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Immature stages of Spodoptera albula (Walker) (Lepidoptera: Noctuidae): Developmental parameters and host plants

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ABSTRACT

This study aimed to detail the temporal and morphological parameters of the immature stages of *Spodoptera albula* (Walker 1857) under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase) and to gather information about their larval host plants. For this purpose, a new rearing method and artificial diet was employed and validated. The viability of the egg, larval, pupal and pre-pupal stages was 94.54, 97.33, 93.84 and 92.34%, respectively. The average duration of the egg, larval, pupal and pre-pupal stages was 4.14, 16.37, 1.69, and 9.34 days, respectively. During the larval stage, 80.85% of females and 93.99% of males passed through six and remaining through seven instars, with significant larval protandry. The larvae that developed through six and seven instars exhibited a mean growth rate of 1.58 and 1.48, respectively. Fifty five host plant species belonging to 29 families are listed. The female pupae were significantly larger, exhibiting protogyny. Both the rearing methods as well as the larval diet proved adequate, providing more detailled observations of the biological cycle, especially the larval stage, and resulting in an overall survival of almost 80%.

Key words: annual crop pest, armyworm, artificial diet, development, life cycle.

INTRODUCTION

The genus *Spodoptera* Guenée, 1852 (Lepidoptera: Noctuidae: Noctuinae) (Lafontaine and Schmidt 2010) is cosmopolitan and includes many of the most important agricultural caterpillars (Pogue 2002). *Spodoptera albula* (Walker 1857) has been recorded from Florida and Southern Texas, throughout the Caribbean, Central America, and from Venezuela south to Paraguay and Southern Brazil (Pogue 2002, Zenker et al. 2010), and Chile (Angulo et al. 2008). *Spodoptera albula* has been erroneously referred to as "*Spodoptera sunia* (Guenée, 1852)" which is currently recognized as Neogalea sunia (Guenée 1852), representative of the Oncocnemidinae (Lafontaine and Schmidt 2010).

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Beside being polyphagous, the larvae of *S. albula* usually migrate to crops, both coming from the various weeds that are between the rows and can be their host plants (Hallman 1979), as well as along the edges (González-B 1966). This species represents a potential risk, making it unfeasible to develop important crops such as tobacco (Stoyan and Machado 1970, Novo Padrino et al. 1984, 1985, Páez Gázquez and Novo Padrino 1987), cotton (Alcaraz Vieco 1962, González-B 1966), tomato (Gloria-B 1975), cabbage (Armstrong (1994), sesame, soybean (Hallman 1979, 1983), peanuts (Teixeira et al. 2001), sunflower (Pruett and Guamán 2001), papaya (Semillas del Caribe 2010) and even seedling production in forestry nurseries (Vázquez et al. 1999).

Beyond its great voracity and reproductive capacity (Stoyan and Machado 1970, Martin Zequeira 1982, Novo Padrino et al. 1984, 1985, Novo Padrino and Martínez Reyes 1985, Páez Gázquez and Novo Padrino 1987, La Rosa et al. 1992), *S. albula* is tolerant to several chemical insecticides (Gloria-B 1975, Savoie 1988) and to the *Bacillus thuringiensis* Cry1Ac gene (Zenner-de-Polanía et al. 2008, Amaya et al. 2009). Its importance as a pest and its tolerance to several chemical products motivated the identification of pheromonal components to assist the integrated pest management of this species in cotton (Bestmann et al. 1988) and in melon crops (Dunkleblum et al. 1995).

Due to the importance of this species, especially in Central America and Cuba, several biological studies were developed to determine biological parameters (Stoyan and Machado 1970, Martin Zequeira 1982, Novo Padrino and Martínez Reyes 1985, La Rosa et al. 1992), and damage potential (Novo Padrino et al. 1984, 1985, Páez Gázquez and Novo Padrino 1987).

Considering the importance of *S. albula* for several crops of economic interest, this study aimed to: (a) detail the various temporal and morphological parameters of the immature stages under controlled conditions, to allow comparisons with previous studies and with other representatives of the same genus; (b) gather and organize information relating to host plants; and (c) validate a rearing method and an artificial larval diet which has already been used to detail the biological parameters of pest noctuids, in the Laboratório de Controle de Pragas of the Universidade de Caxias do Sul.

MATERIALS AND METHODS

Insects

The experiments only used first generation specimens whose progeny initiated with eggs from a single female collected on January 9, 2011, in Jaboticabal, São Paulo (21°16'37.52"S, 48°17'37.54"W, 572m height). Species level identification was accomplished by comparing larvae and adults with descriptions in Pogue (2002).

