

RESEARCH

Sexual Dimorphism and Mating Behavior in *Anomala testaceipennis*Sérgio Roberto Rodrigues,^{1,2} Elias Soares Gomes,¹ and José Maurício Simões Bento³¹Departamento de Entomologia, Universidade Estadual de Mato Grosso do Sul, Rodovia Aquidauana/CERA, km 12, 79200-000, Aquidauana, MS, Brazil²Corresponding author, e-mail: sergio@uemms.br³Departamento de Entomologia e Acarologia, Laboratório de Ecologia Química e Comportamento de Insetos, Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Ave. Pádua Dias, 11, CP 09, 13418-900, Piracicaba, SP, Brazil

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ABSTRACT. The beetle, *Anomala testaceipennis* Blanchard (Coleoptera: Scarabaeidae), occurs in central-western Brazil where larvae feed on the roots of plants causing damage. This research aimed to study sexual dimorphism and mating behavior of *A. testaceipennis*. Adults of *A. testaceipennis* were collected with light traps in the experimental area of the State University of Mato Grosso do Sul, in Aquidauana. Laboratory experiments were performed to describe copulation behavior and adult morphology of males and females. In males the last abdominal segment has a pronounced constriction, which is absent in females, and the male's last segment of the first pair of legs has a ventral projection, which is poorly developed in females. The mating activities of adults begin soon after sunset, when adults leave the soil and fly. When the male encounters a female, he touches her with antennae and tarsi. If accepted, the male climbs on the female and remains on her back, and soon after the copulation begins. When the female does not accept the male for mating, she moves rapidly and can roll on the ground, and by so removing the male. In the field, adults feed and mate on bloomed trees of Oiti, *Licania tomentosa* Benth (Malpighiales: Chrysobalanaceae) and Louro, *Cordia glabrata* Martius (Boraginaceae). In trees without inflorescences no adults of this species were found.

Key Words: morphology, Rutelinae, Scarabaeidae, sexual behavior, white grub

The genus *Anomala* Samouelle (Scarabaeidae) comprises over 1,000 species distributed throughout the world (Jameson et al. 2003). In America, there are ~300 described species (Ramirez-Ponce and Morón 2009). Some of these species are considered pests of economic importance due to damage caused mainly by the larval stage, which consumes roots of cultivated plants, while adults can nourish from leaves and flowers (Morón 1996, Del-Bosque 1998, Micó et al. 2003, Arakaki et al. 2004b; Wenninger and Averill 2006, Rodrigues et al. 2008).

Although scarce, mainly due to the fact that this insect remains much of its life cycle in the soil, some studies on biological and behavioral aspects can be found for *Anomala albopilosa sakishimana* Nomura (Arakaki et al. 2004b); *Anomala cincta* Say, *Anomala forreri* Bates, and *Anomala sticticoptera* Blanchard (Micó et al. 2003); *Anomala cuprea* Hope (Fujiyama 1997); *Anomala dubia* (Scopoli) and *Anomala quadripunctata* (Olivier) (Micó and Galante 2005); *Anomala flavipennis* Burmeister (Del-Bosque 1998) and *Anomala orientalis* (Waterhouse) (Facundo et al. 1999).

Anomala testaceipennis Blanchard occurs in central-western Brazil causing damage to crops such as soybean, *Glycine max*; corn, *Zea mays*; wheat, *Triticum aestivum*; oat, *Avena sativa* (Ávila and Santos 2009); and grass, *Brachiaria* spp. (Rodrigues et al. 2008). The life cycle reported for *Brachiaria decumbens* (egg to adult) lasts an average of 139.4 d, with two generations per year (Rodrigues et al. 2008). Females lay eggs in roots of the host plant, and larval development comprises three instars lasting almost 115 d (Rodrigues et al. 2008). After the pupal period (13.6 d in average) the adults emerge and females last 12.5 d to reach sexual maturity, meanwhile they leave the ground at night but do not mate (Rodrigues et al. 2008).

In the cities of Aquidauana and Caarapó in the state of Mato Grosso do Sul, Brazil, the highest number of adults was normally found leaving the soil and flying out all at once in swarms, which occurred yearly during August (Rodrigues et al. 2008) and October (Ávila and Santos 2009).

