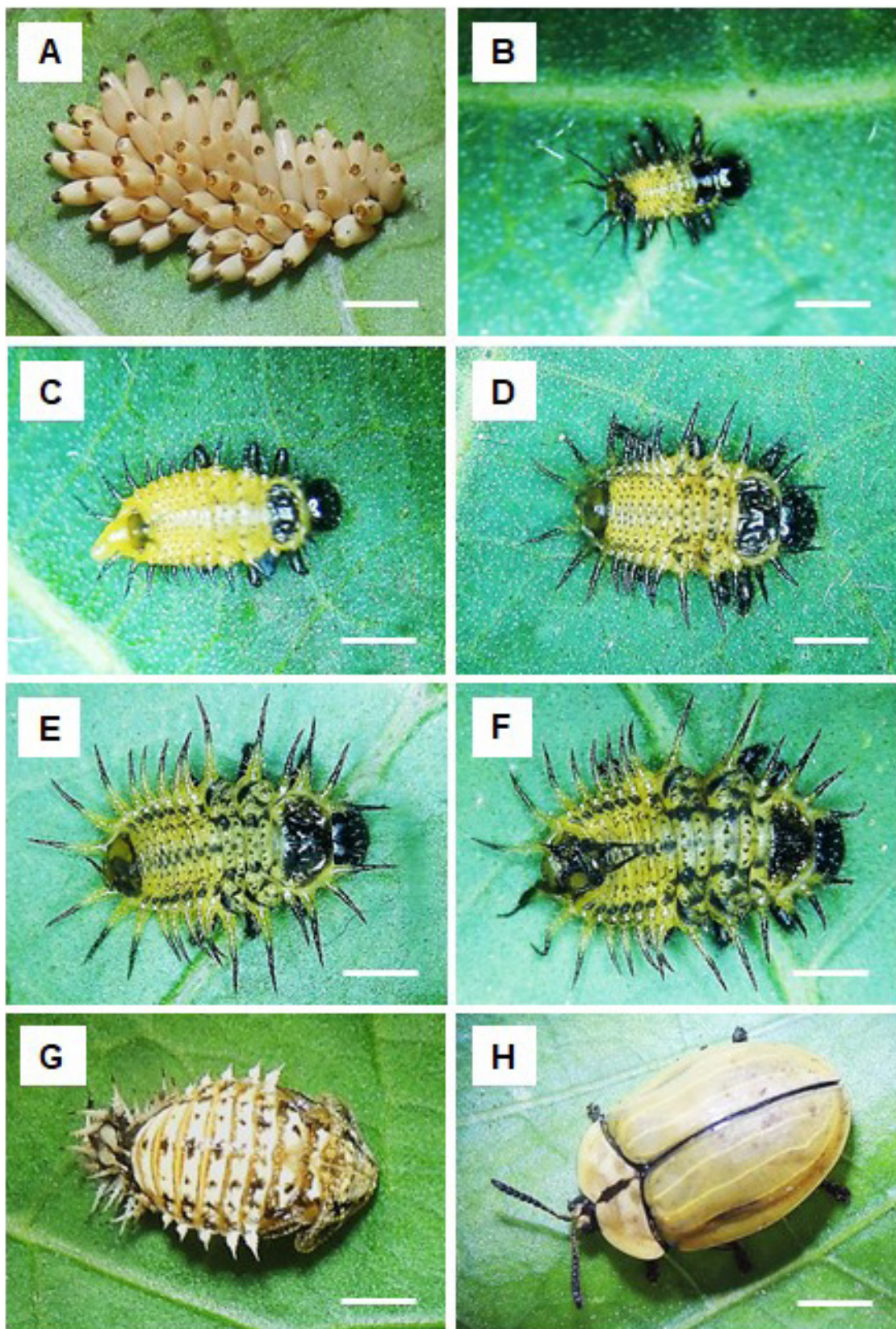


Figure 1. Eggs (A), first (B), second (C), third (D), fourth (E) and fifth (F) instar larvae, pupa (G) and adult (H) of *Zatrephina lineata* (Coleoptera: Chrysomelidae) on a leaf of *Ipomea pes-caprae*. Scale bar = 1 mm.

The first, second, third, fourth and fifth instar of *Z. lineata* are light yellow in color, dorsoventrally flattened, narrowing backwards and forwards from the metathorax and measuring 2.04 ± 0.01 and 1.00 ± 0.00 mm; 2.52 ± 0.01 and 1.22 ± 0.01 mm; 3.07 ± 0.02 and 1.53 ± 0.01 mm; 4.04 ± 0.01 and 2.02 ± 0.01 mm and 5.94 ± 0.03 and 2.96 ± 0.02 mm in length and width, respectively (Figures 1B-F). Fourteen pairs of lateral scoli along the body, six on the thorax and eight on the abdomen, were observed on *Z. lineata* larvae (Buzzi, 1976). The last pair of scoli, in the abdominal segment of larvae of this insect, is connected to the exuvial-fecal shield, which dorsally retains the compacted exuvia mixed with feces.

