Biological Aspects of *Leucothyreus aff conquisitor* (Ohaus, 1924) (Coleoptera: Melolonthidae)

Aspectos biológicos de *Leucothyreus aff conquisitor* (Ohaus, 1924) (Coleoptera: Melolonthidae)

DOI: 10.34188/bjaerv3n2-027

Recebimento dos originais: 20/01/2020
Aceitação para publicação: 30/03/2020

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ABSTRACT
The biological aspects and occurrence of members of the genus *Leucothyreus* Macleay, are little investigated, therefore, studies were conducted in the municipality of Aquidauana, Mato Grosso do Sul State, Brazil, to analyze the occurrence and life-cycle duration of *Leucothyreus aff. Conquisitor* (Ohaus, 1924). Adults were captured in a light trap. Afterwards, they were sexed and couples were isolated in the laboratory for reproduction. The biological studies started from eggs under laboratory conditions. In the field, we observed that adults were collected mostly in warmer and wetter periods, from October to March. The embryonic period of *L. aff. conquisitor* lasted 17.0 days, the 1st, 2nd and 3rd instars lasted, 23.5, 30.4, and 84.5 days. The pupal stage lasted 26.3 days and the egg to adult period was completed in 184.5 days on average. These results suggest that *L. aff. conquisitor* can be characterized a univoltine specie.

Keywords: Biology, Ecology, Geniatini, Scarab beetle, Tropical beetle.

RESUMO
Os aspectos biológicos e ocorrência de membros do gênero *Leucothyreus* Macleay, são pouco investigadas, portanto, estudos foram realizados no município de Aquidauana, Mato Grosso do Sul, Brasil, para analisar a ocorrência e duração do ciclo de vida *Leucothyreus aff. conquisitor* (Ohaus, 1924). Os adultos foram capturados em uma armadilha de luz. Depois, eles foram sexados e casais foram isolados em laboratório para reprodução. Os estudos biológicos começaram a partir de ovos em condições de laboratório. No campo, observamos que nos períodos mais quentes e úmidos as revoadas ocorreram de outubro a março. O período embrionário de *L. aff. conquisitor* durou 17,0 dias, o 1º, 2º e 3º instares duraram 23,5, 30,4 e 84,5 dias, respectivamente. A fase de pupa durou 26,3 dias e o ciclo foi concluído em 184,5 dias, em média. Estes resultados sugerem que *L. aff. conquisitor* pode ser caracterizado como espécie univoltina.
**1 INTRODUCTION**

In the subfamily rutelinae and geniatini tribe, the genus *Leucothyreus* Macleay, 1819 presents the largest number of species, being represented by 164 species (Jameson, 2008). According to Morón (2004), 83 species are reported for this genus.

The association of *Leucothyreus* with plant species is related to some regions of Brazil. In commercial plantation area of *Eucalyptus grandis* (w. Hill. ex Maiden) (Myrtaceae), Oliveira et al. (2001) collected 14 species of *Leucothyreus* in the municipality of Nova Era, MG. Puker et al. (2009) found larvae of *L. dorsalis* (Blanchard, 1850) and *Leucothyreus* sp., associated with the root system of *Acrocomia aculeata* (Jaq.; Lodd. ex Mart.) (Arecaceae) plants in Aquidauana, MS. Puker et al. (2011) collected *L. albopilosus* adults (Ohaus 1917) on *Eucalyptus citriodora* plant stem lesions, and on bee colonies of *Apismellifera* (L.) (Hymenoptera: Apidae) in the municipality of Mundo Novo, MS.