Rearing

All the experiments were preformed in a climate controlled room (25 ±1°C, 70 ±10% RH and a 14 hour photophase), with daily observations.

Egg Stage

Each egg mass was individually placed in a Petri dish lined with filter paper moistened with distilled water, where it remained until the eclosion of the larvae. We evaluated the feasibility (fertility) and the embryonic period, in days, of 16 egg masses (4,454 eggs) taken randomly from four couples, including the first and last ovipositions. It was observed that the evaluated egg masses were from couples, whose females presented two spermatophores in the bursa copulatrix, indicating that they had been fertilized during the experiment.

Larval Stage

Soon after hatching, 300 larvae were individually placed in properly identified 150 mL plastic cups, covered with a transparent plastic cap. A small wad

of cotton wool (~1 cm in diameter), moistened with distilled water to maintain moisture, along with a small dose (~1 cm^3) of artificial diet were included in each cup, as described below. Daily observations were made to verify the survival and development of the larva (with removal of the head capsule), the need to complement or replace the dosage of the diet, and the cotton in order to maintain humidity, always being careful to not interfere and to touch the larva as little as possible. The head capsules were stored, by larvae, in microcentrifuge tubes, for posterior measurement. In some cases, the change of instar was noticed through the development of the larva, but the capsule was not found, most likely because it had been eaten by the larva, which is relatively common among insects. In these cases, the date of ecdysis was recorded, and the size was then compared with the other larvae to confirm ecdysis, and the corresponding duration of each stage.

When the larvae reached the prepupal period, characterized by a decrease in size and the interruption of feeding, the diet and the cotton swab were removed. Thereafter expanded vermiculite, moistened with distilled water, was added to each cup to a height of 0.5 cm to encourage the development of the pupal chamber and to allow the observation of metamorphosis, recording the prepupal period.

This methodology allowed us to record the number of larval instars, the survival and the individual duration of each instar / stage and of the prepupal period, taking into account the sex of each larva. It also allowed us to evaluate growth as a function of the number of larval instars.

As the method of measuring between the frontal setae of the head capsule (Podoler and Klein 1978) is more precise than the traditional method that measures the distance between genas (Pérez et al. 2005), we chose to measure distances between genas only in the first and last instar to permit comparisons with other studies and between the frontal setae for comparisons among instars, for larvae that developed through six and seven instars.

Composition and Preparation of Larval Diet

The artificial diet (adapted from Greene et al. 1976) was composed of: 2,150 mL of distilled water; 35 g of agar; 125 g of type 1 carioca bean; 100 g of wheat germ; 25 g of powdered whole milk; 62.5 g of yeast extract; 6 g of ascorbic acid; 10 mL of Vanderzant vitamin mixture; 250 mg of tetracycline; 6 mL of 40% formaldehyde; 5 g of methyl parahydroxybenzoate (Nipagin); 3 g of sorbic acid; and 50 g of soy protein.

Initially, the beans, placed in an Erlenmeyer flask (500 mL) with distilled water (150 mL) and capped with a wad of hydrophobic cotton wrapped in gauze, were cooked in an autoclave at one atmosphere for 40 min. After that, the flask with the baked beans was removed from the autoclave, capped with aluminum foil and kept on the lab table until the temperature reached 25°C.

Then, the pre-baked beans were ground together with the remaining ingredients (wheat germ, powdered milk, yeast extract, soy protein and agar) that were added slowly along with the distilled water (1,500 mL) in a domestic blender at full power for at least 10 minutes, forming a homogeneous mass. This homogenized mass was transferred to a stainless steel pot and cooked for 5 minutes, counting from the boiling point. After cooking, the mass was removed from the heat, and was cooled to 40°C, by manually mixing it.

At the same time, the ascorbic acid, sorbic acid, Nipagim, tetracycline chlorhydrate, vitamin mixture and formaldehyde solution were manually mixed in a 1 L Beaker with distilled water (500 mL), until the complete homogenization of the ingredients. This solution was added to the cooked mass and both were manually mixed together until completely homogenized.

The finished diet was placed in polyethylene gerbox-type boxes (11 x 11 x 3.5 cm) to the maximum height of 2.5 cm of diet. The Gerboxes were immediately transferred to a laminar flow chamber with

ultraviolet light, until the temperature of 25°C was reached. After that, the Gerboxes were closed and kept under refrigeration (5°C) until the diet was used.