Pupae of *Z. lineata* are opaque pale yellow in color, oval-shaped and flattened dorsoventrally, narrowing backwards and forwards from the first abdominal segment and measuring 8.07 ± 0.09 mm in length by 5.05 ± 0.03 mm wide, without any cover (Figure 1G). The abdominal segments of these pupae are visible with a darker transverse stripe in the posterior portion delimiting the beginning of the subsequent abdominal segment with lighter shade. A caudal process, developed to retain the fifth instar exuvia, was observed in the last abdominal segment. The prothorax covers the head, being the most developed part of the thorax and resembling an isosceles triangle, with a base 2.5 times greater than its height.

Females and males *Z. lineata* are oval with, respectively, 9.79 ± 0.12 mm, 8.14 ± 0.09 mm long, 6.42 ± 0.12 mm, and 5.96 ± 0.06 mm width. The elytra and pronotum of these adults are short after emergence, translucent yellow in color, becoming straw yellow with lighter longitudinal stripes after the sclerotization (Figure 1H).

4. Discussion

4.1. Survival, development and reproduction of *Z. lineata*

The survival, similar between the sexes in the egg, larva, pupa and the five instar stages of *Z. lineata*, may be related to the reduced sexual dimorphism of this insect, with females only slight larger than its males (Stillwell and Fox, 2007; Stillwell et al., 2010).

The greater survival of the egg and pupa of *Z. lineata* compared to its larvae can be attributed to the nutritional needs and energy demands of each developmental stage of this insect (Gillette et al., 2021). On the other hand, the greater survival of first and fifth instar larvae than in second and third instar of *Z. lineata* is probably due to the gregarious behavior of first instars (Paleari, 2021). First-instar larvae of *Z. lineata* increase their feeding rate with group size and the opposite tendency was observed for second- and third-instar larvae, as reported for first-instar larvae of the *Hybosa acutangula* (Spaeth, 1913) (Begha and Oliveira, 2024). On the other hand, the greater mobility and foraging of fifth-instar larvae of *Z. lineata* may explain their greater survival compared to those of early-instar (Morrison and Windsor, 2018).

The similar periods of development of the egg, larval and pupal stages and of the five instars of *Z. lineata* that originated males or females is probably due to the lack

of differences between immature stages of both sexes of this insect (Tamaru and Esperk, 2007; Teder, 2014). These periods were longer than the 7.49, 15.17, 4.69, 2.83, 2.08, 1.99, 3.12 and 5.15 days, respectively, for the egg, larva and pupa and the first, second, third, fourth and fifth instars of *Z. lineata* fed on *Ipomoea asarifolia* leaves at 27 °C and 80% relative humidity (Paleari, 2021), which can be attributed to differences in temperature, relative humidity and plant species used.

The longest period of the larval stage and the shortest one for the pupal stage was expected because, in the first, insects feed and accumulate energy for metamorphosis (Scriber and Slansky Junior, 1981). The pupa, on the other hand, does not feed using the energy accumulated during the larval stage to transform itself into adults (Rolff et al., 2019). These results are similar to those observed for the larval and pupal stages of *Z. lineata* fed on *I. asarifolia* leaves (Paleari, 2021).

The longer period of fifth-instar larvae of *Z. lineata* is due to the greater nutritional needs of this instar, due to their size compared to the initial instars of this insect (Scriber and Slansky Junior, 1981; Ghebremariam et al., 2014).

4.1.1. Description of the immature and adult stages of *Z. lineata*

The imbricate shape of *Z. lineata* eggs may facilitate their grouping and allow the female to protect them with her body, as observed for *Paraselenis (Spaethiechoma) dichroa* (Germar, 1824) (Coleoptera: Cassidinae) (Cuozzo et al., 2017). The coloration of *Z. lineata* eggs is characteristic, but may vary with the embryonic development of this insect, which makes it possible to distinguish the oldest egg clusters from the most recent ones (Gomes et al., 2012). The peduncle, on these eggs, can be a strategy to reduce the contact between the egg and the leaf to reduce plant sensitivity and, consequently, rejection of the postures (Cuozzo et al., 2017).

Larvae of *Z. lineata* are similar to those of *Anacassis cribrum* (Klug, 1829) and *Anacassis fuscata* (Klug, 1829) (Mesomphaliini), but are distinguished from them by the quadridentate mandible, arrangement of chitinous processes and coloration with dorsal dark spots (Buzzi, 1976). The first three instars of this insect are gregarious with synchronous chemo-behavioral defenses, such as buccal regurgitation, anal emissions, gland eversion, fecal shield undulations and cycloalexia (Chaboo et al., 2014). The mixture of exuvia with feces linked to the last pair of scholosis of the abdominal segment is a physical protection against dissection and predation (Nogueira-de-Sá and Trigo, 2005; Chaboo and Engel, 2009).

Pupae of *Z. lineata* are also similar to those of *A. cribrum* and *A. fuscata*, on the abaxial surface of the host plant leaf, losing the fecal shield and increasing the development of the lateral skoli and pronotum, a common characteristic of other Mesomphaliini species (Buzzi and Garcia, 1983; Gomes et al., 2012; Macedo et al., 2015).

The margins of the elytra, proportionally, more expanded on males than on females characterize the sexual dimorphism of *Z. lineata* as the size of females and males of this insect are similar (Gámez and Acconcia, 2019a, b).

Ipomea pes-caprae is an important host of *Z. lineata* and population outbreaks of this Chrysomelidae can cause severe defoliation (Paleari, 2021) and compromise the vegetative propagation of this plant, inducing bioerosion processes in dunes on the Brazilian coast where infestations of this insect are reported.

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