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# Postembryonic development of *Stiphra* sp. X (Orthoptera: Proscopiidae) feeding on *Psidium guajava* L. (Myrtaceae) leaves in the laboratory

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## Abstract

Proscopiidae is an orthopteran family endemic to South America. Some species, such as *Stiphra robusta*, are considered sporadic pests in drought areas in the northeastern region of Brazil. This study establishes, for an undescribed *Stiphra* species, parameters for *stadia* and mortality that accompany its postembryonic development, as well as the sex ratio of females (no. female: total emerged adults) under laboratory conditions (67.4% mean relative humidity and 26.7°C). The adult stages were attained by three different developmental regimes involving different numbers of instars: four (4.8% of the population, males only), five (64.5%, sex ratio 0.24) and six instars (30.7%, sex ratio 0.94). The species is univoltine with the following mean duration for postembryonic development: males 71.8 d to complete four instars, 83.8 d for five instars, 110.7 d for six instars; females 85.6 d for five instars and 103.1 d for six instars. Overall mortality from neonates to adults was 13.1%, highest in the first instar.

## Key words

Pest, sex ratio, life cycle, supernumerary instar

## Introduction

Study of the life history, including life span, development time, mortality rate, reproductive mode, and sex ratio, contributes importantly to an understanding of an insect species (Schowalter 2000). Proscopiidae is an orthopteran family endemic to South America (Mello-Leitão 1939), whose external features are similar to those of the large Phasmatodea: most Proscopiidae are apterous and have developed remarkable camouflage strategies (Richards & Davies 1984), making them very similar to the brown twigs found in the vegetation. They have saltatorial hind legs but rarely jump (Zolessi 1968).

In Brazil the species of this group have many different common names: "mané-magro", "gafanhoto-da-jurema", "maria-mole", "maria-seca", "esperança-de-cavalo", "alma-de-cavalo", "bicho-pau", "cipó-seco", "taquara-seca" (EMBRAPA/CIRAD/FAO 1987, Launois 1984).

One of the most important species of this group in the northeastern region of Brazil is *Stiphra robusta* Mello-Leitão, which is classified as a sporadic pest in drought areas (Duranton *et al.* 1987). Silva *et al.* (1996) consider this species a pest in both the central and the northeastern regions of Brazil. Just like other species of Proscopiidae, this one is found feeding on plants of different families, for instance, Bombacaceae (*Pseudobombax simplicifolium*), Borraginaceae (*Cordia leucocephala*), Burseraceae (*Bursera leptophlocos*), Euphorbiaceae (*Manihot esculenta*, *Ricinus communis*, *Jatropha* sp., *Croton sonderianus*), Poaceae (*Zea mays*), Leguminosae (*Caesalpinia pyramidalis*, *Mimosa hostilis*, *Prosopis juliflora*, *Vigna unguiculata*), Malvaceae (*Gossypium hirsutumn*), Anacardiaceae (*Anacardium occidentale*, *Mangifera indica*, *Schinopsis brasiliensis*), Myrtaceae (*Eucalyptus spp.*, *Psidium guajava*), Lauraceae (*Persea gratissima*) (Silva *et al.* 1968, Launois 1984). As this behavior is considered dangerous to crops, the government of Brazil asks growers to promptly report each new host plant they encounter (EMBRAPA/CIRAD/FAO 1987).

Launois (1984) is the only reference on Proscopiidae that discusses some general biological aspects of *Cephalocoema protopeirae* Amedegnato, a species closely related to *Stiphra*, including its morphometric patterns.

As studies of biological features are rare in Proscopiidae, the purpose of the present study was to establish the duration, in days, of the postembryonic developmental periods, mortality, sex ratio (proportion of females to the total number of individuals, as described in Silveira-Neto *et al.* 1976 for ecological and biological studies), and sex proportion (females: males). Despite their having similar meanings, the last two parameters are both presented due to the fact that they are separately depicted in the literature, the former comparing the number of females to the population in the study, and the latter the number of females to that of males). In addition, some aspects about reproductive behavior for *Stiphra* sp. X (Orthoptera: Proscopiidae) are presented because this species is a potential pest of *Psidium guajava* (Myrtaceae) (guava) trees, both in backyards and commercial orchards in the northeastern region of Brazil. According to Prof. Alba Bentos-Pereira (pers. comm.), a specialist in taxonomy of Proscopiidae at the Uruguay College of Sciences, this is a new species that belongs to the genus *Stiphra*. She will describe and name it.