The diet was cut with a stainless steel spatula, previously cleaned with 70% alcohol, and individually offered to each caterpillar, in cubes of approximately 1 cm^3, during the daily maintenance activities.

Considering the polyphagous habit and lack of organization of information relating to larval host plants, a survey of the plants cited in literature and in the internet was performed, gathering information on the botanical family, specific name, common name and bibliographic reference. The nomenclature of the plants has been updated mainly using Backes and Nardino (2001).

Pupal Stage

The pupae were kept without food, under the same conditions and containers of the prepupa. On the second day after pupation, when the cuticle was further hardened, the sex was determined comparing with the drawings of Angulo and Jana-Sáens (1982). In addition to duration, the mass was measured using a semi analytical balance, accurate to one hundredth of a gram. As the sex can only be precisely identified during the pupal stage, the identity number of each larva was maintained until pupation to know whether it was male or female, allowing comparisons between sexes, even during the larval stage. The daily maintenance activities consisted of maintaining the moisture, with a few drops of distilled water, and detecting the emergence of the adult.

Temporal and Morphometric Parameters

The temporal and morphometric parameters were analyzed using descriptive statistics with the calculation of means and standard deviations. When necessary, the means were compared using a t-test assuming unequal variances, at a significance level of 95%.

RESULTS AND DISCUSSION

The duration of the immature stages of *S. albula* (Table I) resembled many of the results already described for the same species, under similar conditions of temperature and fed with tobacco (Novo Padrino and Martínez Reyes 1985) and tomato (La Rosa et al. 1992).

These results also resemble those described for other species of the same genus, reared under similar conditions of temperature and whose larvae were fed with various host plants, such as: *S. eridania* (Stoll, 1782) on sweet potato leaves (Foerster and Dionisio 1989); *Spodoptera frugiperda* (Smith 1797) on corn (Pinheiro et al. 2008), and cassava leaves (Lopes et al. 2008); *S. cosmioides* (Walker 1858) on artificial diet (Bavaresco et al. 2002); and *S. exigua* (Hübner 1808) on cabbage leaves (Azidah and Sofian-Azirun 2006). Despite these similarities, it should be noted that several authors have shown a great variation in the duration of the life cycle of the of the *Spodoptera* representatives, as a function of the larval diet (i.e. Parra et al. 1977, Yoshida and Parrella 1992, Greenberg et al. 2001 , Bavaresco et al. 2003, Azidah and Sofian-Azirun 2006, Sá et al 2009, Barros et al. 2010, Saeed et al. 2010, Farahani et al. 2011), which may vary even among biotypes of the same species (i.e. Giolo et al. 2002, Busato et al. 2005).

TABLE I

Survival and duration of life cycle of *S. albula* during different developmental stages, under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase).

Stage	N initial-final	Survival (%)	Duration (days)	Range (days)
Egg	4454 - 4211	94.544	4.141 ± 0.043	3-5
Larvae	300 - 292	97.333	16.367 ± 0.593	14- 21
Prepupae	292 - 274	93.836	1.691 ± 0.751	1 - 4
Pupae	274 - 253	92.336	9.336 ± 1.051	7 - 12
Total	-----	**79.732**	**31.535**	-----

The incubation period (Table I) is similar to the 3.5 to 4.0 days described for the same species, under similar temperatures (Novo Padrino and Martínez Reyes 1985, Novo Padrino et al. 1985, La Rosa et

al. 1992). However, under mean temperatures of 21.8 and 20.7°C, Martin Zequeira (1982) described periods of 3.4 and 3.0 days, respectively. Without indicating temperature, Stoyan and Machado (1970) described an embryonic period of 6.4 days. The embryonic period of *S. albula* is similar to that described for most of the species of this genus, under similar conditions of temperature (i.e. Foerster and Dionísio 1989, Bavaresco et al. 2003, Azidah and Sofian-Azirun 2006, Barros et al. 2010).

The relatively high egg viability (Table I) corresponds to the 94-98% described by Novo Padrino and Martínez Reyes (1985). This viability, above 90%, is only observed in a few studies of representatives of the genus (i.e. Mattana and Foerster 1988 - *S. eridania* ~ 90%, Tisdale and Sappington 2001 - *S. exigua* > 90%; Santos et al. 2005 - *S. frugiperda* ~ 80%,). However, higher rates of fertility for *Spodoptera* are most likely to be observed when using several couples per cage (Milano et al. 2008). Along these lines, Saeed et al. (2010) even demonstrated that food (host plant) can negatively influence the fecundity and fertility of *S. exigua* during each generation.