Details on the biological aspects of *Leucothyreus* species are still unknown. Studies on the biology of *L. dorsalis* demonstrated that the embryo period lasted on average 15.5 days, the first, second and third instars lasted 32.4, 38.9 and 52.7 days, respectively. The pre-pupal phase lasted 130.7 days, the pupal phase 23.5 days and the adult longevity was 18 days. The cycle was completed in 273.5 days, on average (Rodrigues et al., 2010). For *L. ambrosius* (Blanchard, 1850) the duration of the embryo phase was 14.6 days, the first, second and third instars lasted 21.6, 19.6 and 85.6 days, respectively. The pupa lasted 35.5 days and egg to adult lasted 173.3 days, on average (Gomes et al., 2014). According to Pereira et al. (2013) adults of *L. alvarengai* (Frey, 1976) and *L. aff. semipruinosus* (Ohaus, 1917) begin the flock in the rainy season, where they perform copulations and postures, and the immature phases of both species complete the biological cycle in less than a year.

Despite the diversity of species and the importance of the genus *Leucothyreus*, there are few information on this group. Thus, studies on the biological aspects of *L. conquisitor* were carried out in the city of Aquidauana, Mato Grosso do Sul, Brazil.

**2 MATERIAL AND METHODS**

**Place of collection**

The study was conducted at the Universidade Estadual de Mato Grosso do Sul (UEMS), in the municipality of Aquidauana, Mato Grosso do Sul, Brazil. Adults of *L. aff. conquisitor* were
sampled and collected from January 2010 to February 2012 using a light trap model “Luiz de Queiroz” equipped with a 20-watt fluorescent lamp, and installed between the pasture (*Brachiaria decumbens*) and crop areas (soybean, bean, corn, cotton and sugarcane). Every day the light was turned at 18:00 h to 6:00 h the following day and *Leucothyreus* species were attracted.

**Creation of insects in laboratory**

Males and females of *L. aff. Conquisitor* were placed in plastic containers (30 x 19 x 12 cm, 4L capacity) with a 5 cm deep soil layer for oviposition and seedlings of *Brachiaria decumbens* (Trin.) Griseb (Poaceae). In Mato Grosso do Sul, the pastures occupy an area of 11,970,000.00 hectares (Sano et al., 1999) and *B. decumbens* is the major species cultivated. In function of the occurrence of *Leucothyreus* on pasture and as *B. decumbens* is the main gramineae cultivated, the experiment was conducted using this species as a food source to the larvae.

Two pairs of adults were isolated per container, which remained in the laboratory at room temperature. The plastic containers were properly identified with numbers, and covered with polyester veil to provide air circulation and subsequently checked daily for the presence of eggs. Because of a lack of information on the alimentation of adults of *L. aff. conquisitor*, it was offered a variety of food sources to the adults such as leaves, flowers and fruits of vegetal (guava, cashew, grape, acerola, mango and banana), as a result, adults preferred to feed on bananas, so the last one was used as a food source during the experiment. To measure egg development, eggs (n=25) oviposited were collected every day and placed in Petri dishes. We put a layer of 2-3 cm of soil in the Petri dishes to receive the eggs. The Petri dishes were then kept in a climatic chamber with the temperature-controlled (26 ± 2°C and L: D 12:12 h photoperiod). Every three days the eggs were inspected, and the newly hatched, larvae separated, and were placed individually in 500 ml plastic pots containing soil and seedling of *B. decumbens* using root as a food source.

Larvae were maintained in an acclimated chamber with the same conditions for the eggs. Due to moisture loss and seedlings wilt, the soil was moistened weekly and *B. decumbens* replanted every 15 days to supply roots for larval feeding. Each three or four days, the rearing pots were checked and the soil and seedlings of *B. decumbens* were carefully removed to visualize the larvae. This procedure allowed to measure the cephalic capsule, and after measuring its width, the larval growth and instar change were determined.

The larvae are gray, when feeding; however, the pre-pupal stage, they show a white color and not feed on the roots of *B. decumbens*. After pre-pupae were formed, seedlings of *B. decumbens* were no longer supplied. To check the duration of the pupa, pupal chambers in the openings were
made, allowing the visualization of the pupa inside. After the emergence, adult couples were kept in plastic containers (30 x 19 x 12 cm, 4 L capacity) as described above. Life-cycle was determined starting from newly oviposited eggs until adult death. Longevity and survivorship data from different developmental stages of *L. aff. Conquisitor* were recorded.