## Methods

Rearing began when 5 females and 2 males were hand-picked from guava trees in Maceió, Alagoas, Brazil. They were carried to the laboratory in a plastic container (a box with dimensions 18 X 13 X 12 cm) covered with organdy and held by a rubber band, containing fresh guava leaves. Direct sunlight was avoided.

The bioassay was carried out under an average relative humidity of 67.4% and a temperature of 26.7°C, and a 12:12h photoperiod. These field specimens were maintained in a cylindrical glass container (25 cm diameter, 35 cm high) feeding on fresh stems of guava with these held in a jar of water to maintain turgidity. To obtain eggs for

**Table 1**. Frequency of male and female adults and sex ratio of females per different numbers of nymphal instars in *Stiphra* sp. X (Orthoptera: Proscopiidae) feeding on the leaves of *P. guajava* (Myrtaceae) (guava) in the laboratory (67.4% relative humidity, 26.7°C, 12:12h photoperiod).

| Number of nymphal instars | Frequency | | Total | | Sex ratio (females) |
|---|---|---|---|---|---|
| | Males | Females | Absolute | Relative (%) | |
| 4 | 8 | 0 | 8 | 4.8 | 0.00 |
| 5 | 81 | 26 | 107 | 64.5 | 0.24 |
| 6 | 3 | 48 | 51 | 30.7 | 0.94 |
| Total | 92 | 74 | 166 | 100.0 | 0.45 |

the bioassay, a plastic vial (9 cm diameter, 7 cm high), filled with slightly wet sand, was put in the glass container for oviposition.

The first instar specimens preferred to eat dehydrated leaves, and the biggest nymphs fresh ones. The food was replaced daily. All nymphal stages were reared independently in glass vials (300–500 ml) covered with an organdy cloth, securely fixed with a rubber band.

Daily observations were made in order to record the date of ecdysis. Both sexes could be determined, even in early instars, by analyzing their terminalia (Launois 1984). The sex ratio was calculated as shown by Silveira–Neto *et al.* (1976).

Additional observations on reproductive behavior were made on adults (a total of 37 specimens: 27 males and 10 females, sex ratio = 0.27). They were reared in a 0.2 $m^3$–volume cage (0.50 X 0.34 X 1.14 m) with a large living guava branch (0.70 m) inside. To prevent the dehydration of the fresh leaves, the branch was put in a container with water.

A voucher sample of adult specimens is deposited in the Entomological Collections of the Museu Nacional do Rio de Janeiro and the Uruguay College of Sciences. Descriptive statistics were determined for each period.

## Results and Discussion

Even under confined rearing conditions, ecdysis occurred normally. A few specimens had trouble shedding the leg cuticle, but did achieve the adult stage.

*Number of instars and sex ratio.*—An overview of the distribution of instars in the population is shown in Table 1. Among the specimens that attained the adult stage, 64.5% had 5 nymphal instars, 30.7% showed a 6th supernumerary instar, and in 4.8% only 4 instars were observed. The number of instars may vary, and in some species the number of male instars might be different from the female number (Romoser & Stoffolano 1994). Launois (1984), while studying the field population structure of *C. protopeirae*, concluded that males had an average of 5 instars and females 6: the 4–instar cycle was not detected.

According to Parra (2000), variation in the number of instars in a given species can be related to such factors as heredity, rearing method, temperature, insect nourishment, gender, and parasitism. Since this bioassay was carried out under a careful routine involving adequate and abundant food and low fluctuation in daily temperature, both sexes developed with 5 or 6 instars, predominantly male in the first case and female in the second. A fourth instar development was observed only in males.