Larval Stage

The high level of larval survival (Table I) indicates that both the diet and the rearing conditions were satisfactory for the development of *S. albula* in the laboratory. La Rosa et al. (1992) described a higher larval survival (90.5%) for the same species fed with tomato at a mean temperature of 26.7°C. However, several authors (Stoyan and Machado 1970, Martin Zequeira 1982, Novo Padrino and Martínez Reyes 1985, Novo Padrino et al. 1985, La Rosa et al. 1992) have reared this species under controlled conditions. This demonstrates that this species, like other representatives of *Spodoptera* (i.e. Bavaresco et al. 2004, Santos et al. 2005, Azidah and Sofian-Azirun 2006, Barros et al. 2010, Saeed et al. 2010, Xue et al. 2010, Farahani et al. 2011) is adaptable to laboratory conditions.

Most of the larvae (87.226%) developed during six instars and the remainder (12.774%) for seven instars. Published records describe five (Stoyan and Machado 1970) and six (Martin Zequeira 1982, Novo Padrino and Martínez Reyes 1985, Novo Padrino et al. 1985, La Rosa et al. 1992) larval instars for this species. However, several authors have described different numbers and proportions of larval instars. Along these lines, Azidah and Sofian- Azirun (2006), after testing host plants for *S. exigua*, found five and six instars for larvae feeding on cabbage (*Brassica oleracea* var. *capitata* variety KK cross) and on cowpea (*Vigna unguiculata*); six, seven and eight instar for larvae feeding on shallot (*Allium cepa* var. Indian Rose); and five, six, seven and eight instars for those feeding on lady's finger (*Abelmoschus esculenta*). Bavaresco et al. (2004) discovered the existence of different proportions of *S. cosmioides* larvae that went through six and seven larval instars as a function of three artificial diets. In both studies, the highest proportion of larvae that developed through a greater number of larval instars were in the groups that were fed on plants or less appropriate diets.

Our results (Table II) indicate that the number of females that were developed through seven instars (19.149%) was much higher than males (6.015%), an aspect still unexplored for representatives of Noctuidae. According to Esperk et al. (2007), the most common factors influencing instar number include temperature, photoperiod, food quantity and quality, humidity, injuries, inheritance, and sex. Typically, instar number tends to increase under adverse rather than favorable conditions and this conclusion is consistent with the compensations scenario, according to which additional instars are inserted in poor conditions when larvae fail to reach a species-specific threshold-size with the "normal" instar number.

As this study was carried out under controlled conditions, the variation of the number of instars between the sexes can be attributed, at least in part,

TABLE II
Mean larval duration (days) of *S. albula*, during each instar, including the larvae of each sex which developed for six and seven instars, fed with an artificial diet, under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase).

	Six instars			Seven instars				
Instar	**Females (114)**	**Males (125)**	**A**	**Females (27)**	**Males (8)**	**B**	**C**	**D**
I	2.841 ± 0.368	2.694 ± 0.463	**	2.667 ± 0.480	2.500 ± 0.534	ns	ns	ns
II	2.239 ± 0.449	2.145 ± 0.376	ns	2.185 ± 0.557	2.125 ± 0.641	ns	ns	ns
III	2.522 ± 0.536	2.435 ± 0.574	ns	2.370 ± 0.492	2.250 ± 0.463	ns	ns	ns
IV	2.726 ± 0.448	2.589 ± 0.494	*	2.407 ± 0.501	2.250 ± 0.463	ns	**	*
V	2.920 ± 0.426	2.839 ± 0.467	ns	2.481 ± 0.509	2.250 ± 0.463	ns	**	**
VI	3.336 ± 0.689	3.258 ± 0.901	ns	2.556 ± 0.506	2.625 ± 0.517	ns	**	*
VII	-----	-----		2.667 ± 0.734	2.375 ± 1.061	ns	---	---
Prepupae	1.726 ± 0.848	1.581 ± 0.903	ns	1.963 ± 0.706	2.000 ± 0.756	ns	ns	ns
Total	18.310 ± 1.763	17.540 ± 2.038	**	19.296 ± 1.815	18.375 ± 1.188	ns	*	ns
Total [1]	17.910 ± 1.721			19.085 ± 1.946			**	

1 Mean value including males and females which developed during the same number of instars. Comparisons of means using a Student t-test, considering different variances, at a significance level of 95% (Ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$) **A** - comparison between six instar females and males; **B** - comparison between seven instar females and males; **C** - comparison between six and seven instar females; **D** - comparison between six and seven instar males.

to the larger size of females (see pupal stage section). In this sense, in species that a pronounced sexual dimorphism and with larger females, the development of larvae which originate females often demands an additional instar (Parra 1991). Thus, considering that the absolute size of caterpillars at the end of their development triggers the process of metamorphosis (Nijhout 1975), due to their larger size, some females of *S. albula* require an additional instar to reach the size required for transformation into a pupae.

