

Biological aspects of *Leucothyreus ambrosius* Blanchard (Coleoptera, Melolonthidae, Rutelinae)

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ABSTRACT. Biological aspects of *Leucothyreus ambrosius* Blanchard (Coleoptera, Melolonthidae, Rutelinae). Coleopterans of the family Melolonthidae comprise a large group of species that feed on different food sources, including plant roots, stems, and leaves, in addition to plant materials at different decomposition stages. Several species are found in the genus *Leucothyreus*, occurring in different regions of Brazil, including the various biomes in the country. Information on the biology of species of the genus *Leucothyreus* is scarce, therefore, we conducted studies on the biological aspects of *Leucothyreus ambrosius* Blanchard, 1850. The period of adult occurrence was determined with a light trap installed between a cropped and pasture area in the municipality of Aquidauana, Mato Grosso do Sul State, Brazil. Adults collected in the field were used to form insect pairs and the studies were initiated in the entomology laboratory as the adults began ovipositing. Adults were observed flying in the field from October to December. Eggs were obtained as pairs were formed and a colony was established, the embryonic period lasting 14.6 days on average. The larval period in the 1st instar lasted 21.6 days, in the 2nd instar 19.6 days, and in the 3rd instar, 85.6 days. The head capsule width was 1.48 mm in the 1st instar, 2.44 mm in the 2nd, and 3.83 mm in 3rd larval instar. The pupal stage had an average duration of 35.5 days. The egg to adult period lasted 173.3 days. Morphometric information for the larval and adult stages is presented in this study.

KEYWORDS. Geniatiini; Insecta; rizophagous insect; Scarabaeoidea; white grub.

The genus *Leucothyreus* MacLeay comprises 164 species occurring in several countries (Jameson 2008). The larval stages of species of *Leucothyreus* feed on plant roots, damaging them. Puker *et al.* (2009) found larvae of *L. dorsalis* Blanchard, 1850 and *Leucothyreus* sp. in the root systems of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart (Arecaceae) in the state of Mato Grosso do Sul, Brazil. Pereira *et al.* (2013) sampled larvae of *L. alvarengai* Frey, 1976 and *L. aff. semipruinosus* Ohaus, 1917 developed in succession of soybean and corn systems in Tangará da Serra, Mato Grosso State, Brazil. In Colombia, larvae of *L. femoratus* Burmeister, 1844 and other species of this genus are listed as major pests of crops and pastures (Pardo-Locarno *et al.* 2003, 2005, 2006). In Chiapas, Mexico, species of *Leucothyreus* are considered important pests in agricultural areas because, according to Ramírez-Salinas & Castro-Ramírez (2000), the larvae cause damage to roots of corn plants.

Adults of *Leucothyreus* may also be associated with different plant species. While studying Scarabaeidae species in the state of Minas Gerais, Brazil, Oliveira *et al.* (2001) recorded the occurrence of 14 species of *Leucothyreus* in a plantation of *Eucalyptus grandis* Hill ex Maiden. Puker *et al.* (2011) studied adult individuals of *Leucothyreus albopilosus* Ohaus, 1917, in the central-western region of Brazil. The adults were

found in trunk lesions of *Eucalyptus citriodora* Hook and in hives of Africanized bees, *Apis mellifera* Linnaeus, 1758 (Hymenoptera, Apidae), where the adults remained in propolis layers. In Colombia, adults of *L. femoratus* feed on leaves of *Elaeis guineensis* Jacquin and daily consumption reaches 13 mm²/insect (Martínez *et al.* 2013), causing severe defoliation.

Some information on the biological characteristics of the species of *Leucothyreus* is available. Rodrigues *et al.* (2010a) studied biological aspects of *L. dorsalis* occurring in the central-western region of Brazil, and observed that the egg to adult period is completed in 273.5 days. According to Pereira *et al.* (2013), adults of *L. alvarengai* and *L. aff. semipruinosus* are found in the field at the beginning of the rainy season, and during this period, the reproductive period begins and the larval stages are completed in less than a year. In Colombia, the egg-to-adult period of *L. femoratus* lasts 170.4 days (Martínez & Plata-Rueda 2013).