Further studies should be carried out in order to establish the cause of this phenomenon. The predominance of a 6–instar development in females is expected "due to their reproductive role, which demands a longer development time"; moreover, in competing to gain mates, and also to facilitate copulation (Parra 1991), "males tend to emerge earlier" (Parra 2000). According to this same author, "no correlation exists between cycle duration and the number of instars, but depending on the insect habit, an extra instar could be required ".

Launois (1984), studying the population structure of *C. protopeirae*, detected two different instances of egg eclosion, both soon after rain spells: eggs hatching in December produced adults between April and May; eggs hatching in March produced adults in July or August.

As one of the characteristics that can be measured and described in populations (Romoser & Stoffolano 1994, Daly *et al.* 1998), the population sex ratio at eclosion (number of females/n) was 0.45 (1: 1.24 proportion of females to males). Throughout their development, however, three different ratios were registered for the three different instar regimes. Among the individuals that went through 4 instars in their development, only males were detected (sex ratio = zero). In the case of the five–instar cycle, the sex ratio was 0.24 (1: 3.12), and in the supernumerary instar the sex ratio was 0.94 (1:16).

The choice of this parameter is important because the proportion of females is a sign of the reproductive potential of a population (the importance of sexual reproduction, mating system, and capacity for reproduction): a mean sex ratio near 50:50 generally indicates equally important roles for males and females, in view of the fact that selection would minimize the less reproductive sex; this sex ratio maximizes availability of males to females, and hence maximizes genetic heterogeneity (Schowalter 2000).

*Length of the nymphal stadia.*—Time to attain the adult stadium was assessed in two dimensions: absolute and relative. The relative view (percentage) permits further comparisons with data from other related species, even under different laboratory conditions (humidity, temperature, and food). Launois (1984), in a study with *C. protopeirae*, established the duration (approximate data), but did not present a statistical analysis, rather a panoramic view with a graphic, showing that it is a univoltine species with two egg–eclosion phases, coinciding with the rainy periods: the first one occurring at the end of the year, and the other 3 mo later.

Duration (absolute values) for the 3 instar regimes per sex is given in Tables 2 and 3. The supernumerary instar enlarged the cycle within the species by approximately 30 d. The first instar is longest in male cycles, while in female cycles its length is similar to the last instar (either 5th or 6th). This can be justified because

**Table 2.** Duration (days) of nymphal instars for males of *Stiphra* sp. X, feeding on the leaves of *P. guajava* (Myrtaceae) (guava) (67.4% relative humidity, 26.7°C and 12:12h photoperiod).

| Development Regimes | Stage | Duration (days) | | | CV** (%) | Confidence Interval of the Mean |
|---|---|---|---|---|---|---|
| | | Mean ± $s_{\bar{x}}$* | Mode/Median | Amplitude (Min—Max) | | |
| 4 nymphal Instars (n=8) | N1 | 21.9 ± 1.7 | 22/22 | 15(16–31) | 22.3 | 17.8–26.0 |
| | N2 | 16.4 ± 0.7 | 15, 16/16 | 6(14–20) | 11.7 | 14.8–17.9 |
| | N3 | 15.6 ± 0.9 | 14/14.5 | 7(13–20) | 16.4 | 13.5–17.8 |
| | N4 | 17.9 ± 0.8 | 16, 19/18 | 6(15–21) | 12.1 | 16.1–19.7 |
| | Total | 71.8 ± 3.4 | 85/71 | 25(60–85) | 13.5 | 63.7–79.8 |
| 5 nymphal Instars (n=81) | N1 | 20.6 ± 0.4 | 18/20 | 18(16–34) | 17.5 | 19.8–21.4 |
| | N2 | 15.5 ± 0.2 | 16/16 | 7(12–19) | 10.4 | 15.2–15.9 |
| | N3 | 14.7 ± 0.2 | 15/15 | 13(11–24) | 14.1 | 14.2–15.1 |
| | N4 | 15.5 ± 0.2 | 14/15 | 11(11–22) | 13.8 | 15.0–15.9 |
| | N5 | 17.5 ± 0.2 | 17/17 | 12(14–26) | 11.7 | 17.0–17.9 |
| | Total | 83.8 ± 0.8 | 77/83 | 31(73–104) | 8.7 | 82.2–85.4 |
| 6 nymphal Instars (n=3) | N1 | 25.3 ± 2.9 | –/26 | 10(20–30) | 19.9 | 12.8–37.8 |
| | N2 | 16.7 ± 0.9 | –/17 | 3(15–18) | 9.2 | 12.9–20.5 |
| | N3 | 17.3 ± 0.7 | 18/18 | 2(16–18) | 6.7 | 14.5–20.2 |
| | N4 | 18.0 ± 1.0 | 19/19 | 3(16–19) | 9.6 | 13.7–22.3 |
| | N5 | 17.3 ± 0.7 | 18/18 | 2(16–18) | 6.7 | 14.4–20.2 |
| | N6 | 16.0 ± 2.5 | –/18 | 8(11–19) | 27.2 | 5.2–26.8 |
| | Total | 110.78 ± 7.5 | –/115 | 25(96–121) | 11.8 | 78.3–143.1 |

