Bioecological aspects of the common black field cricket, *Gryllus assimilis* (Orthoptera: Gryllidae) in the laboratory and in *Eucalyptus* (Myrtaceae) plantations

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Abstract

The common black field cricket, *Gryllus assimilis* (Orthoptera: Gryllidae), damages young plants of red cedar, *Juniperus virginiana* (Cupressaceae); strawberry, *Fragaria* × *ananassa* (Rosaceae); sugarcane, *Saccharum officinarum* (Poaceae); teak, *Tectona grandis* (Lamiaceae); upland cotton, *Gossypium hirsutum* (Malvaceae); and, mainly, *Eucalyptus* spp. (Myrtaceae). The objective of this study was to investigate the biological and behavioral parameters of this insect in the laboratory and in *Eucalyptus* spp. plantations in Inhambupe, Bahia State, Brazil. The incubation period and the viability of *G. assimilis* eggs were 11.87 days and approximately 22%, respectively. The duration of the nymphal stage was 62.34 days with approximately 60% of the nymphs obtained in the laboratory being females. The average number of egg batches per female, eggs per female, and eggs per batch per female of this insect were 25.50, 862.17, and 34.65, respectively. *G. assimilis* females lived for 76.50 days in the adult stage, and 138.34 days in total, from egg through nymph to adult. Males produced three characteristic sounds: one for the marking of territory, one for courtship, and one when alone. *G. assimilis* fed primarily on weeds but, in their absence, it damaged young *Eucalyptus* spp. plants. This paper presents important data on the biology and behavior of *G. assimilis*; this information may encourage additional biological research, laboratory rearing, and integrated management of this pest.

Keywords

bioecology, field observation, forest pest, Gryllides, Grylloidea, laboratory rearing

Introduction

The common black field cricket, *Gryllus assimilis* (F., 1775) (Orthoptera: Gryllidae), is a pest of *Eucalyptus* spp. (Myrtaceae) (Severin 1926, Barbosa et al. 2009, Silva et al. 2013). Adults and nymphs commonly damage outdoor plants, mainly nursery seedlings and young plants in the field (Spann 1934, Barbosa et al. 2009, Weissman et al. 2009). Occasional damage to seeds shortly after sowing and in adult *Eucalyptus* spp. trees has been reported (Grodzki 1972, Barbosa et al. 2009, Silveira et al. 2014). Damage by nymphs and adults (Hutchins and Langston 1953) may necessitate the replanting of *Eucalyptus* spp. to maintain the desired number of plants per hectare (Folsom 1931, Thomas and Reed 1937, Doggett et al. 1980). *G. assimilis* has been reported from Canada to Argentina and from the Atlantic to the Pacific Oceans (Rehn and Hebard 1915, Alexander and Walker 1962, Weissman et al. 1980, 2009).

Eucalyptus spp. planting is done in the months prior to and during the rainy season in Brazil when irrigation is, generally, not necessary (Stape et al. 2001, Sampaio et al. 2016), making conditions favorable for *G. assimilis*, which prefers moist soil and a dark environment (Grodzki 1972, David et al. 2003, Barbosa et al. 2009). Nymphs and adults of this pest feed on the stem, leaves, roots, and branches of young *Eucalyptus* spp. plants during the night and remain hidden during the day in holes or between clods of earth and vegetation on the ground (Grodzki 1972, David et al. 2003, Barbosa et al. 2009). Damage by *Gryllus* sp. adults was recorded only on the lower third of *Eucalyptus grandis* seedlings in a laboratory in Brazil, and damage was greater to plants on the ground than in those grown in raised beds in a nursery (Barbosa et al. 2009), probably due to this insect's inability to jump or fly to elevated heights (Guerra and Pollack 2007).

Management strategies for *G. assimilis* in *Eucalyptus* spp. and other forest trees in the nursery and the field depend on studies of the biology and behavioral aspects of this insect (Mello et al. 1980, Hall 1988, Bertram and Rook 2012). An online system for rapid identification of insect pests in commercial teak plantations,

Tectona grandis (Lamiaceae), including *G. assimilis*, using smartphones as the inference mechanism, was developed (Nascimento et al. 2016). *G. assimilis* was found to inflict damage to *E. grandis* seedlings up to 40 days after planting, and this damage was lower in plantations near native forest areas (Barbosa et al. 2009), probably due to the presence of *G. assimilis* predators in the forest areas (Severin 1926). The objective of the present study was to evaluate the biology and behavior of *G. assimilis* in the laboratory and *Eucalyptus* spp. plantations in Inhambupe, Bahia State, Brazil.