In order to control scarabaeid pests in Brazilian crops, some techniques are employed, such as different methods in preparing the soil that decrease the density of larvae in the field (Silva et al. 1994, Oliveira et al. 2000), as well as the use of chemical products applied in the seeding groove or seed treatment (Santos et al. 2008).

An important aspect for the appropriate management of this group of insects, refers to the understanding of their biology and behavior. For Scarabaeidae pests occurring in Brazil, except for *Phyllophaga cuyabana* Moser (Oliveira et al. 1996, Oliveira and Garcia 2003, Zarbin et al. 2007), little is known about biology and sexual behavior. Thus, the aim of this research was to study the sexual dimorphism and mating behavior of *A. testaceipennis*, seeking for future identification of the sex pheromone for management of this pest.

Materials and Methods

Flight Time. The flight time of *A. testaceipennis* was observed using a light trap (20 W), model 'Luiz de Queiroz', from 18:00 to 6:00 h (Brazil daylight saving time), between 6 and 17 February 2012, at the Experimental Farm, State University of Mato Grosso do Sul (UEMS) in Aquidauana, MS (latitude 20° 28', longitude 55° 48'). At 60-min intervals, the trap was inspected and the captured insects were collected according to methodology used by Rodrigues et al. (2010). The trap was installed next to a pasture of *B. decumbens* and plots of other crops such as sugarcane (*Sacharum officinarum*), corn (*Z. mays*) and soybean (*G. max*). Data relating to the capture of adults were transformed into $\sqrt{x+1}$, and subjected to analysis of variance and means were compared with the Scott-Knott test ($P < 0.05$), using ASSISTAT software (Silva and Azevedo 2002). Mean temperature (°C), rainfall (mm), and solar radiation (kJ/m²) data in Aquidauana-MS were obtained from the National Institute of Meteorology (INMET, Brasília-DF).

Mating Behavior in Laboratory. Adults of *A. testaceipennis* obtained from the light trap were sexed and placed on individual 1-liter plastic boxes, containing three fourth of soil (750 ml), and closed on its