To monitor larval growth and of instars differentiation, we measured the cephalic capsule of larvae, at the greatest width, using a digital caliper (precision 0.05 mm) at intervals of 3 to 4 days. Eggs, larvae and pupae were measured the length and width with digital caliper, and the weight obtained with analytical balance (precision 0.0001 gramme). Some adults of *L. aff. conquisitor* collected with light trap, were measured, sexed, subsequently killed and kept in an oven for 48 hours (60 ± 5°C), and then weighed.

**Species identification**

Adults of *L. aff. Conquisitor* were identified by Dr. Miguel Angel Morón, Institute of Ecology, Xalapa, Veracruz, Mexico (IEXA). Ten adults were mounted and deposited in the insect collection of the Entomology Laboratory at UEMS and 8 in the Institute of Ecology, Xalapa, Veracruz, Mexico.

**3 RESULTS**

Adults were collected in January, February and May 2010, in October, November and December 2011, and in January and February 2012 (Figure 1) during the warmer and wetter periods of the region of Aquidauana.

**Figure 1.** *Leucothyreus* aff. *Conquisitor* adults captured using light traps from January 2010 to February 2012 in the municipality of Aquidauana, Mato Grosso do Sul State, Brazil.
Eggs were laid in March in the laboratory and individually deposited on the soil near one another. The egg longevity was 17.0 days (Table 1). Newly oviposited eggs were white and elliptic with 2.5 mm long x 1.9 mm wide and weighed 0.0033 g (Table 2).

The first instar lasted 23.5 days (Table 1) and developed from April to early May (Fig. 2). Larvae dimensions are 8.2 mm long x 1.9 mm thorax width and weight 0.018 g (Table 2). Mean cephalic capsule width was 1.57 mm. The second instar lasted 30.4 days between May and early June (Fig. 2).

**Figure 2.** Seasonality of the life stages of *Leucothyreus* aff. *conquisitor* in plastic containers with soil and seedlings of *Brachiaria decumbens* under laboratory conditions (26 ± 2°C, 12: 12 h photoperiod).

Larva has length of 15.9 mm x 2.9 mm thorax width and weight 0.136 g, thereby, increasing 7.6 times its weight in relation to the first instar (Table 2). The cephalic capsule width of the second instar was 2.68 mm.

**Table 1.** Duration (Means ± SE) of developmental stages of *Leucothyreus* aff. *conquisitor*, under laboratory conditions (26 ± 2°C, 12: 12 h photoperiod).

| Stage        | Duration (days) | Range (days) | Individuals (n) |
|--------------|-----------------|--------------|-----------------|
| Egg          | 17.0 ± 1.15     | 15 – 19      | 25              |
| 1st instar   | 23.5 ± 1.60     | 20 – 32      | 13              |
| 2nd instar   | 30.4 ± 1.53     | 21 – 34      | 13              |
| 3rd instar   | 84.5 ± 3.50     | 62 – 104     | 05              |
| Larva        | 140.5 ± 11.90   | 119 – 162    | 05              |
| Pupa         | 26.3 ± 1.50     | 23 – 30      | 5               |
| Egg to adult | 184.5 ± 4.00    | 177 – 192    | 5               |

The development of third instar was from June to late September (Fig. 2) and longevity of 84.5 days (Table 1). The third instar was 30.4 mm long x 5.6 mm thorax width with 0.73 g weight (Table 2), and cephalic 3.43 mm width. From the first to the third instar, larva increased the weight.
at 40.6 times. In late August and early September, the third instar acquired a whitish coloration and reaching the pre-pupa stage. The larvae constructed a chamber with soil, decaying matter, and saliva for the pupal stage protection, until late September (Fig. 2). The pupal chambers are structures with smooth inner lining and that hardly broke when handled in the laboratory. The mean duration of \textit{L. aff. conquistor} larva was 140.5 days (Table 1).

From late September until early November, the mean duration of pupa, was 26.3 days (Table 1, Fig. 2). Pupa of \textit{L. aff. conquistor} are yellow on the dorsal region and brown on ventral region. The pupa was 16.9 mm long x 7.8 mm wide and 0.40 g weight (Table 2). The adults emerged in November and December (Fig. 2).