Material and methods

Study site.—The study was carried out in the Laboratório de Proteção Florestal (LPF) at 26.5 ± 0.5 °C, 61.0 ± 0.5% RH, and 12h:12h (L:D) photoperiod, and in *Eucalyptus* spp. plantations (11°47'S × 38°21'W, 292 m above sea level) of Bracell Ltd. in Inhambupe, Bahia State, Brazil, with a temperature and RH of 26.5 ± 0.5 °C and 62 ± 15%, respectively. Meteorological data were obtained from the company weather station located about 5 km from the study site. The municipality is located on the northern coast of Bahia State, where *Eucalyptus* spp. are planted for the production of special soluble cellulose (basically two types: rayon-grade and specialty-grade) with α-cellulose content above 98.5%. *Eucalyptus* spp. pests, including *G. assimilis*, have been reported in Inhambupe and in another 20 municipalities of Bahia State (Masson et al. 2017a, b).

Collecting insects of the parental generation in the field.—*G. assimilis* adults were collected from the study site at night during outbreaks on recently planted *Eucalyptus* spp. seedlings and brought to the laboratory in individual plastic containers (500 mL) lined with hydrophilic cotton. For mating, each pair was placed in a glass container (1.5 L) closed with polyvinyl chloride (PVC) fabric. Into each glass container was placed a plastic container (4 mL) with freshly harvested, crushed *Brassica oleracea* group *acephala* (Brassicaceae) leaves as food, another container (4 mL) with hydrophilic cotton soaked in distilled water as a moisture source, and a third container (60 mL) with oviposition substrate (3 cm of sieved and sterilized fine sand) (Mello et al. 1980). This sand was sieved using a Granutest n° 35 sieve (São Paulo State, Brazil). Corrugated and perforated cardboard was also placed in the container for shelter and shade (Mello et al. 1980), an important addition for *G. assimilis* as it is nocturnal (Ackert and Wadley 1921) as well as cannibalistic and an omnivore (Blatchley 1901).

Insect identification.—*G. assimilis* was identified by Dr. Evoneo Berti Filho of the Departamento de Entomologia e Acarologia at the Universidade de São Paulo in Piracicaba, São Paulo State, Brazil. Five adult males and five adult females collected in *Eucalyptus* spp. plantations in Inhambupe were identified by comparing external morphology with that described in Weissman et al. (1980).

Rearing eggs, nymphs, and adults of the F1 generation.—*G. assimilis* eggs obtained from adults collected in the field were placed in Petri dishes (9 cm diameter) lined with sterilized fine sand. Nymphs obtained from these eggs were each put in separate Petri dishes (15 cm diameter) using a fine-tipped brush. The nymphs were given the same food and moisture source as the adults were given.

Forty healthy *G. assimilis* adults (20 males and 20 females) of a larger size were selected from the nymphs reared in the laboratory, which were in turn obtained from individuals collected in the field and mated as described. The mating and maintenance of adults and the collection and maintenance of *G. assimilis* eggs were performed as for the parental generation. If one half a pair died, it was replaced with a healthy individual of the same sex.

Biological evaluations of the F2 generation nymphs in the laboratory.—*G. assimilis* nymphs were obtained from eggs laid by the F1 generation adults in the laboratory. First-instar nymphs were kept individually in Petri dishes (15 cm diameter) until the end of the last instar. The number of instars and the duration of the nymph stage (days) were quantified by counting the number of exuviae observed on the base of the rearing containers during daily evaluations.

Behavioral assessments of nymphs in the laboratory (F2 generation) and field.—The behavioral aspects of *G. assimilis* were determined via visual observation throughout the insects' life cycle in the laboratory, as well as day and night visits (two visits per month in the morning, afternoon, and night periods for 12 months) and notes in the field. The following behavioral parameters were measured and basic statistics obtained: beginning, ending, and hatching peaks of the nymphs; the dispersal behavior of the nymphs shortly after hatching in the rearing containers; their coloration two hours after hatching and the changes in coloration with development; their feeding start time; and the acts of cannibalism and the percentage of each body part attacked. The percentage of first instar nymphs adhered to eggshells and the percentage of these nymphs that died were quantified. The percentage of nymphs that fed on their exuviae was also assessed.

In the field, the depth (cm) of galleries with aggregated first instar nymphs, and the nymphs' dispersion characteristics, according to their development, were registered from 20 randomly selected galleries excavated between 04:00 and 08:00 AM, which is the period of greatest occurrence of this insect in *Eucalyptus* spp. plantations.