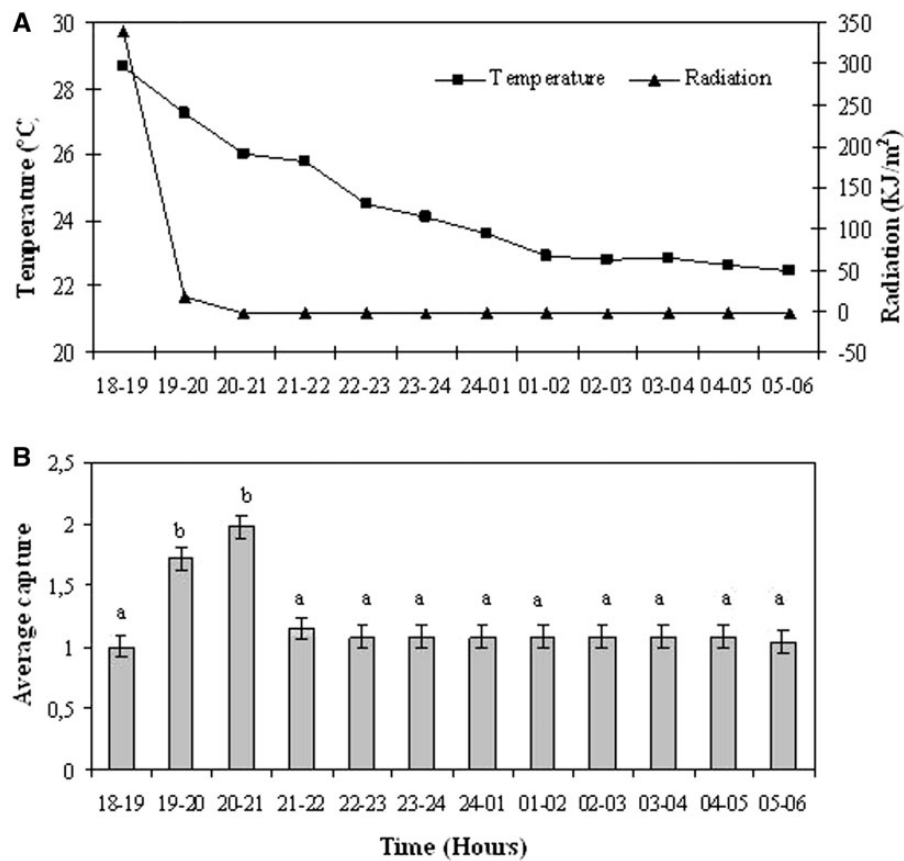


Fig. 1. Radiation (kJ/m^2) and average temperature ($^{\circ}\text{C}$) obtained from the weather station (INMET) in Aquidauana, MS, Brazil, from 6 to 17 February 2013 (A). Adults of *A. testaceipennis* collected with a light trap in the same period (B). Bars with the same letter are not statistically different ($\pm\text{SD}$) ($P < 0.05$, Scott-Knott test).

surface with voile fabric. The next day, after 17:00 h in the containers the time when the adults emerged from the ground and engaged in mating was observed. Thereafter, the couples were formed and transferred to a 4-liter plastic box containing soil and seedlings of *B. decumbens* which were placed in a dark room for mating behavior remarks, according to adaptations from Facundo et al. (1999). To observe and record the behavior exhibited by males and females, we used a Sony camcorder, model DCR-SX21 STD. In total, 40 couples were observed separately detailing all their behavior. These studies were conducted from 6 to 25 February and from 13 August to 8 September 2012.

Field-Mating Behavior. The field-mating behavior of *A. testaceipennis* was observed on seven and three trees of Oiti, *Licania tomentosa* (Chrysobalanaceae) and Louro, *Cordia glabrata* (Boraginaceae), respectively, between 17:30 and 22:00 h in the UEMS farm, from 22 August to 6 September 2012. In nature, *A. testaceipennis* was seen flying in "swarms" around these trees and copulations were recorded with a Sony camcorder, model DCR-SX21 STD.

Sexual Dimorphism. During mating, 20 males and 20 females were identified according to their position in copula. Individuals of each sex were then sacrificed by freezing and the external structures were observed under Zeiss Stemi SV6 stereoscope at $40\times$ magnification with the aid of an ocular micrometer, searching for sexual differentiation characters. The identified material was deposited in the entomological collection of the UEMS.

Results

Flight Time. Adults of *A. testaceipennis* began flight activity between 18:00 and 19:00 h, and continued until 05:00 to 06:00 h on the next day, coinciding with the beginning and end of scotophase (Fig. 1).

The amount of captured insects was greater during the first 2 h of the scotophase ($F_{11:108} = 8.74$; $P = 0.001$), between 19:00 and 21:00 h evidenced by the sharp fall in average solar radiation of 338.2 kJ/m^2 at 18:00 h, to 16.9 kJ/m^2 at 19:00 h and -3.54 kJ/m^2 at 20:00 h. The average temperature during the experiment ranged from 28.6 to 22.4°C between 18:00 to 5:00 h, respectively (Fig. 1).

Mating Behavior in Laboratory. During the photophase, males and females of *A. testaceipennis* sheltered in soil chambers built by themselves the night before inside the plastic containers. Just before leaving the ground, part of the males ($n = 28$) and females ($n = 31$) exhibited a portion of the clypeus out of the soil surface and moved the lamellae of the antennae in different directions for an average of 10.9 ± 1.5 min (3–20 min), and then completely emerged from the ground and took flight, unlike the other males ($n = 12$) and females ($n = 9$) which left the soil rapidly, and initiated flight.

Once out of the ground all individuals ($n = 80$) flew actively and after an average of 16.6 ± 2.3 min (6–43 min), stopped flying and walked or remained motionless with antennae erected and slides open. Thereafter, the couples formed and mating began. From 40 observed couples, 22 underwent a series of steps related to mating behavior, whereas the remaining 18 did not engage in mating (Fig. 2).