The objective of this study was to investigate on the biological aspects of *Leucothyreus ambrosius* Blanchard, 1850 in the municipality of Aquidauana, Mato Grosso do Sul, Brazil. According to Morón (2004), in Brazil, 83 species of *Leucothyreus* have been reported and the occurrence of *L. ambrosius* has been observed only in the state of Mato Grosso, Brazil.

MATERIAL AND METHODS

The studies were conducted at *Universidade Estadual de Mato Grosso do Sul* (UEMS), in the municipality of Aquidauana, Mato Grosso do Sul State, Brazil. To collect *L. ambrosius* adults, a light trap model “Luiz de Queiroz” equipped with a fluorescent light bulb (20 watts) was installed between pastures and cropped areas, from January 2010 to December 2011. The light bulb in the trap was turned on every day at 6 p.m. and off at 6 a.m. the next day.

The adults of *L. ambrosius* collected were kept in 4 L plastic tray (20x30x12 cm), with a 5 cm layer of soil and seedlings of *Brachiaria decumbens* Stapf. Each tray was identified and covered with voile fabric to prevent the insects from escaping (Rodrigues *et al.* 2008a). Two insect pairs were kept per tray for copulation and oviposition. The trays were inspected daily to observe the presence of eggs.

After oviposition, the eggs were transferred to Petri dishes containing moist soil and the dishes were kept in an incubator at $26 \pm 1^\circ\text{C}$ and photophase of 12 h. The material was inspected at intervals of 3–4 days to monitor embryonic development and the emergence of larvae (Rodrigues *et al.* 2010a).

After hatching, the larvae were separated in plastic jars (500 mL) containing soil and seedlings of *B. decumbens*. The soil was moistened weekly and the seedlings were replaced by fresh ones every 15 days to supply roots for the larvae to feed on.

As the pre-pupae started to appear, seedlings of *B. decumbens* were no longer supplied. Observations were made on the egg to adult period. The pupae were kept under the same conditions as the pre-pupae.

The head capsule measurements were obtained for the larvae ($n = 20$) at the largest widths, similar to procedures of Rodrigues *et al.* (2008a) during their study on the biological aspects of *Anomala testaceipennis* Blanchard, 1856.

Adults of *L. ambrosius* were sexed, and some of them were measured, killed and kept in a drying oven for 48 hours ($60 \pm 5^\circ\text{C}$) to be weighed. The biological variables evaluated included the duration of the embryonic, larval, and pupal periods, and adult longevity.

The adults were identified by the third author by means of comparisons with material identified and deposited at the *Zoologische Museum der Humboldt Universität zu Berlin*, Germany, and with original descriptions by Blanchard (1851) and Ohaus (1917, 1918, 1924, 1931).

Twenty adults of *L. ambrosius* were deposited in the collection of the Entomology Laboratory, *Universidade Estadual de Mato Grosso do Sul*, in the municipality of Aquidauana, Mato Grosso do Sul, Brazil, and eight adults were deposited in the *Colección Entomológica, Instituto de Ecología, A.C.* Xalapa, Veracruz, Mexico (IEXA).

RESULTS

From January to September 2010, no adults of *L. ambrosius* were collected with the light trap. However, eight adults were collected in October, three in November, and five

in December. In 2011, similarly to 2010, no adults were collected in the field from January to September. Twenty-two adults were collected in October and seven in November. The flight period of adults in both years occurred during the rainy season. Thus, the occurrence of adults in the field refers to the period from October to December (Fig. 1).