* Standard error of the mean.

** Coefficient of variation.

**Table 3.** Duration (days) of nymphal instars for females of *Stiphra* sp. X, feeding on the leaves of *P. guajava* (Myrtaceae) (guava) (67.4% relative humidity, 26.7°C and 12:12h photoperiod).

| Development Regimes | Stage | Duration (days) | | | CV** (%) | Confidence Interval of the Mean |
|---|---|---|---|---|---|---|
| | | Mean ± $s_{\bar{x}}$* | Mode/Median | Amplitude (Min–Max) | | |
| 5 Nymphal Instars (n=27) | N1 | 19.5 ± 0.4 | 18/19 | 9(15–24) | 11.0 | 18.7–20.4 |
| | N2 | 15.7 ± 0.2 | 16/16 | 4(14–18) | 7.8 | 15.2–16.2 |
| | N3 | 13.7 ± 0.2 | 13/14 | 3(12–15) | 7.4 | 13.3–14.1 |
| | N4 | 16.1 ± 0.3 | 17/16 | 6(13–19) | 9.6 | 15.5–16.7 |
| | N5 | 20.0 ± 0.4 | 19, 20/20 | 9(17–26) | 11.0 | 19.1–20.9 |
| | Total | 85.6 ± 0.9 | 85/85 | 18(79–97) | 5.5 | 83.7–87.5 |
| 6 Nymphal Instars (n=48) | N1 | 21.2 ± 0.6 | 20/20 | 21(10–37) | 19.3 | 20.1–22.4 |
| | N2 | 16.4 ± 0.2 | 15/16 | 7(13–20) | 10.2 | 15.9–16.9 |
| | N3 | 14.2 ± 0.3 | 13/14 | 9(11–20) | 14.7 | 13.6–14.8 |
| | N4 | 15.9 ± 0.5 | 14/15 | 19(11–30) | 22.4 | 14.9–16.9 |
| | N5 | 16.4 ± 0.3 | 17/16 | 10(12–22) | 13.7 | 15.8–17.1 |
| | N6 | 19.0 ± 0.3 | 18/19 | 13(15–28) | 11.1 | 18.4–19.6 |
| | Total | 103.1 ± 1.4 | 101, 102/101 | 38(89–127) | 9.4 | 100.3–105.9 |

* Standard error of the mean.

** Coefficient of variation

**Table 4.** Mean duration (days) and relative duration of nymphal instars of *Stiphra* sp. X feeding on the leaves of *P. guajava* (Myrtaceae) (guava) (67.4% relative humidity, 26.7 °C and 12:12h photoperiod).