For successful copula the male typically approaches the female from behind ($n = 16$) or frontally ($n = 6$), and the antennae and first pair of legs touch the anal plate (pygidium) or the final portion of the female's elytra, for a period of 45.8 ± 21.5 s (2–480 s). Once accepted by the female ($n = 12$) the male climbs on her body and remains there for an average of 83.6 ± 43.5 s (10–540 s), holding her with all three pairs of legs, performing rhythmic up and down movements of the

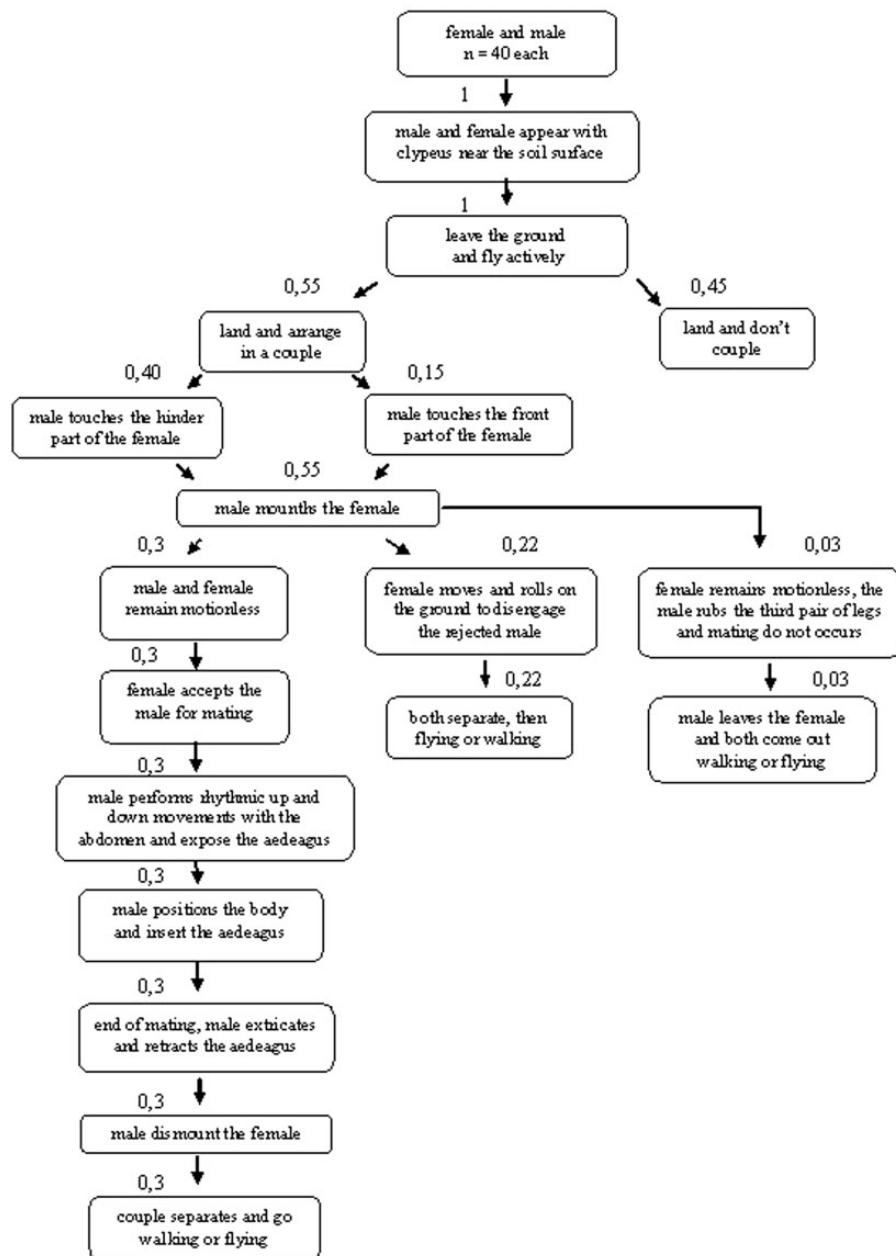


Fig. 2. Ethogram of mating behavior of *A. testaceipennis* in the laboratory ($n = 40$ couples). Numbers above behavioral descriptions represent percentages that progressed to a subsequent step in the mating behavior sequence.

abdominal segments and exposing the aedeagus until reaching the female pygidium, initiating the copula.