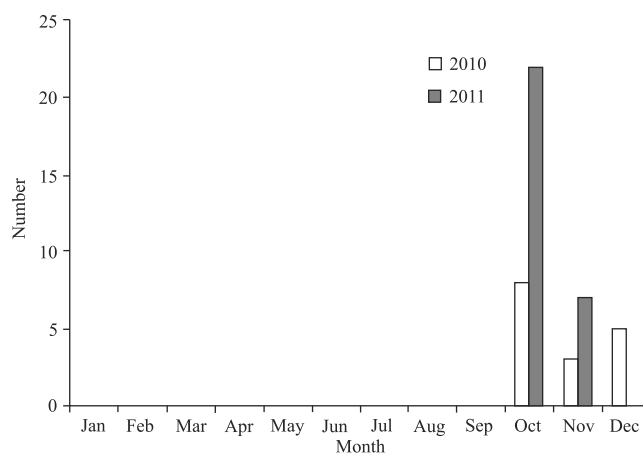


Fig. 1. Number of adults of *Leucothyreus ambrosius* Blanchard, 1850 collected in light traps from January 2010 to January 2011 in Aquidauana, Mato Grosso do Sul State, Brazil.

Eggs of *L. ambrosius* were laid during the months of October and November in the rearing trays in the laboratory (Fig. 2). The eggs were white, placed individually in chambers made in the soil, internally measuring 2.5 mm (length) by 2.1 mm (width) ($n = 25$). Freshly laid eggs were oval, measuring 2.0 mm in length and 1.9 mm wide on average. As the embryonic development progressed, the eggs reached 2.4 mm in length and 2.0 mm wide on average ($n = 36$). The embryonic period lasted 14.6 days on average (Table I).

Table I. Duration (mean \pm SE¹) of the developmental stages of *Leucothyreus ambrosius* Blanchard, 1850 under laboratory conditions ($26 \pm 1^\circ\text{C}$, photoperiod of 12 h).

Stage	Duration (days)	Interval (days)	N	Variability (%)
Egg	14.6 \pm 0.9 ¹	13 – 16	37	83.78
1 st instar	21.6 \pm 3.2	18 – 28	29	93.54
2 nd instar	19.6 \pm 0.9	18 – 21	23	79.31
3 rd instar	85.6 \pm 3.2	81 – 90	23	100.0
Larval duration	123.0 \pm 1.8	121 – 127	21	91.3
Pupae	35.5 \pm 5.5	30 – 41	21	100.0
Egg to adult	173.3 \pm 2.4	170 – 178	20	95.2

¹SE = Standard error of the mean.

The duration of the 1st instar of the larval stage was 21.6 days on average. The larval stage was observed in the rearing trays in October and November (Table I; Fig. 2). Newly hatched larvae measured 5.3 mm in length and 1.2 mm wide at thorax, on average, and they were slightly grey (Table II). The head capsule width was 1.48 mm, on average.

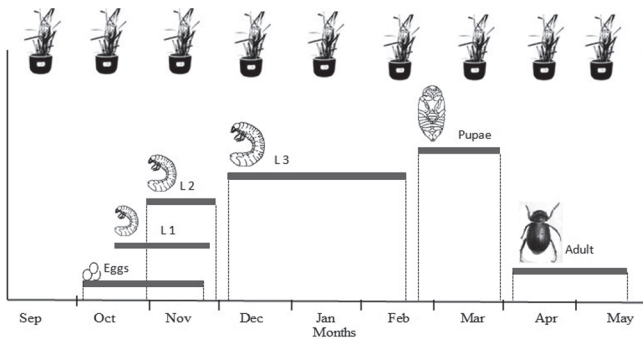


Fig. 2. Development of immature stages (eggs, larvae, pupae) and adults of *Leucothyreus ambrosius* in trays with seedlings of *Brachiaria decumbens*, reared in the laboratory ($26 \pm 1^\circ\text{C}$, photoperiod of 12 h). (L1 = first-instar larvae, L2 = second-instar larvae, and L3 = third-instar larvae).

The duration of the 2nd instar of the larval stage was 19.6 days, occurring in November (Table I; Fig. 2). The head capsule width in the 2nd instar was 2.44 mm and the larvae measured 16.6 mm in length and 3.8 mm wide at thorax on average (Table II).

