An Acad Bras Cienc (2023) 95(4): e20191357 DOI 10.1590/0001-3765202320191357
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Biological aspects and first record of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) in sugarcane (*Saccharum officinarum L*) (Poaceae) fields of Mato Grosso do Sul state, Brazil

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Abstract: Larvae of *Leucothyreus* spp. have been reported causing damage to several crops in Brazil. From May 2012 to April 2013, adults and immatures *of Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) samples were obtained from sugarcane (*Saccharum officinarum* L.) (Poaceae) fields in the municipalities of Naviraí and Nova Andradina, Mato Grosso do Sul, Brazil. Adults were collected with the aid of light traps, whereas immatures were sampled by opening two trench sizes (30 x 30 x 30 cm and 80 x 50 x 40 cm) in the soil, beside the plants. The collected larvae were reared in laboratory conditions, fed by sugarcane seedlings. The biological cycle of *L. alvarengai* was completed in 230 days, displaying three larval instars with mean size of the cephalic capsule of 1.68, 2.46, and 3.00 mm for the first, second, and third instar, respectively. Adults were collected in greater numbers in December 2012, and first-instar larvae were observed as of January 2013. Conversely, pupae were observed in the field from July 2012 to January 2013. This is the first record of larvae and adults of *L. alvarengai* in sugarcane fields in Mato Grosso do Sul state, contributing with the knowledge about its biology and temporal distribution in the field.

Key words: biology of insects, temporal distribution, white grubs, root-feeding pests, Scarabaeoidea.

INTRODUCTION

The genus *Leucothyreus* MacLeay (Coleoptera: Melolonthidae: Rutelinae) includes 164 species that are found from southern Mexico to Uruguay (Jameson & Hawkins 2005). Adults are classified as small or medium (8.0 - 18.0 mm) and have dark brown, black or dark green coloration, with or without metallic luster (Jameson & Hawkins 2005). Grossi & Vaz-de-Mello (2019) reported the occurrence of 91 species and two subspecies of *Leucothyreus* in Brazil, and the following species are known in Mato Grosso do Sul state: *L. marginaticollis* Blanchard (Ferreira & Rodrigues 2017), *L. suturalis* Castelnau (Puker et al. 2015), *L.* aff. *semipruinosus* Ohaus (Pereira et al. 2013), *L. alvarengai* Frey (Pereira et al. 2013), *L. ambrosius* Blanchard (Gomes et al. 2014), *L. albopilosus* Ohaus (Puker et al. 2011, Ferreira et al. 2016), and *L. dorsalis* Blanchard (Puker et al. 2009, Rodrigues et al. 2010).

Studies on *Leucothyreus* species have been conducted mostly in South America, especially in Brazil (Rodrigues et al. 2010, Gomes et al. 2014, Ferreira et al. 2016, Ferreira & Rodrigues 2017) and Colombia (Pardo-Locarno et al. 2005, Martinez & Plata-Rueda 2013, Martinez et al. 2013). Empirical

data have been obtained in these countries and could be used in the management of species associated with agricultural crops, although pest status of this group requires further investigation (Puker et al. 2015).

Larvae of *Leucothyreus* species are important soil pests (Macedo & Macedo 2006). Considering the importance of some *Leucothyreus* species for Brazilian crops, Leite et al. (2012) conducted experiments on biological and chemical control of larvae of an unidentified species of *Leucothyreus* in commercial sugarcane (*Saccharum officinarum* L.) (Poaceae) plantations. Larvae of *L. alvarengai* have been found at high densities in soybean (*Glycine max* (L.) Merr.) (Fabaceae) crops, and some information about their biology is already know, such as: length, average body width and duration of the developmental stages (larva, pupa and adult) and widths of the cephalic capsules in the three larval instars (Pereira et al. 2013).

This study aimed to show information about its biology, including the duration of development from larva to adult as well as the temporal distribution in the field and the first record of immatures and adults of *L. alvarengai* in sugarcane fields in the state of Mato Grosso do Sul, Brazil.

MATERIALS AND METHODS

The study was conducted in sugarcane fields in the municipalities of Naviraí (23°07'S, 54°12'W) and Nova Andradina (21°5'S, 53°25'W), Mato Grosso do Sul, Brazil, which are 156 kilometers away one from the other. The climate of the region, according to the Köppen classification is Aw, that is, tropical hot, with a rainy summer and a dry winter (Alvares et al. 2014). In each of the municipalities a sugarcane (variety RB 935744) area was selected, one belonging to Infinity Bio-Energy (USINAVI) in Naviraí and another to Santa Helena Bio-Energy in Nova Andradina (sugarcane mills). Within a radius of approximately 10 km from the industrial area of the mills, 24 plots were randomly selected, totaling 48 plots sampled in the two sampling environments. Every plot showed the following approximately 1 ha (10.000 m^2).