A. testaceipennis mating behavior always occurred between the first and second hour of scotophase, and only one mating per each female was observed. During copulation, the male and female remained motionless for an average of 419.2 ± 52.0 s (120–720 s).

After copulation, the male disengaged from the female, retracting the aedeagus in 3–4 s, and moving or flying away from her. Females in turn, remained motionless at the same location between 1 and 6 h ($n = 9$) or dug tunnels and took shelter on the ground ($n = 13$). On one occasion the male after copulation and having retracted the aedeagus, remained on the female for 6 min.

On the occasions where females rejected males for mating ($n = 9$), it was observed that such females walked ($n = 4$) or rolled on the ground ($n = 5$) for an average of 34.7 ± 7.2 s (20–90 s) before flying or

completely moving away from males. In these situations the males did not achieve aedeagus intromission.

Field-Mating Behavior. In field studies, adults of *A. testaceipennis* were found on trees *L. tomentosa* and *C. glabrata*, feeding on flowers and performing copulations on stems, leaves, or flowers.

In *L. tomentosa*, the insects were found exclusively in flowering trees. At dusk, males and females of *A. testaceipennis* showed intense flight activity around the inflorescences, leaves, and branches of *L. tomentosa* trees and were seen distributed mainly through the basal and middle canopy of the plant, that were ~ 8 m high.

In *L. tomentosa* copulations occurred after the encounter of males and females on leaves ($n = 15$), branches ($n = 4$), and inflorescences ($n = 35$), and the sequence was similar to that described previously in the laboratory. On several occasions, adults ($n = 18$) collided with the branches and leaves of the tree and fell to the ground, but quickly