| Sex | Development | Instar | Duration (days) | |
|---|---|---|---|---|
| | | | Absolute | Relative (%) |
| Male | 4 nymphal Instars (n = 8) | N1 | 21.9 | 30.5 |
| | | N2 | 16.4 | 22.8 |
| | | N3 | 15.6 | 21.8 |
| | | N4 | 17.9 | 24.9 |
| | | Total* | 71.8 | 100.0 |
| | 5 nymphal Instars (n = 81) | N1 | 20.6 | 24.6 |
| | | N2 | 15.5 | 18.5 |
| | | N3 | 14.7 | 17.5 |
| | | N4 | 15.5 | 18.5 |
| | | N5 | 17.5 | 20.9 |
| | | Total* | 83.8 | 100.0 |
| | 6 nymphal Instars (n = 3) | N1 | 25.3 | 22.9 |
| | | N2 | 16.7 | 15.1 |
| | | N3 | 17.3 | 15.7 |
| | | N4 | 18.0 | 16.3 |
| | | N5 | 17.3 | 15.6 |
| | | N6 | 16.0 | 14.4 |
| | | Total* | 110.6 | 100.0 |
| Female | 5 nymphal Instars (n = 27) | N1 | 19.5 | 23.0 |
| | | N2 | 15.7 | 18.5 |
| | | N3 | 13.7 | 16.1 |
| | | N4 | 16.1 | 18.9 |
| | | N5 | 20.0 | 23.5 |
| | | Total* | 85.0 | 100.0 |
| | 6 nymphal Instars (n = 48) | N1 | 21.2 | 20.6 |
| | | N2 | 16.4 | 15.9 |
| | | N3 | 14.2 | 13.8 |
| | | N4 | 15.9 | 15.4 |
| | | N5 | 16.4 | 15.9 |
| | | N6 | 19.0 | 18.5 |
| | | Total* | 103.1 | 100.0 |

* Value obtained by summing the means of the duration of each instar.

females have to accumulate more energy to produce and lay eggs. Despite different mean values (in each different development cycle), the duration data did not differ statistically (*t* test, 5%). Data on relative duration are in Table 4.

*Mortality during development*.—The initial number of insects hatching was 191. During the course of development, 25 individuals died before achieving the adult stage, a 13.1% mortality rate. Eighty–four percent (21) of the deaths occurred at the first instar (highest mortality), 4% (1 individual) at the 2nd and 4th instars, and 8% (2) at the 5th nymphal instar. Deaths were not registered for the 3rd instar.

*Copulation and oviposition behavior.*—Although the focus of this investigation was upon postembryonic development, some remarks pertaining to adult specimens are reported. During mating behavior the male remains on top of the female most of the time (Fig. 1A), mating frequently (Fig. 1B). The male dismounts only to feed.

The number of males was higher than the number of females (27:10, sex ratio 0.30) which triggered fights in their midst. Both males and females suffered injuries that, in some instances, led to their bleeding to death – a great amount of hemolymph (dark green) was produced from lesions on the abdomen. The fights notwithstanding, copulation did occur, though at times interrupted by the