The duration of the larval stage, including the prepupal period (Table I) is similar to descriptions for the same species reared under similar temperatures (Novo Padrino and Martínez Reyes 1985, Novo Padrino et al. 1985, La Rosa et al. 1992). However, several temporal differences were detected between sexes and numbers of larval instars. In general, the duration of the first stage was longer than the subsequent three, this longer duration of the first stage is also described for the same species (Martin Zequeira 1982) and for several noctuids including *S. eridania* (i.e. Santos et al. 2005), *S. exigua* (i.e. Azidah and Sofian-Azirun 2006) and *S. frugiperda* (i.e. Santos et al. 2003). Authors such as Novo Padrino and Martínez Reyes (1985), Novo Padrino et al. (1985) and La Rosa et al. (1992) describe a longer duration for the first, followed by the second, third and last instar. The mean total duration of the larvae that were developed through seven instars was significantly higher than through six instars(Table II). Such differences were also noticed among females, but not among males, probably due to the small number of males that were developed through seven instars. The longer duration of larvae that were developed through seven instars in this study is consistent with experiments of other *Spodoptera* species in which longer larval period was associated with an increased number of instars (e.g. Santos et al. 2005, Azidah and Sofian-Azirun 2006).

The difference in the developmental time of female and male larvae that underwent six instars was also significant (Table II). The differences between the duration of the stages was more

pronounced (significant) from the fourth instar on, when it was observed that the duration of the larval stages that went through seven instars was reduced compared with those who had six instars. Although there are no studies that individualize the observations by the number of larval instars and by sex, a similar behavior is described in the study by Azidah and Sofian-Azirun (2006) where, in Table I, the greatest periods of development and differences between S. exigua larvae that went through five, six or seven instars, are at the end of their development, especially during the last instar, including the prepupal period of our results (Table II).

The mean width of the head capsule ranged from 0.285 ± 0.025 mm, in the first instar, to 2.693 ± 0.121 in the last instar, very similar to descriptions by Martin Zequeira (1982), Novo Padrino and Martínez Reyes (1985) and La Rosa et al. (1992) for the same species in Cuba. Also like that of *S. eridania*, which has a similar size (Mayer and Babers 1944 - first instar 0.26 - 0.29 mm; last instar 2.41 - 2.77 mm). However, as demonstrated for several species, depending on the diet, the size of the capsules, especially at the end of development, can vary greatly (eg, Parra et al. 1977, Mattana and Foerster 1988, Santos et al. 2003).

The measurement between the frontal setae (Table III) demonstrated that both in larvae that had six instars and those that went through seven instars showed higher growth rates among the first instars, decreasing progressively until the last, especially noticeable in larvae that underwent seven instars. Similar behavior is obtained by analyzing data described for the same species by Martin Zequeira (1982), Novo Padrino and Martínez Reyes (1985) and La Rosa et al. (1992) and for *S. eridania* by (Mayer and Babers (1944) and Parra et al. (1977).

The largest mean growth rate recorded for larvae that develop through fewer number of instars (Table III) is described for other noctuids, including *S. eridania* (Parra et al. 1977, Mattana and Foerster 1988), and is certainly related to the principle

TABLE III

Distance between frontal setae of *S. albula* larvae at each instar and their respective growth rates, including larvae which developed for six (15 females and 15 males) and seven instars (15 females and 8 males), fed with an artificial diet, under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase).