Immatures and adults of *L. alvarengai* were sampled every 15 days from May 2012 to April 2013. For the collection of the immature specimens, 20 small trenches (30 x 30 x 30 cm) and four large trenches (80 x 50 x 40 cm) were made in the soil in each of the 48 sampled plots, totaling 1,152 trenches during the study period. The trenches were done in and between the plant rows, the first about 50 m from the edge of the field, while the others followed a zigzag walking protocol to cover the entire sampling area.

The day before the collection of the immature specimens, two "Luiz de Queiroz" model light traps were installed per area, with a 45 w UV lamp for the collection of adults. The traps were installed about 1 m from the ground level next to the previously selected field for sampling, with the trap lamp being switched on from 6:00 p.m. on the previous day until 6:00 a.m. on the following day. The captured specimens were counted and packed in vials containing 96% alcohol.

Leucothyreus alvarengai Rearing

Sugarcane seedlings of variety RB 935744 were planted in transparent pots of 500 or 1000 mL, and 30-day-old plants were used to feed *L. alvarengai* larvae obtained from sugarcane fields. The stress caused due to the handling of the rearing is responsible for high mortality of the first instar larvae (Pereira et al. 2013). Therefore, of a total of 186 first-instar larvae collected from sugarcane, 10 of them were kept in a single plastic container (1000 mL) containing moist soil and one sugarcane seedling and were not handled for about 90 days. All other larvae (n = 176) were individually kept in plastic containers (500 mL)

containing moist soil and one sugarcane seedling according to the methodology used by Coutinho et al. (2011).

The transparent containers were inspected weekly to evaluate the cephalic capsule width, body length, and larval biomass, aiming, thus, to monitor the larval development. The instar changes were characterized by the abrupt growth of the cephalic capsule associated to ecdysis. The sugarcane seedlings were kept until the larvae had built their pupal chambers (when they ceased feeding), which was weekly monitored. The emerging adults were killed and prepared as voucher specimens. Which were deposited in the museum of the Entomology laboratory of Embrapa Agropecuária Oeste.

Specimens of *L. alvarengai* (Fig. 1) were identified with original descriptions (Frey 1976) and compared with previously identified specimens (adults and larvae) in the study by Pereira et al. (2013).

RESULTS

Sampling of soil trenches in sugarcane fields yielded larvae (n = 186), pupae (n = 3), and adults (n = 5) of *L. alvarengai* around the roots of the plants. The larvae were found in the three stages of development, with 71 first-instar larvae, 69 second-instar larvae, and 46 third-instar larvae (Fig. 2). Absence of *L. alvarengai* larvae was recorded only during the month of September, whilst a total of 129 larvae (69%) was obtained between January and April, when first-instar larvae predominated (Fig. 2). The first-instar larvae had a mean cephalic capsule width of 1.68 mm, length of 11.71 mm, and biomass of 59.80 mg (Table I). The second-instar larvae had a mean cephalic capsule width of 2.46 mm, a length of 16.64 mm, and a biomass of 110.30 mg, while third-instar larvae remained active on average 89.86 days and had a cephalic capsule width of 3.00 mm, length of 19.46 mm, and biomass of 251.5 mg (Table I). During the third (last) larval instar there was a gradual growth in the cephalic capsule and larval biomass, reaching in the pre-pupal phase an average width of 3.15 mm and biomass of 259.60 mg. The pre-pupae remained inactive for 23 days until they transformed into pupae, when they reduced their biomass to 205.00 mg and remained in this stage for 21.4 days until adult emergence. The larvae collected in the first instar with minimal handling, that were not measured the biological characters such as size of head capsule, weight and body length completed the biological cycle in 230 days. However those of first instar that suffered manipulation did not survive.

However, adults of *L. alvarengai* were collected in the light traps during all the months of sampling (Fig. 3) (n = 402). In the sugarcane fields of Nova Andradina there was a peak in adults in December, when 79.5% of the beetles were found (Fig. 3).