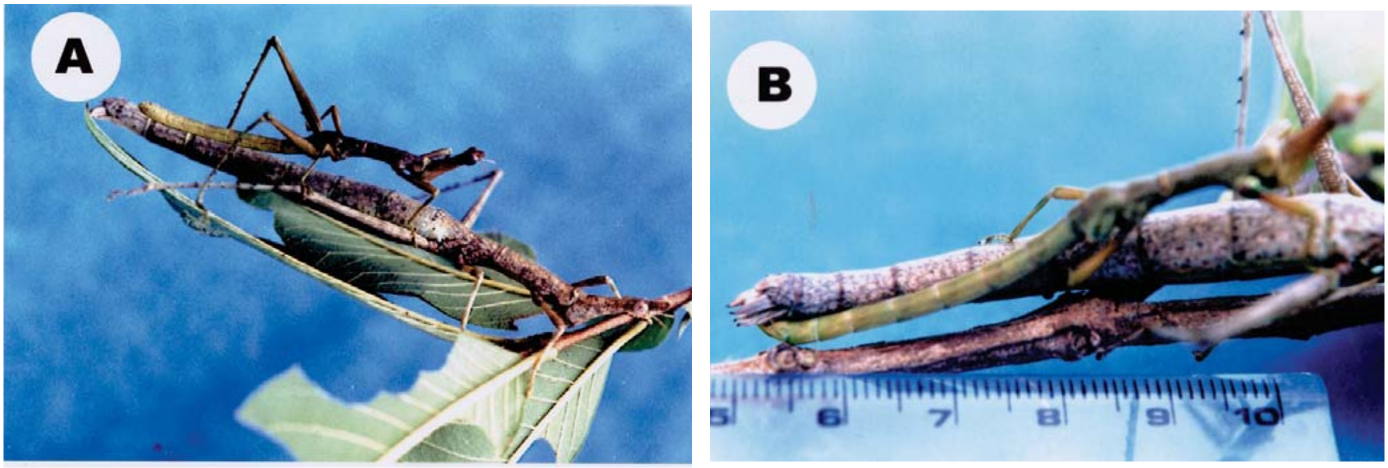


**Fig. 1.** *Stiphra* sp. X (Orthoptera: Proscopiidae). A. Pair with the male atop the female. B. Closer view of engaged genitalia.

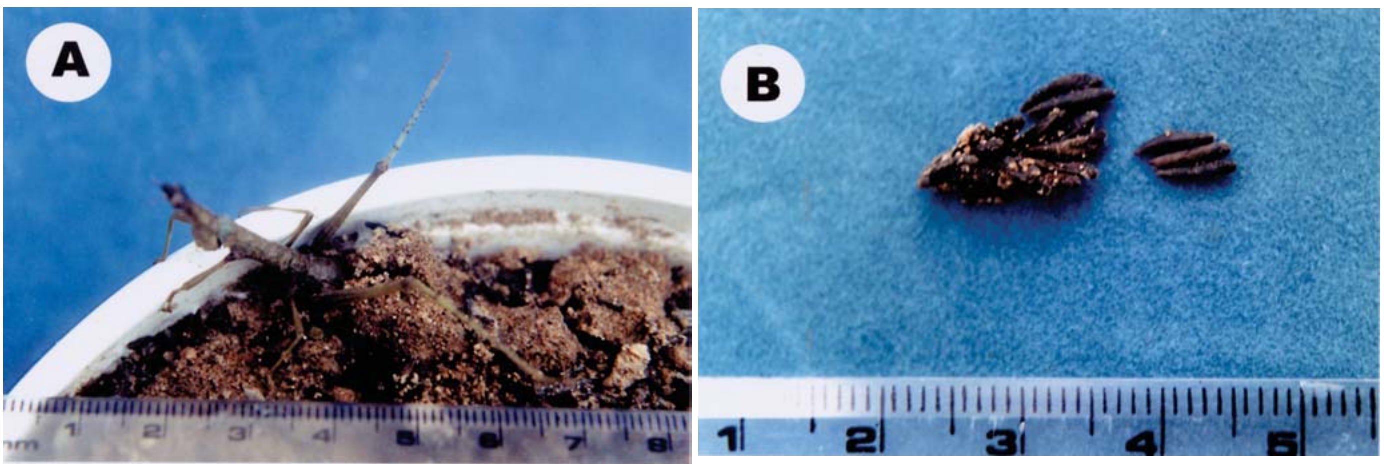


**Fig. 2.** *Stiphra* sp. X (Orthoptera: Proscopiidae). A. Female laying eggs in the soil. B. Egg batch.

arrival of one or more males. Aggressive behavior was not observed in monogamous couples. These observations were made within a laboratory setting. There are no available field data to allow for a comparison. Launois (1984) reported that 2 or 3 males could be carried by a single female, when there is a great number of the male sex in a population.

Oviposition takes place only in the soil, not unlike *S. robusta* and *C. protopeirae* (Launois 1984). The female remains still with the whole of the abdomen buried (Fig. 2A). The eggs are pale when laid, but turn dark brown in 24 h, and are glued in pyramidal batches (Fig. 2B).

## Conclusions

This is a univoltine species, with a long life cycle, including extended longevity in the adults: 10 mo after the eclosion of the eggs, most adults were alive and mating, with the females still laying eggs. For the first time a 4–instar cycle for males is registered for this orthopteran family. Most of the males emerged earlier: all of the 4–instar cycle individuals (sex ratio = 0) and the predominant sex among the 5–instar specimens (sex ratio = 0.24). Females presented 5, though more frequently, 6 instars (sex ratio = 0.94).

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