Instar	Six instars		T	Seven instars	
	Distance between frontal setae (mm)	Growth rate		Distance between frontal setae (mm)	Growth rate
I	0.091 ± 0.015	-----	ns	0.096 ± 0.014	-----
II	0.149 ± 0.022	1.632	ns	0.155 ± 0.025	1.625
III	0.252 ± 0.038	1.688	ns	0.259 ± 0.028	1.664
IV	0.401 ± 0.046	1.592	*	0.379 ± 0.025	1.466
V	0.597 ± 0.060	1.488	**	0.551 ± 0.042	1.453
VI	0.884 ± 0.078	1.481	**	0.817 ± 0.087	1.483
VII	-----	-----	-----	0.953 ± 0.043	1.166
Mean	-----	1.576	-----	-----	1.476

Comparison of means using a Student *t*-test, considering different variances, at a 95% significance level (Ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$)

that the absolute size of caterpillars at the end of development triggers the process of metamorphosis (Nijhout 1975). This also explains the low growth rate between the penultimate and last larval instar of specimens that have undergone additional instars (Table III), also described by Parra et al. (1977) and Mattana and Foerster 1988).

During the prepupal period (Tables I, II), which corresponds to the time when the larvae do not feed and prepare for the pupa stage, a relatively high survival was observed, along with a relatively short duration, without any significant differences between sexes and individuals that underwent six or seven larval instars. Nevertheless, the only data referring to prepupal survival in the literature (La Rosa et al. 1992) indicates 100% survival for this period, regardless of large larval mortality. In any case, *S. albula* was very well adapted to rearing conditions, even during this period, usually considered critical for holometabolous insects due to metamorphosis (Parra 1991).

When observing the number of individuals (N), in Tables II and IV, it appears that (during prepupa) many more individuals from larvae that went through seven instars died (28.571%), than those that went through six instars (4.603%). As in the present study all larvae received the same treatment we consider to be plausible that, within the population, individual variations produced insects that have development difficulties, even in conditions that for most individuals could be considered good. These individuals have behaved differently as observed in other studies with other insects in which additional instars are inserted in poor conditions when larvae fail to reach a species-specific threshold size with the "normal" instar number (Esperk et al. 2007), and that larvae that produced viable adults reached pupation as much as three days earlier than those that eventually failed to successfully complete emergence (Nagoshi 2011).

Examination of different information sources showed that the larvae of *S. albula* already recorded them feeding on at least 55 plant species, from 29 families (Table IV). In addition to the larvae feeding on a wide variety of host plants, they also exhibit some preference for several weeds from which they can migrate to cultivated plants (González-B 1966, Hallman 1979, 1983), and for Fabaceae species that can be used as trap plants (Savoie 1988). The behavior of migrating to host plants other than those where they were born, known as polyphagia at the individual level, is relatively unusual among Lepidoptera, although reported for other representatives of the genus, particularly *S. frugiperda* (Bernarys and Singer 2002).

TABLE IV

Host plants of *Spodoptera albula* larvae from several authors.

Plant Family	Scientific name	Common name	References
1. Aizoaceae	*Trianthema portulacastrum* Linn.	Trianthema	19
2. Amaranthaceae	*Amaranthus dubius* Mart. ex Thell.	Spleen amaranth	6
3.	*Amaranthus spinosus* Linn.	Spiny amaranth	5, 12
4. Apiaceae	*Apium graveolens* Linn.	Celery	2
5.	*Daucus carota* Linn.	Carrot	16
6. Arecaceae	*Elaeis guineensis* J.	Oil palm	14
7. Asteraceae	*Acanthospermum hispidum* DC.	Hispid star bur	6
8.	*Helianthus annuus* Linn.	Sunflower	17
9.	*Lactuca sativa* Linn.	Lettuce	16
10. Bignoniaceae	*Tabebuia* spp.		15
11. Brassicaceae	*Brassica oleracea* var. *capitata* Linn.	Cabbage	8, 11, 13, 16, 18
12. Caricaceae	*Carica papaya* Linn.	Papaya	21
13. Casuarinaceae	*Casuarina* sp.	Casuarina	15
14. Convolvulaceae	*Ipomoea batatas* (Linn.) Lam.	Sweet potato	18
15.	*Ipomoea triloba* Linn.	Littlebell morning glory	6
16. Cucurbitaceae	*Cucumis melo* Linn	Melon	13, 18
17.	*Cucurbita pepo* Linn.	Pumpkin	11, 18
18.	*Citrullus vulgaris* Schrad. Ex Eckl. & Zeyh	Watermelon	11, 18
19. Euphorbiaceae	*Croton hirtus* L'Her	Croton	6
20.	*Manihot esculenta* Crantz	Cassava	18
21. Fabaceae	*Arachis hypogaea* Linn.	Peanut	10, 18
22.	*Cassia tora* Linn.	Tora	5
23.	*Glycine max* (Linn.) Merril.	Soybean	6, 7, 8, 9, 11, 13, 18