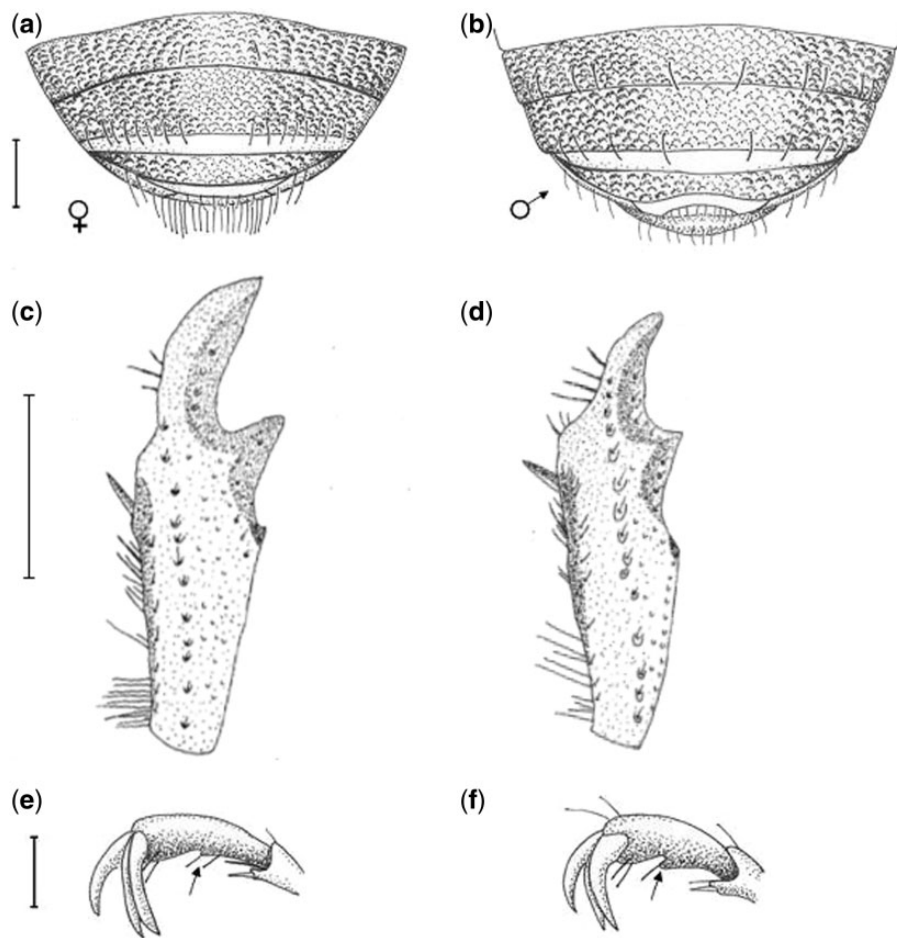


Fig. 3. *Anomala testaceipennis*. Ventrite VI. (a) ♀; (b) ♂. Protibia and last tarsomere of the protibia. (c and e) ♀; (d and f) ♂. Scale of 1 mm.

resumed flight. As well as in the laboratory, one single mating per female was observed. When males and females landed on the branches, leaves, or flowers, and didn't find mating partners, they rapidly walked over the plant structures, abandoning the site and flying again. The average copulation time was 420 ± 24.1 s (300–600 s) ($n = 13$), and after mating the couple separated but remained close and then flew ($n = 8$), but when it remained on the inflorescence they could remain feeding ($n = 5$).

Both *L. tomentosa* and *C. glabrata* inflorescences, adults of *A. testaceipennis* consumed petals, anthers, fillets, stigmas, and stylus. In the inner portion of the flowers where the floral nectaries are found, feeding adults were also observed. On other 15 trees of *L. tomentosa* which were not in bloom, adults of *A. testaceipennis* were not observed, suggesting that the inflorescences played an important role in the attraction of these insects.

Sexual Dimorphism. It was possible to morphologically separate males and females of *A. testaceipennis* through observation of abdominal segments and the first pair of legs (Fig. 3). The last abdominal segment in males is constricted, whereas it is not in females. The tibia of the first pair of legs in males is 2.31 mm (2.12–2.43) long and 0.58 mm (0.56–0.61) width. The tibia has two apical spurs and a small one in the basis, on the external edge. On the inner edge, there is a preapical spur which is acute, straight, short, and is 0.32 mm (0.29–0.36) long ($n = 10$). The last tarsomere has a pronounced ventral projection. The tibia of the first female pair of legs is 2.47 mm (2.19–2.80) long and 0.59 mm (0.56–0.66) width. Tibia has two large apical spurs and a basal small one on the external edge. On the inner edge, the spur is acute, straight and short being 0.31 mm (0.29–0.34) long ($n = 10$). The last

tarsomere hasn't a ventral projection. Female tibia is longer, wider, and has two apical spurs which are larger than in the male.

Discussion

Flight time of *A. testaceipennis* started at dusk and lasted the entire scotophase with higher activity during the first 2 h (19:00–21:00 h), as reported previously by Rodrigues et al. (2008). The same pattern of flight was also found in other Scarabaeidae species, such as *Leucothyreus dorsalis* (Rutelinae) (Rodrigues et al. 2010) and *Dasylepida ishigakiensis* (Anomalinae) (Arakaki et al. 2004a).

In the laboratory, *A. testaceipennis* adults actively flew, landed, and formed couples as they left the soil early in the scotophase. Under natural conditions, the adults also displayed the same mating behavior, with the couples forming on the canopies of trees in full bloom. According to Facundo et al. (1999), the adults of *A. (= Exomala) orientalis* leave the soil but do not fly, and the females about to perform calling bury themselves partially in the soil keeping the abdomen out to attract the males to mate. When males of *A. testaceipennis* find the females, they use their antennae and first pair of legs to touch and recognized them prior to being accepted. The same behavioral pattern was observed for *A. cuprea* whose males have different types of olfactory sensilla on the lamellae surface that detect the sexual pheromone released by females (Leal and Mochizuki 1993). Therefore, it is possible that the male *A. testaceipennis* also use this mechanism for mate recognition. During our observations, the females of this species mated only once in the laboratory and field, but this do not exclude the possibility that mating occurs more times. Similarly, *D. ishigakiensis* females mate multiple times between 19:50 and 20:25 h (Harano et al. 2010).