Male and female were distinguished for the tarsomeres width in forelegs. In males and females in the elytra and legs, we observed the yellow-brown coloration, while the head and pronotum have dark green-metallic coloration. The mean female size was 14.1 mm length x 7.1 mm wide and 0.080 g weight, while mean male size was 13.0 mm length x 6.6 mm wide and 0.076 g weight (Table 2). The egg to adult life cycle of \textit{L. aff. conquistor} was 184.5 days (Table 1).

### Table 2. Mean (± SE) length, width, and weight of developmental stages of \textit{Leucothyreus} aff. \textit{conquistor} under laboratory conditions (26 ± 2 °C, 12: 12 h photoperiod).

| Stage | Length (mm) mean range | Width (mm) mean range | Weight (g) mean range |
|-------|------------------------|-----------------------|-----------------------|
| Egg   | 2.5 ± 0.12 2.2 – 3.0 1.9 ± 0.03 1.8 – 2.0 | 0.0033 ± 0.001 0.0016 – 0.0075 |
| 1\textsuperscript{st} instar | 8.2 ± 0.31 7.2 – 9.6 1.9 ± 0.06 1.8 – 2.3 | 0.018 ± 0.001 0.012 – 0.026 |
| 2\textsuperscript{nd} instar | 15.9 ± 1.57 12.0 – 19.9 2.9 ± 0.28 2.2 – 3.8 | 0.136 ± 0.02 0.052 – 0.219 |
| 3\textsuperscript{rd} instar | 30.4 ± 0.34 29.5 – 31.2 5.6 ± 0.29 4.9 – 6.3 | 0.73 ± 0.06 0.42 – 0.88 |
| Pupa | 16.9 ± 0.71 15.3 – 18.3 7.8 ± 0.43 7.2 – 8.6 | 0.40 ± 0.05 0.32 – 0.48 |
| Adult ♂ | 13.0 ± 0.07 12.9 – 13.2 6.6 ± 0.18 6.2 – 6.8 | 0.076 ± 0.00 0.071 – 0.086 |
| Adult ♀ | 14.1 ± 0.29 12.8 – 14.6 7.1 ± 0.69 5.7 – 7.8 | 0.080 ± 0.019 0.052 – 0.091 |

(\textit{L1} = 1st instar, \textit{L2} = 2nd instar and \textit{L3} = 3rd instar).

4 DISCUSSION

The emergence of adults of \textit{L. aff. conquistor} occurs from November to December, which is a hot and humid period in the region of Aquidauana, Mato Grosso do Sul State, Brazil. These species showed a similar occurrence with other species \textit{Leucothyreus} in this region. Puker et al. (2011) recorded the occurrence of \textit{L. albopilosus} in January, in the municipality of Mundo Novo, Mato Grosso do Sul. Adults of \textit{L. dorsalis} were observed from September to December and from January to March (Rodrigues et al., 2010). Similar results are found for \textit{L. albopilosus} in which
adults were collected during the hottest and wettest periods of the year in Aquidauana (August to March) and \textit{L. ambrosius} with occurrence from October to December (Ferreira et al., 2016; Gomes et al., 2014).

Adults of \textit{L. aff. conquisitor} were not observed feeding on \textit{B. decumbens} plants, the main gramineae on pasture in the region of Aquidauana, MS. However, adults fed on fruits of banana in the laboratory. According to Martínez et al. (2013), adults of \textit{L. femoratus} feed on leaves of the palm \textit{Elaeisguineenses} Jacquin (Arecales, Arecaceae).

During the flying period, female adults perform the oviposition, and so the eggs start being found. Some females build small chambers in the soil where one or more eggs are conditioned in order to promote a system to protect the eggs until the larvae eclosion, as observed to \textit{L. dorsalis} (Rodrigues et al., 2010) and \textit{L. ambrosius} Blanchard, 1850 (Gomes et al., 2014). Neither \textit{L. aff. conquisitor} performed the construction of chambers in order to protect and condition the eggs, of each respectively species.