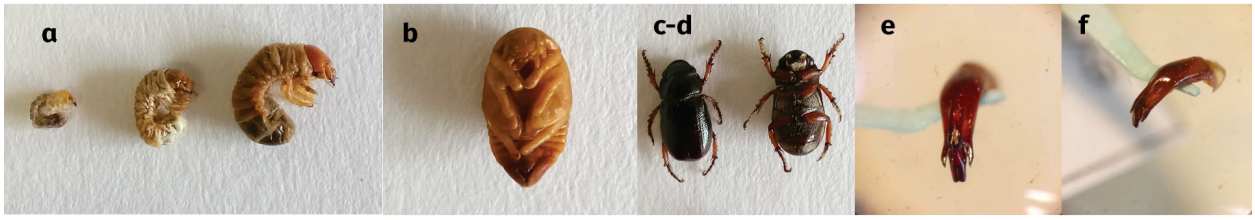

Figure 1. Details of the larvae in the three instars in lateral (L1; L2 and L3) view (a), pupa (b) and of the adult in ventral (c) and dorsal (d) view and aedeagus from front (e) in lateral (f) of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) (scale 1 mm).

DISCUSSION

Larvae, pupae and adults of *L. alvarengai* occurred throughout the sampling period, demonstrating their good adaptability to the environmental conditions of sugarcane cultivation in the state of Mato Grosso do Sul. Sugarcane plants have a dense rhizosphere that provides shelter and food for the specimens that were found just below the plants up to 20 cm deep. Among these species we highlight seven species of Melolontidae belonging to the subfamilies Melolonthinae (n = 2), Dinastinae (n = 4) and Rutelinae (n = 1).

Adults of *L. alvarengai* occurred mainly during the rainy season. The same was observed with *L. ambrosius* in Aquidauana, MS (Gomes et al. 2014) and by Rodrigues et al. (2010), in which *L. dorsalis* occurred in *Brachiaria decumbens* Stapf (Poaceae) fields during the first rains. The collection of insects in the light traps, installed in a seasonal way in short periods, can suffer great influence of climatic conditions (rain, winds and temperature). According to Pereira et al. (2013), the beginning of the reproductive period of *L. alvarengai* and *L. aff. semipruinosus* coincided with the beginning of the rainy season in the

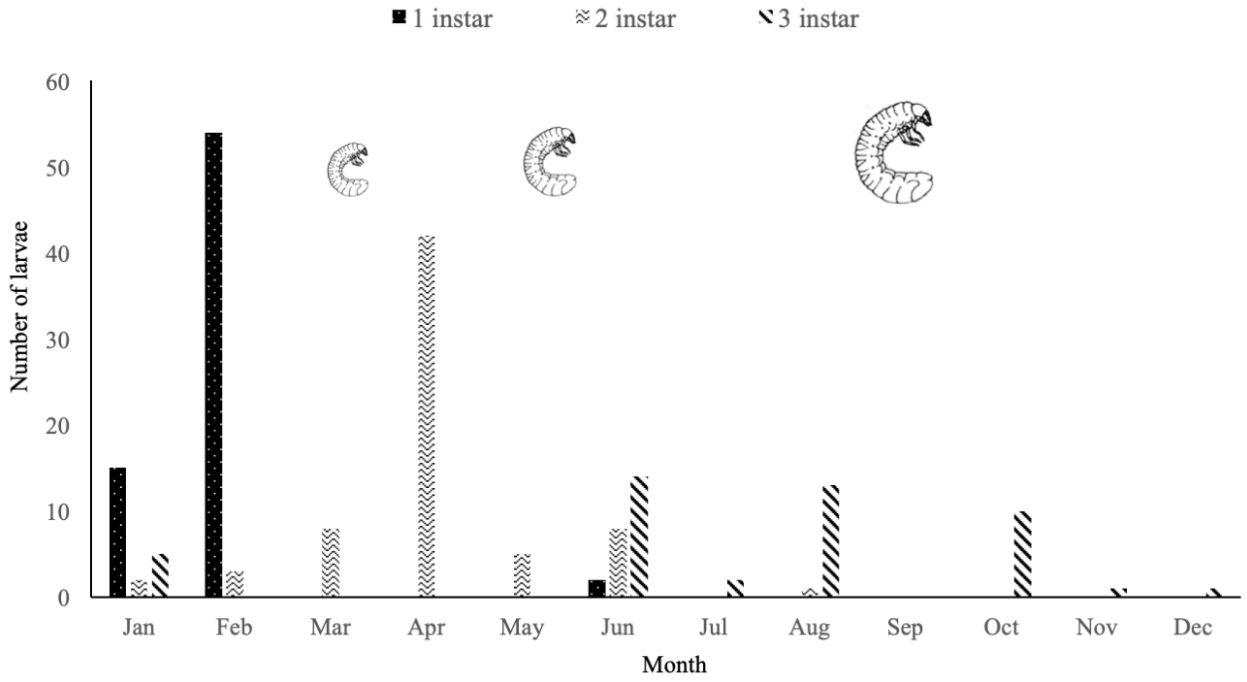

Figure 2. Number of first, second, and third-instar larvae of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) found in sugarcane (*Saccharum officinarum* L.) (Poaceae) fields of Naviraí and Nova Andradina, Mato Grosso do Sul, Brazil, in the period from May 2012 to April 2013.