On several occasions, female *A. testaceipennis* did not accept males for mating and disengaged from mounts. In this case, it is possible that females had already mated in the field and, as a consequence, according to Avila et al. (2011), their physiology and behavior may have changed, such as a decrease in receptivity for an additional mating. Another possible reason for females not to accept males for mating may be related to differences in the couple's sexual maturity, according to studies of Favila (1988) on sexual behavior of *Canthon cyanellus cyanellus* (Scarabaeidae, Scarabaeinae).

Adults of *A. testaceipennis* fed on *L. tomentosa* and *C. glabrata* inflorescences consuming petals, anthers, fillets, stigmas, and stylus. Adult feeding of *Anomala* spp. has already been reported by other researchers. Micó et al. (2003) showed that adults of *A. cincta* Say feed on leaves of *Pithecolobium dulce* (Leguminosae), *Acacia pennatula* (Mimosaceae), and *Vernonia patens* (Asteraceae), and according to Morón (1996) adults of *Anomala discoidalis* Bates, *A. cincta* Say, and *A. xantholea* Bates feed on *Hibiscus rosa sinensis* (L.) (Malvaceae) flowers.

L. tomentosa trees are ornamentals and do not occur naturally in Brazilian Tropical Savannas where the present study was performed. However, it demonstrated to be an important food source and mating substrate for *A. testaceipennis* adults. *C. glabrata* trees, in turn, are abundant and naturally occur in the region of the present study (Pott and Pott 1994). The flight and mating time displayed by *A. testaceipennis* adults, from August to September, matched the flowering season of *C. glabrata*, showing that this tree might also be important as mating substrate and food source for this insect species.

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References Cited

Arakaki, N., M. Kishita, A. Nagayama, M. Fukaya, H. Yasui, T. Akino, Y. Hirai, and S. Wakamura. 2004a. Precopulatory mate guarding by the male green chafer, *Anomala albopilosa sakishimana* Nomura (Coleoptera: Scarabaeidae). *Appl. J. Zool.* 39: 455–462.

Arakaki, N., Y. Sandoyama, M. Kishita, A. Nagayama, A. Oyafuso, M. Ishimine, M. Ota, T. Akino, M. Fukaya, Y. Hirai, et al. 2004b. Mating behavior of the scarab beetle *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae). *Appl. J. Zool.* 39: 669–674.

Ávila, C. J., and V. Santos. 2009. Corós associados ao sistema de plantio direto no estado de Mato Grosso do Sul. Dourados, MS: Embrapa Agropecuária Oeste, 2009. 32p. (Documentos/Embrapa Agropecuária Oeste, n. 101).

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner. 2011. Insect seminal fluid proteins: identification and function. *Annu. Rev. Entomol.* 56: 21–40.

Del-Bosque, L. A. R. 1998. A sixteen-year study on the bivoltinism of *Anomala flavipennis* (Coleoptera: Scarabaeidae) in México. *Environ. Entomol.* 27: 248–252.

Facundo, H. T., C. E. Linn, M. G. Villani, and W. L. Roelofs. 1999. Emergence, mating, and postmating behaviors of the oriental beetle (Coleoptera: Scarabaeidae). *J. Insect Behav.* 12: 175–192.

Favila, M. E. 1988. Comportamiento durante el período de maduración gonádica em um escarabajo rodador (Coleoptera: Scarabaeidae; Scarabaeinae). *Folia Entomol. Mex.* 76: 55–64.

Fujiyama, S. 1997. Life cycle of *Anomala cuprea* Hope (Coleoptera: Scarabaeidae) in field experiments on adaptation to different thermal environments. *Japan. J. Appl. Entomol. Zool.* 41: 121–131.

Harano, K. I., S. Tanaka, H. Yasui, S. Wakamura, A. Nagayama, Y. Hokama, and N. Arakaki. 2010. Multiple mating, prolonged copulation and male substance in a scarab beetle *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae). *Int. J. Trop. Insect Sci.* 30: 119–126.