Bioecological evaluations of F2 generation adults and their eggs in the laboratory.—The sex ratio (females: males) was evaluated with the females identified by the ovipositor at the abdomen extremity (Weissman et al. 1980). Copulation was reported and described. The number of copulations per couple and the copula time (h) were obtained daily by visual observations during the adult stage. The period (days) of pre-oviposition, oviposition, and post-oviposition, the number of egg batches (eggs laid per oviposition act) and eggs per female, the number and viability (%) of eggs per batch, and the incubation period (days) of eggs laid per female were evaluated. Adult female longevity (days) and the duration (days) of the full life cycle (from egg oviposition to death of the F2 generation females) were also evaluated. The duration (min) of the oviposition period and oviposition behavior, considering egg batch oviposition, the introduction of the ovipositor in the rearing container sand (depth in cm), and the distance (mm) between eggs of the same batch were evaluated in five randomly selected females. The geometric shape and the egg diameter (mm) were measured. The egg diameter was measured in five randomly selected eggs per female from egg batches laid at the intermediate time. The color of the newly deposited eggs, those close to hatching, and of the unviable eggs were also evaluated.

Sound observations under laboratory conditions.— The number and types of sounds, the sex of individuals emitting them, and the reaction of conspecific males and females when hearing these sounds were registered. Three trials were set for mating males and females in three different combinations and appraising the

sounds emitted in each situation. The first trial consisted of four crickets: two males and two females. The second trial consisted of one male and one female, and the third trial consisted of a single individual male. In all trials, only two-day-old virgin crickets were used. The largest and healthiest F1 and F2 generation insects from the laboratory colony were chosen for the sound tests. Three replicates per trial were conducted, and the crickets' behavior in each trial was observed and recorded for 24 hours. Each trial was conducted in one of three glass containers, each at the far end of an insect rearing room (25 m^2) to minimize the possibility of trials interfering with each other. The containers were placed on a bench at a height of 1.5 m, 26.5 ± 0.5°C, 61.0 ± 0.5% RH, and 12h:12h (L:D) photoperiod.

Host plants in the field.—Plants preferred by *G. assimilis* nymphs and adults for feeding, including *Eucalyptus* spp. and weeds, were evaluated visually in two commercial plots of *Eucalyptus* in Inhambupe, one without weed removal and another with manual weed removal at plots establishment. *Eucalyptus* spp. and weeds were examined daily for damage from planting to 30 days.

Results and discussion

Biological evaluations of F2 generation nymphs in the laboratory.—*G. assimilis* nymphs passed through five instars in 53 to 66 days (average 62.34 days) (Table 1). These results are similar to previous reports on *Gryllus abbreviatus* Serville, 1838, in Illinois (McNeill 1891) and the fall field cricket, *Gryllus pennsylvanicus* Burmeister, 1838, in Indiana, USA (Blatchley 1901). However, our results differed from those found in other studies of *G. assimilis*. For example, *G. assimilis* from *Eucalyptus saligna* plantations in Brazil also had five instars but spent three to five weeks as nymphs (Grodzki 1972); *G. assimilis* collected in *T. grandis* plantations (Silva et al. 2013) and those collected in *Eucalyptus* spp. plantations in Piracicaba, São Paulo State, Brazil (Mello et al. 1980), both spent 45 days as nymphs. These differences may be due to variation in RH in the laboratories (61.0 ± 0.5% in this study vs. 70.0 ± 10.0% in the others).

Behavioral assessment of nymphs in the laboratory (F2 generation) and field.—In the laboratory, *G. assimilis* nymphs emerged between 05:00 and 10:00 AM, with a peak around 06:00 AM (Table 1). This time corresponds to sunrise in Inhambupe, with milder temperatures and rains or dew during the rainy season. The newly emerged, yellowish-white *G. assimilis* nymphs, which spread through the rearing container shortly after hatching in the laboratory, turned brown after about two hours and then turned darker brown to black as they developed. Cannibalism was observed only on moribund or dead nymphs in both the absence and presence of food and a moisture source; the abdomen of the victim was the most attacked body part. First and second instar nymphs are more agile than the other instars. During the daytime, nymphs remained in dark places and began foraging in the rearing containers at about 05:00 PM. No preferential feeding time was observed. About 2% of the nymphs were trapped in the eggshell and died, probably from injuries to their bodies. The nymphs fed on their exuviae after molting making it difficult to evaluate the number of exuviae. Cannibalism behavior on weak or dying individuals had rarely been observed among laboratory-living individuals (Ackert and Wadley 1921).