Table I. Mean (± SE) of cephalic capsule width (mm), length (mm), and biomass (mg) of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) in laboratory (26 ± 1°C, relative humidity of 70 % ± 10, 12-hour photophase).

Instar	Cephalic capsule Mean ± SE	Amplitude	Length Mean ± SE	Amplitude	Biomass Mean ± SE	Amplitude
First	1.68± 0,06	1.48 – 1.89	11.71± 0.68	10.10 – 12.91	59.80± 1.73	56.90 – 62.90
Second	2.46± 0.10	2.29 – 2.76	16.64± 1.02	15.31 – 18.65	110.30± 7.8	96.30 – 123.20
Third	3.00± 0.02	2.80 – 3.19	19.46± 0.48	14.25 – 23.69	251.5± 18.30	102.40 – 424.10

SE=standard error.

study region, corroborating the information obtained in this study. This information suggests that the beginning of the adult flight period of *Leucothyreus* is associated with the rainy season.

The adults of *L. alvarengai* were caught in a light trap after sundown, as was also observed with *L. ambrosius* (Gomes et al. 2014) and *L. dorsalis* (Rodrigues et al. 2010). According to Pardo-Locarno et al. (2006), *L. femoratus* adults have crepuscular habits. Adults of *L. alvarengai* occurred in all months of the year, but the peak (80%) was observed in December. Similar results were found for *L. albopilosus* in which adults were collected during the hottest and wettest periods of the year in Aquidauana (from August to March) and *L. ambrosius* with occurrence from October to December (Gomes et al. 2014, Ferreira et al. 2016).

In areas of soybean and maize (*Zea mays* L.) (Poaceae) cultivation in the summer in the state of Mato Grosso, Pereira et al. (2013) observed the occurrence of adults and larval instars of *L. alvarengai* as of September, and affirmed that the reduction in the number of larvae in the month of July may be related to soil moisture or to the biological cycle of this species. In the studied plots in the municipality of Naviraí, larvae of *L. alvarengai* were found to reach 15.8 larvae m^{-2}. In Brazil, larvae of *L. alvarengai* and *L. semipruinosus* were found in soybean and maize fields in the state of Mato Grosso, with up to 23 larvae m^2 (Pereira et al. 2013).

In this study, the cephalic capsule widths of *L. alvarengai* were 1.68 mm, 2.46 mm, and 3.00 mm for the 1st, 2nd and 3rd instars, respectively. Different values were found for other *Leucothyreus* species. For *L. dorsalis*, the widths of the cephalic capsules for the 1st, 2nd and 3rd larval instars were 1.0, 1.8, and 2.8 mm, respectively (Rodrigues et al. 2010). The widths of the cephalic capsules of *L. ambrosius* were 1.48 mm, 2.44 mm, and 3.14 mm for the 1st, 2nd and 3rd instars, respectively (Ferreira et al. 2016) whereas for *L. ambrosius* these values were 1.32 mm, 2.13 mm, and 3.83