TABLE IV (CONTINUATION)

Plant Family	Scientific name	Common name	References
24.	Medicago sativa Linn.	Alfalfa	2
25.	Phaseolus vulgaris Linn.	Bean	3, 9, 10, 11, 12, 13
26.	Pisum sativum Linn.	Pea	2, 9, 13, 16, 18
27.	Vigna unguiculata (Linn.) Walp.	Cowpea	13
28. Iridaceae	Cipura campanulata Ravenna	-----	20
29. Lamiaceae	Mentha arvensis Linn. var. piperacens Malinvaud.	Peppermint	22
30. Liliaceae	Allium cepa Linn	Onion	13, 16, 18
31.	Allium porrum Linn.	Leek	18
32.	Allium sativum Linn.	Garlic	16, 18
33.	Asparagus officinalis Linn.	Asparagus	2, 13, 16
34. Linaceae	Linum usitatissimum Linn.	Flax	8
35. Malvaceae	Gossypium hirsutum Linn.	Cotton	2, 3, 5, 8, 10, 11, 13, 18
36.	Hibiscus spp.	Hibiscus	15
37. Musaceae	Musa paradisiaca Linn.	Banana	18
38. Myrtaceae	Eucalyptus sp.	Eucalyptus	15
39. Nyctaginaceae	Boerhavia erecta Linn.	Erect spiderling	6, 19
40. Pedaliaceae	Sesamum indicum Linn.	Sesame	6, 11, 13
41. Pinaceae	Pinus caribaea Morelet (viveiros)	Caribbean pine	15
42.	Pinus tropicalis Morelet (viveiros)	Tropical pine	15
43. Poaceae	Echinochloa colonum (Linn.) Link	Shama millet	6
44.	Oryza sativa Linn.	Rice	8, 11
45.	Sorghum bicolor (Linn.) Moench	Sorghum	8, 9, 11, 13, 18
46.	Zea mays Linn.	Corn	5, 9, 11, 13, 18
47. Portulacaceae	Portulaca oleracea Linn.	Purslane	7, 12, 19
48. Quenopodiaceae	Beta vulgaris Linn. var. cicla Linn.	Swiss chard	1, 2, 13, 16, 18
49. Scrophulariaceae	Antirrhinum majus Linn.	Snapdragons	8
50. Solanaceae	Capsicum annuum Linn.	Pepper	11, 13, 16, 18
51.	Solanum tuberosum Linn.	Potato	1, 2, 11, 16
52.	Nicotiana tabacum Linn.	Tobacco	11, 18
53.	Solanum melongena Linn.	Brinjal	11
54.	Solanum lycopersicum Linn.	Tomato	2, 8, 9, 10, 11, 13, 16, 18
55. Zygophyllaceae	Kallstroemia maxima (L.) Hook. & Arn.	Big caltrop	19

References: 1 - Wolcott 1936; 2 - Wolcott 1951; 3 - González 1959; 4 - Herrera 1961; 5 - Alcaraz Vieco 1962; 6 - Hallman 1979; 7 - Hallman 1983; 8 - Passoa 1983; 9 - Saunders et al. 1983; 10 - Rosset et al. 1985; 11 - Maes and Tellez Robleto 1988; 12 - Savoie 1988; 13- Coto et al. 1995; 14 Sául-S and Ortiz-G 1998; 15 - Vázquez et al. 1999; 16 - OIRSA 2001; 17 - Pruett and Guamán 2001; 18 - Branch et al. 2003; 19 - Lastres 2007; 20 - Janzen and Hallwachs 2009; 21 - Semillas del Caribe 2010; 22 - Mendonza et al. 2011

Pupal Stage

The obtained sex ratio was 0.515, not differing significantly from a 1:1 ratio ($\chi^2 = 0.227$; $p < 0.05$). In this study, the pupal survival of *S. albula* (Table I), despite relatively high, was lower than that obtained by La Rosa et al. (1992) which indicated 100% pupal survival for the same species whose larvae were fed with tomato at 20°, 25° and 26.7°C and 90.0% at 30°C. Our results are larger than those described for *S. cosmioides*, whose larvae were fed with three artificial diets (Bavaresco et al. 2004 to

59.1 to 86.8%). However, studies that use natural diets described very different values (less than 50 to 100%), depending on the suitability of the plant for each species (i.e. Parra et al. 1977, Bavaresco et al. 2003, Santos et al. 2005, Lopes et al. 2008, Pinheiro et al. 2008).