The eggs of \textit{L. aff. conquisitor} lasted 17 days, are white and deposited individually on the ground near each other. For \textit{L. dorsalis}, the eggs are white and the embryonic period is 15.5 days (Rodrigues et al., 2010). The embryonic period of \textit{L. femoratus} is 8.73 days (Martínezand Plata-Rueda 2013). The embryonic period of \textit{L. aff. conquisitor} and \textit{L. albopilosus} is longer than that observed for \textit{L. dorsalis} and \textit{L. femoratus}.

The larvae of \textit{L. aff. conquisitor} reared in laboratory, fed on roots of \textit{B. decumbens}, completed their biological cycle, what indicates that the food source used to the larvae comprises an important food source. Larvae of \textit{L. dorsalis}, \textit{L. alvarengai}, \textit{L. aff. semipruinosus} and \textit{L. ambrosius} we also reared in laboratory and fed on roots of \textit{B. decumbens} (Gomes et al., 2014; Pereira et al., 2013; Rodrigues et al., 2010).

Pardo-Locarno et al. (2006) report that \textit{L. femoratus} can feed of the roots of \textit{Steinichismalaxa} (Sw) Zuloaga (Poaceae) in grassland. Martínez and Plata-Rueda (2013) reared larvae of \textit{L. femoratus}, feeding them on roots of corn. Pardo-Locarno et al. (2005) found \textit{Leucothyreus} sp. feeding on grass pastures, cassava and coffee roots in forest areas. Puker et al. (2009) collected larvae of \textit{L. dorsalis} and \textit{Leucothyreus} sp. feeding on macaw palm trees, \textit{A. aculeata}. Pereira et al. (2013) found larvae of \textit{L. alvarengai} and \textit{L. aff. semipruinosus} feeding on roots of soybean and corn in Tangará da Serra, MT, Brazil.

The egg-to-adult period of \textit{L. aff. conquisitor} (184.5 days) falls within the range values reported for other species of \textit{Leucothyreus} in the region. Development of \textit{L. dorsalis} was determined to be 273.5 days (Rodrigues et al., 2010). \textit{Leucothyreus alvarengai} and \textit{L. aff. semipruinosus} feeding on roots of soybean and corn in Tangará da Serra, MT, Brazil.
semipruinosus, was less than 1-yr (Pereira et al., 2013), and L. femoratus was 170.4 days (Martínez and Plata-Rueda2013), characterizing them as univoltine.

Observing the stages of development of L. aff. conquistor it appears that during the drier and colder period of the year, between July and September, the third instar larvae achieve the pre-pupa stage. Normally, in this stage, the larvae build chambers in the soil where they remain safe, reaching the pupa phase and adulthood, afterwards. The pre-pupa of L. alvarengai, L. aff. semipuinosus and L. ambrosius also remain safe in the soil inside chambers during dry and cold period of the year (Gomes et al., 2014; Pereira et al., 2013). The sexual dimorphism of L. dorsalis was also reported by Rodrigues et al. (2010), and the dilated tarsomeres were observed in male forelegs. Puiker et al. (2011) also described this feature in L. albopilosus. Thus, several Leucothyreus species can be sexed observing the tarsomeres of forelegs in males.

5 CONCLUSION

Leucothyreus aff. conquistor is a univoltine species and its life cycle from egg to adult was 185.4 days. In addition, in Mato Grosso do Sul, Brazil, the areas of pasture containing B. decumbens, occupy an extensive cultivated area, therefore, the aspects related to the alimentation of immature of L. aff. conquistor provide an important information on the ecological associations that these species maintain with that vegetal species, and with the pasture environment, demonstrating that species of the genre Leucothyreus can use this source as an important food source.

ACKNOWLEDGEMENTS

We thank to the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (Brasil) for the granting of scientific initiation scholarship to the first author. S.R.R. was supported by CNPq (305260/2014-6). To Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul FUNDECT (Brasil) for the financial support.

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