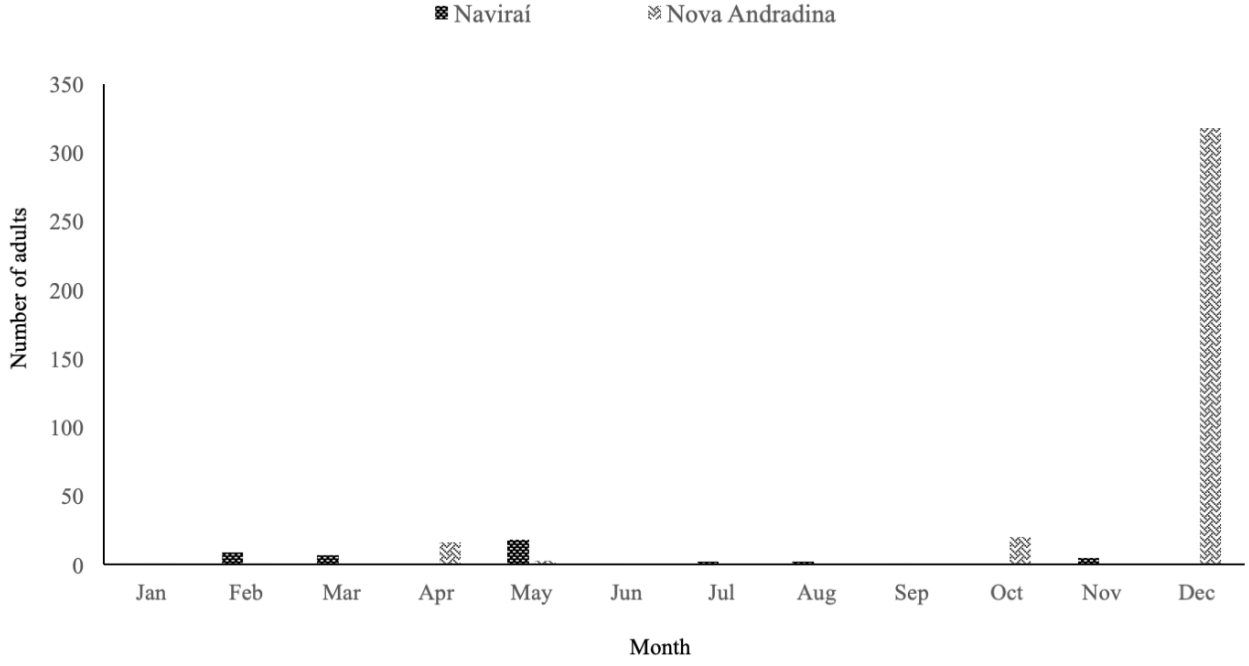

Figure 3. Number of adults of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) caught with light trap in sugarcane (*Saccharum officinarum* L.) (Poaceae) fields of Naviraí and Nova Andradina, Mato Grosso do Sul, Brazil, in the period from May 2012 to April 2013.

mm for the 1st, 2nd and 3rd instars, respectively (Gomes et al. 2014). Thus, larvae of *L. alvarengai* are larger than *L. dorsalis*, but similar to larvae of *L. albopilosus* and *L. ambrosius*.

According to Pardo-Locarno et al. (2006), *L. femoratus* third instar larvae have cephalic capsule width ranging from 3.0 to 3.2 mm. The third larval instar of *L. alvarengai* lasted 90 days and weighed on average 252 mg. Although the third instar larvae of *L. albopilosus* and *L. ambrosius* display similar duration to that found in this study, they differed in larval weight, with a mean of 290 and 324 mg, respectively, being, therefore, heavier than *L. alvarengai* (Gomes et al. 2014, Ferreira et al. 2016). The egg to adult period of *L. alvarengai* was completed in 230 days, confirming what has been described by Pereira et al. (2013) to *L. alvarengai* and *L. aff. semipruinosus* and similar to the cycle of *L. dorsalis,* which lasts on average 273.5 days (Rodrigues et al. 2010). During the rearing of the larvae in the laboratory, it was verifide the change from the second to the third instar, with an average duration of 133.9 days until the emergence of adults. Considering the 230 day cycle, this difference of 96.1 day is consistent with the duration of the first and second instars larvae together.

In the sugarcane fields of Mato Grosso do Sul, *L. alvarengai* showed a univoltine biological cycle with three larval instars, with December being the main month of the occurrence of adults. This work provided information bioecological of *L. alvarengai* that provide support for the monitoring and population management of this species.

Acknowledgments

We thank Brazilian Agricultural Research Corporation for the financial support, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Finance Code 001 for granting scholarships to the authors, and Federal University of Grande Dourados, which allowed the development of this study.

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How to cite

COUTINHO GV, ÁVILA CJ, GOMES ES, COSTA EN, RODRIGUES SR & SILVA IF. 2023. Biological aspects and first record of *Leucothyreus alvarengai* Frey (Coleoptera: Melolonthidae: Rutelinae) in sugarcane (*Saccharum officinarum L*) (Poaceae) fields of Mato Grosso do Sul state, Brazil. An Acad Bras Cienc 95: e20191357. DOI 10.1590/0001-3765202320191357.

Manuscript received on November 11, 2019;
accepted for publication on March 26, 2020

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Author contributions

Gilmar Vieira Coutinho and Elias Soares Gomes performed the experiments, analyzed the data, prepared figures and tables. Gilmar Vieira Coutinho, Crébio José Ávila, Elias Soares Gomes, Eduardo Neves Costa, Sérgio R. Rodrigues and Ivana Fernandes da Silva analyzed the data and wrote the paper. Gilmar V. Coutinho, Crébio J. Ávila and Elias S. Gomes conducted the statistic, data analysis and Gilmar Vieira Coutinho, Crébio José Ávila, Elias Soares Gomes, Eduardo Neves Costa, Sérgio R. Rodrigues and Ivana Fernandes da Silva reviewed drafts of the paper. All authors critically revised the manuscript and approved the final version.