Female pupae from larvae that underwent six instars developed significantly faster than their male counterparts (Table V), and a similar trend was observed for the insects that underwent seven instars (not significant). These observations of protogyny in *S. albula* pupae are consistent with observations reported for several *Spodoptera* representatives, in which this phenomenon is well documented (eg Santos et al. 1980, Bavaresco et al. 2004, Farahani et al.2011, Nagoshi 2011). However, our results suggest that pupal protogyny in *S. albula* may emerge as a compensation for larval growth, where the duration of female larvae was significantly longer than male larvae (Table II). When the data on the duration of the larval and pupal stages are brought together, there are no significant differences for the duration of the entire immature period between males and females, both for specimens that had six or seven instars (Table V). The duration of larval + pupal development was markedly higher in individuals that had an additional instar, being statistically significant for females (Table V). However, when analyzed together, the larval and pupal duration is not significantly different ($p < 0.05$) between females (n = 128. 27.614 ± 2.323 days) and males (n = 125. 27.064 ± 2.335 days). Thus, these results indicate the importance of biological studies detailing results by sex and by number of larval instars.

TABLE V

***Spodoptera albula* - Mean duration, in days, of pupal stage and larval plus pupal stage and mean weight of the pupae, whose larvae were fed with an artificial diet, under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase), arranged by sex and number of larval instars.**

	Six instars			Seven instars				
Stage(s)	**Females (108)**	**Males (120)**	**A**	**Females (20)**	**Males (5)**	**B**	**C**	**D**
				Duration				
Pupal	9.037 ± 1.013	9.517± 0.944	**	9.650 ± 1.268	10.200 ± 1.789	ns	*	ns
Larval + Pupal	27.346 ± 3.425	27.000 ± 2.312	ns	29.050 ± 2.481	28.600 ± 2.302	ns	*	ns
				Weight				
Pupal	0.217 ± 0.037	0.182 ± 0.431	**	0.221 ± 0.021	0.198 ± 0.031	*	ns	ns

Female pupae were significantly heavier than male, both among individuals who have had six, as with those with seven larval instars. This sexual dimorphism is relatively well documented among representatives of *Spodoptera* (i.e. Habib et al. 1983, Mattana and Foerster 1988, Bavaresco et al. 2004, Santos et al. 2005, Xue et al. 2010), and other Lepidoptera.

The artificial diet and the proposed rearing methodology allowed an overall survival of almost 80% (Table I), above the 75% recommended by Singh (1983). A detailed description of the preparation process will permit that the diet can be repeated in many future studies.

The methodology proposed in this study, specifically enabled a more complete detailing of several biological parameters of *S. albula* with minimal interference in its development. This allowed several unknown inferences such as the duration and the survival of larval instars and sex determination, along with the duration of larval and pupal stages. On the other hand, several comparisons

with parameters of other species were not possible due to the lack of standardization and, especially, lack of detail in the available information.

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RESUMO

Este estudo objetivou detalhar parâmetros biológicos dos estágios imaturos de *Spodoptera albula* (Walker 1857) em condições controladas (25 ± 1°C, 70 ± 10% UR e fotofase de 14 horas) e reunir informações sobre as plantas hospedeiras de suas larvas. Para tanto foram empregadas e validadas novas metodologias de criação e dieta artificial. A viabilidade das fases de ovo, larva, pré-pupa e pupa foi de 94,54; 97,33; 93,84 e 92,34%, respectivamente. A duração média das fases de ovo, larva, pré-pupa e pupa foi de 4,14; 16,37; 1,69; e 9,34 dias, respectivamente. Na fase de larva observou-se que 80,85% das fêmeas e 93,99% dos machos passaram por seis instares e os demais por sete, com protandria larval significativa. As larvas que passaram por seis e sete instares apresentaram razão média de crescimento de 1,58 e 1,48, respectivamente. Foram relacionadas 55 plantas pertencentes a 29 famílias botânicas. As pupas femininas foram significativamente maiores, observando-se protoginia. Tanto a metodologia de criação quanto a dieta larval mostraram-se adequadas, pois permitiram sobrevivência total de praticamente 80% e um detalhamento muito maior das observações relacionadas ao ciclo biológico, especialmente do estágio larval.

Palavras-chave: praga de culturas anuais, lagarta-militar, dieta artificial, desenvolvimento, ciclo de vida.

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