In the laboratory, *G. assimilis* eggs were laid in the container provided with sterilized fine sand as oviposition substrate. Females leaned on their anterior legs and lowered their abdomen, introducing the ovipositor in the sand to a depth of up to 1.5 cm. During the oviposition process, females moved the abdomen down and upwards rapidly several times in a single location. In an egg batch, the distance between each egg was about 1 mm. The number of eggs laid was lower on drier substrates, and no eggs were laid on very moist substrates. The oviposition period was 5 min per egg batch, and a higher number of eggs were laid at night. Feeding habits of adults were similar to that of the nymphs, with cannibalism on dying or dead individuals being observed regardless of the lack or presence of food and a moisture source. As cannibalism was performed, aggressiveness among individuals was observed.

In the field, first instar nymphs were observed aggregated in the interior of galleries they excavated. These nymphs separated from each other according to their development and remained in the galleries at an average depth of 20 cm. This depth probably increases according to nymphs' development and soil moisture, which are important parameters for nymph hatching and development during the rainy season.

Bioecological evaluations of F2 generation adults and their eggs in the laboratory.—The *G. assimilis* female: male ratio was 6:4. Copula occurred during the daytime (04:30 AM to 04:00 PM), coinciding with a reduction in foraging activity (Table 1). The number of copulations per couple varied from zero to four (average 1.34). The pre-oviposition, oviposition, and post-oviposition periods ranged from 16 to 37, 11 to 66, and from 0 to 12 days with averages of 35.20, 36.80, and 4.50 days, respectively (Fig. 1). The number of egg batches, total eggs, and eggs per batch per female ranged from 8 to 55 (average 25.50), 260 to 1,918 (average 862.17), and 23.64 to 46.13 (average 34.65 eggs), respectively. Egg viability was 8 to 38% (average 22%). The egg incubation period ranged from 11.38 to 12.40 days (average 11.87). The F2 adult females' longevity ranged from 27 to 115 days (average 76.50 days); while the entire F2 adult females' life cycle duration (from the egg stage to death) was 138.34 days (Table 1).

Our results generally agreed with earlier works. For example, Veazey et al. (1976) indicate that between 30 to 80% of the total individuals are males for *Gryllus* spp. in the field in Florida, USA. Variation from zero to four in the copulation number per *G. assimilis* couple confirms the fact that multiple mating is frequent and

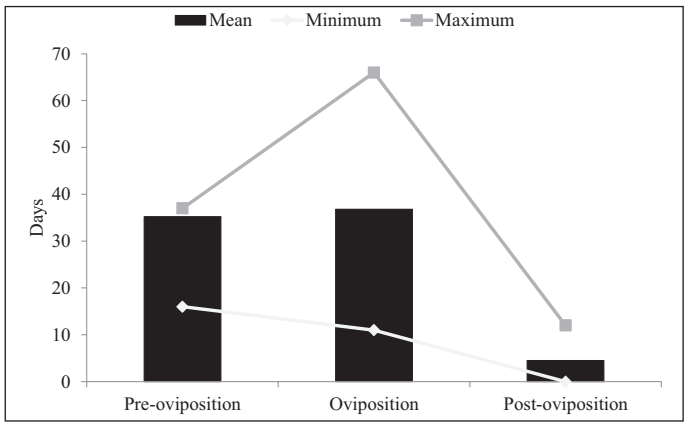

Fig. 1. Minimum, maximum, and mean duration of the pre-oviposition, oviposition, and post-oviposition periods of *Gryllus assimilis* (Orthoptera: Gryllidae) in the laboratory (N = 50 couples).

Table 1. *Gryllus assimilis* biological and behavioral parameters under laboratory conditions: minimum (Min.), maximum (Max.), mean (Mean), total (Total), and sample size (N).

Parameters	Min.	Max.	Mean	Total	N
Number of instars	–	–	–	5	500
Duration nymphal stage (days)	53	66	62.34	–	500
Sex ratio females: males	–	–	–	6:4	100
Number of copulations per couple	0	4	1.34	–	50*
Time at copulation	04:30 AM	04:00 PM	–	–	50*
Number of egg batches per female	8	55	25.50	–	50*
Total number of eggs per female	260	1,918	862.17	–	50*
Eggs per batch per female	23.64	46.13	34.65	–	50*
Viability of eggs (%)	8.40	38.24	22.37	–	20**
Incubation period of eggs (days)	11.38	12.40	11.87	–	20**
Longevity of adult females (days)	27	115	76.50		50*
Female life cycle duration (egg, nymph, and adult) (days)	–	–	138.34	–	50*
Egg diameter (mm)	2.8	3.2	3.0	–	250
Time of nymph emergence	05:00 AM	10:00 AM	06:00 AM	–	20***
Dead nymphs adhered to egg shell (%)	–	–	–	2	20***
Cannibalism on abdomen (%)	–	–	–	100	20***
Time nymphs began foraging	–	–	–	05:00 PM	20***
Depth of galleries excavated by nymphs (cm)	–	–	20	–	20
Depth of ovipositor introduction into the sand (cm)	–	1.5	–	–	10****
Distance between eggs in the same batch (mm)	–	–	1.0	–	2*****
Duration of egg batch oviposition (min)	–	–	5	–	2*****