Jameson, M. L., A. Paucar-Cabrera, and A. Solis. 2003. Synopsis of the new world genera of Anomalini (Coleoptera: Scarabaeidae: Rutelinae) and description of a new genus from Costa Rica and Nicaragua. *Ann. Entomol. Soc. Am.* 96: 415–432.

Leal, W. S., and F. Mochizuki. 1993. Sex pheromone reception in the scarab beetle *Anomala cuprea*: enantiomeric discrimination by sensilla placodea. *Naturwissenschaften* 80: 278–281.

Micó, E., and E. Galante. 2005. Larval morphology and biology of some European Anomalini (Coleoptera: Scarabaeidae: Rutelidae: Anomalinae). A phylogenetical approach. *Insect Syst. Evol.* 36: 183–198.

Micó, E., M. A. Morón, and E. Galante. 2003. New larval descriptions and biology of some new world Anomalini beetles (Scarabaeidae: Rutelinae). *Ann. Entomol. Soc. Am.* 96: 597–614.

Morón, M. A. 1996. Coleoptera Melolonthidae asociados con las flores de *Hibiscus rosa-sinensis* L. (Malvaceae) en la región de Xalapa, Veracruz, México. *G. It. Ent.* 8: 111–123.

Oliveira, L. J., and M. A. Garcia. 2003. Flight, feeding and reproductive behavior of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) adults. *Pesq. Agropec. Bras.* 38: 179–186.

Oliveira, L. J., C. B. Hoffmann-Campo, and M. A. Garcia. 2000. Effect of soil management on the white grub population and damage in soybean. *Pesq. Agropec. Bras.* 35: 887–894.

Oliveira, L. J., B. Santos, J. R. P. Parra, M. L. B. Amaral, and D. C. Magril. 1996. Ciclo biológico de *Phyllophaga cuyabana* (Moser) (Scarabaeidae: Melolonthinae). *An. Soc. Entomol. Bras.* 25: 431–437.

Pott, A., and V. J. Pott. 1994. Plantas do Pantanal. Embrapa Informação Tecnológica.

Ramírez-Ponce, A., and M. A. Morón. 2009. Relaciones filogenéticas del género *Anomala* (Coleoptera: Melolonthidae: Rutelinae). *Rev. Mex. Biodiv.* 80: 357–394.

Rodrigues, S. R., A. Puker, A. R. Abot, C. L. Barbosa, S. Ide, and G. V. Coutinho. 2008. Ocorrência e aspectos biológicos de *Anomala testaceipennis* Blanchard (Coleoptera, Scarabaeidae). *Rev. Bras. Entomol.* 52: 68–71.

Rodrigues, S. R., A. Puker, and E. F. Tiago. 2010. Aspectos biológicos de *Leucothyreus dorsalis* Blanchard (Coleoptera, Scarabaeidae, Rutelinae). *Rev. Bras. Entomol.* 54: 431–435.

Santos, A. C., A. F. Bueno, R. C. O. Bueno, and S. S. Vieira. 2008. Chemical control of white grub *Liogenys fuscus* (Blanchard 1851) (Coleoptera: Melolonthidae) in cornfields. *Bioassay* 3: 1–6.

Silva, F. A. Z., and C. A. V. Azevedo. 2002. Versão do programa computacional Assistat para o sistema operacional Windows. *Rev. Bras. Prod. Agroindustr.* 4: 71–78.

Silva, M. T. B., V. A. Kein, D. Link, and D. J. Reinert. 1994. Influência de sistemas de manejo de solos na oviposição de *Diloboderus abderus* Sturm (Coleoptera: Melolonthidae). *An. Soc. Entomol. Bras.* 23: 543–548.

Wenninger, E. J., and A. L. Averill. 2006. Effects of delayed mating on reproductive output of female oriental beetle *Anomala orientalis* (Coleoptera: Scarabaeidae). *Agric. Forest Entomol.* 8: 221–231.

Zarbin, P. H. G., W. S. Leal, C. J. Ávila, and L. J. Oliveira. 2007. Identification of the sex pheromone of *Phyllophaga cuyabana* (Coleoptera: Melolonthidae). *Tetrahedron Lett.* 48: 1991–1992.

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