The larvae remained in the 3rd instar for 85.6 days on average, occurring from early December to late February (Table I; Fig. 2). The head capsule width was 3.83 mm and the average larval length was 22.1 mm, with an average of 4.5 mm wide at thorax. The entire larval period lasted 123.0 days, on average (Table I).

At the end of 3rd instar, the larvae made a pupal chamber at the bottom of the rearing tray, measuring 2.1 x 1.5 cm internally and 3.5 x 2.1 cm externally ($n = 6$). The chambers probably consisted of soil and saliva, and the pupae remained in the chamber without feeding until they reached the adult stage. The pupal chamber served as shelter for the larvae, since the chamber structure hardly collapsed when handled in the laboratory. This microenvironment provides appropriate conditions for development.

Regarding the larval weight of *L. ambrosius*, larvae in the 1st instar weighed 4.4 mg, in the 2nd instar 162.0 mg, and in the 3rd instar 324.0 mg (Table II). The larvae increased their weights 73.6 times from the 1st to the 3rd instar.

In January, larvae in the 3rd instar started to signal that they were reaching the pre-pupal stage as their activities decreased, feeding stopped (Fig. 2), and the color changed gradually going from grey to white.

Pupae of *L. ambrosius* were observed from late February through late March (Fig. 2). In the laboratory, the pupal stage had an average duration of 35.5 days (Table I). The pupae measured 18.0 mm in length and 7.4 mm wide at thorax, with an average weight of 342.0 mg (Table II). Typically, the pupae were dark yellow on their dorsal region and light yellow on the ventral region. The adults started to emerge under laboratory conditions in early April and were observed until late May (Fig. 2).

Regarding adult size, females were on average 13.2 mm in length and 7.0 mm wide and they were larger than males, which measured 12.3 mm in length and 6.2 mm wide. Adult females were also heavier than adult males, weighing 56.9 mg and 35.7 mg, respectively (Table II).

Adults of *L. ambrosius* can be sexed based on the tarsomers of the first pair of legs, which in males are wider, thus male individuals can be easily identified (Fig. 3). The duration of the egg to adult period of *L. ambrosius* is 173.3 days (Table I; Fig. 2).



Fig. 3. Adults of *Leucothyreus ambrosius*: female (left) and male (right). Scale = 1 cm.

DISCUSSION

Adults of *L. ambrosius* in Aquidauana, MS, occurred during the rainy season. According to Rodrigues *et al.* (2010a), swarms of *L. dorsalis* apparently start to occur in the field during the first rains. Thus, in September 2006, after an accu-

Table II. Average values (\pm SE¹) for length, width, and weight of the developmental stages of *L. ambrosius* Blanchard, 1850 in the laboratory ($26 \pm 1^\circ\text{C}$, photoperiod of 12 h).

Stage	Length (mm)		Width (mm)		Weight (mg)	
	Mean \pm SE	Interval	Mean \pm SE	Interval	Mean \pm SE	Interval
Egg	2.0 \pm 0.07	1.9 – 2.1	1.9 \pm 0.27	1.80 – 2.35	–	–
1 st instar	5.3 \pm 0.24	4.4 – 6.1	1.2 \pm 0.02	1.12 – 1.3	4.4 \pm 0.0	3.9 – 5.1
2 nd instar	16.6 \pm 0.77	13.8 – 18.4	3.8 \pm 0.13	3.47 – 4.19	162.0 \pm 0.01	137.4 – 191.0
3 rd instar	22.1 \pm 1.16	14.2 – 29.6	4.5 \pm 0.10	3.82 – 5.1	324.0 \pm 0.03	161.8 – 525.0
Pupa	18.0 \pm 0.28	17.5 – 18.5	7.40 \pm 0.20	7.0 – 7.7	342.0 \pm 0.01	34.0 – 344.0
Adult ♂	12.3 \pm 0.12	12.0 – 12.6	6.2 \pm 0.13	5.98 – 6.61	35.7 \pm 0.02	29.8 – 40.6
Adult ♀	13.2 \pm 0.15	12.9 – 13.5	7.0 \pm 0.23	6.8 – 7.52	56.9 \pm 0.04	47.1 – 63.3

¹SE = Standard error of the mean.

mulated rainfall of 51.6 mm, adults began to be collected. In the following year, adults were first collected in October 2007, after an accumulated rainfall of 56.2 mm. According to Pereira *et al.* (2013), at the beginning of the reproductive period of *L. alvarengai* and *L. aff. semipruinosus*, adults began to fly and soon after they started ovipositing in the field in September. Possibly, the beginning of the rainy season in the central-western region of Brazil may indicate the beginning of the flight period of adults of *Leucothyreus*.

Possibly, the occurrence of adults of *L. ambrosius* and their reproductive cycle are well adapted to the hot and humid climate conditions in the region of Aquidauana, MS, where the studies were carried out. Adults were captured with a light trap, thus, the insects swarmed at night. Adults of *L. dorsalis* were collected from 7 p.m. to 5:30 a.m. (Rodrigues *et al.* 2010a). Pardo-Locarno *et al.* (2006) stated that *L. femoratus* adults have crepuscular habits.

Eggs of *L. ambrosius* are larger than those of *L. dorsalis* (1.8 mm in length and 1.5 mm wide) (Rodrigues *et al.* 2010a), however, the embryonic periods in both species are similar. The embryonic period of *L. femoratus* is 8.73 days (Martínez & Plata-Rueda 2013), therefore, shorter than that of *L. ambrosius*.

The behavior of adults of *L. ambrosius* laying eggs in chambers built in the soil has also been observed in other species of Melolonthidae, such as *Liogenys fusca* Blanchard, 1850, *L. dorsalis*, and *Cyclocephala verticalis* Burmeister, 1847 (Rodrigues *et al.* 2008b, 2010a, b). The chamber made for egg laying can be used as shelter to hatch larvae.

The head capsule widths of larvae of *L. ambrosius* were 1.48 mm, 2.44 mm, and 3.83 mm for the 1st, 2nd, and 3rd instars, respectively. In *L. dorsalis*, the head capsule widths for the 1st, 2nd, and 3rd larval instars were 1.0, 1.8, and 2.8 mm, respectively (Rodrigues *et al.* 2010a). Thus, larvae of *L. ambrosius* are larger than *L. dorsalis* and *L. femoratus* because, according to Pardo-Locarno *et al.* (2006), larvae in the 3rd instar have maximum head capsule widths ranging from 3.0 to 3.2 mm.

The larvae of the 3rd instar of *L. ambrosius*, which are about to pupate, built a chamber in the soil where they remain sheltered. This behavior has been observed in several species of Melolonthidae, such as *L. femoratus* (Martínez & Plata-Rueda 2013), *Anomala testaceipennis* Blanchard, 1850 and *L. fuscus* (Rodrigues *et al.* 2008ab), *C. verticalis* (Rodrigues *et al.* 2010b), and *P. fulva* (Rodrigues & Falco 2011). Pupae of *L. ambrosius* are larger than those of *L. femoratus*, which are light yellow and measure about 14–16 mm in length and 7–12 mm wide (Pardo-Locarno *et al.* 2006).

The egg to adult period in *L. ambrosius* is completed in 173.3 days, in *L. femoratus* in 170.4 days (Martínez & Plata-Rueda 2013), and much shorter than *L. dorsalis*, which lasts 273.5 days, according to Rodrigues *et al.* (2010a). In *L. alvarengai* and *L. aff. semipruinosus*, the egg to adult period is completed in less than a year (Pereira *et al.* 2013).

There are no previous records on the occurrence of *L. ambrosius* for the Mato Grosso do Sul State, Brazil. There-

fore, in addition to the knowledge about its biological and morphometric aspects, this species is recorded in this State for the first time.

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