* Number of couples, ** number of eggs per first, intermediate, and final egg batch laid per couple, *** number of nymphs obtained from eggs per first, intermediate, and final batch per couple, **** number of ovipositor introductions per first, intermediate, and final batch per couple, and ***** number of egg batches for first, intermediate, and final batch laid per couple.

beneficial for female crickets (Arnqvist and Nisson 2000). The female Mediterranean field cricket, *Gryllus bimaculatus* De Geer, 1773 (Bretman and Tregenza 2005), and the tropical house cricket, *Gryllodes sigillatus* Walker, 1869 (Orthoptera: Gryllidae) (Sakaluk et al. 2002), can mate up to 7 and 15 times, respectively. The number of matings increases fecundity and the number of eggs produced by vocal field cricket females, *Gryllus vocalis* Scudder, 1901 (Gershman 2009). Regarding the number of eggs laid per female, we found a higher number than Mello et al. (1980), who worked on a population of *G. assimilis* females collected in a *Eucalyptus* sp. plantation and subsequently reared in the laboratory in Piracicaba, São Paulo State, Brazil. Nonetheless, egg viability was higher (86%) in the Piracicaba population than in our present study (22%). This variation may be attributed to differences in the rearing environment, e.g., RH. The low viability of the eggs may be associated with a failure in copulation and the rejection of the female by the second male after the exchange of the first one due to its premature death; i.e., non-mating females may lay infertile eggs. Eggs from the first and final batches laid showed variable viability, while batches laid at the intermediate time had the most fertile eggs. This suggests that females require periodic copulations to maintain the viability of their eggs. It was noticed that in those females that laid a low number of eggs, the eggs had high viability, suggesting a negative correlation between the total number of eggs laid per female and their viability. Out of the viable eggs laid within a single batch, 95% hatched within 24 h.

We found a similar incubation period of *G. assimilis* eggs compared with the population from *Eucalyptus* spp. plantations in Piracicaba, São Paulo State, Brazil (Mello et al. 1980). However, in a study in Manhattan, Kansas, USA, Ackert and Wadley (1921) found that the incubation period of eggs from young *G. assimilis* females mated in the field and brought to the laboratory was three weeks. The longevity of *G. assimilis* females in this study was higher than the longevity of the same species of undetermined sex from the study of Silva et al. (2013), which was about 60 days, and higher than those of undetermined sex from the study by Mello et al. (1980), of about 45 days. The complete life cycle of *G. assimilis* females (from the egg stage to the females' death) was 138.34 days, suggesting the potential of two or three generations per year in Inhambupe, Bahia State, Brazil. However, two is more likely because the rainy season in this municipality lasts about five months. In contrast, the life cycle of *G. assimilis* individuals collected on *E. saligna* plantations in Brazil was about three months in summer and shorter in winter (Grodzki 1972).

G. assimilis eggs were rod-shaped and 3.0 ± 0.2 mm diameter. Eggs were white-opaque soon after oviposition, becoming straw-yellow except for the apex, which darkened as it neared hatching. Inviable eggs were translucent white soon after deposition, making it easier to distinguish them. Some viable eggs became dark yellow and wrinkly at low RH, rendering them inviable.

Sound observations.—*G. assimilis* males emitted three types of sound. These sounds were emitted at different times in the laboratory, each during a specific situation. The first sound was for marking territory, the second for courtship, and the third could be heard when the insect was alone. Each sound provoked a particular reaction in both conspecific females and males. The sound for marking territory was emitted intensely by the males in containers with two female and two male individuals, indicating territorial disputes. The wings were quickly raised, and the characteristic sound was emitted. One male retreated from the other and returned or not to the dispute after a few minutes, ending with the death or injury of the smaller, weaker insect. In most cases, the winner changed his song to courtship.

The sound for courtship was alternating, soft, strident, low frequency, and was done while the male slowly walked behind the female and turned his back to her. Then, the male lowered his abdomen to the female, which mounted the male, then lowered her

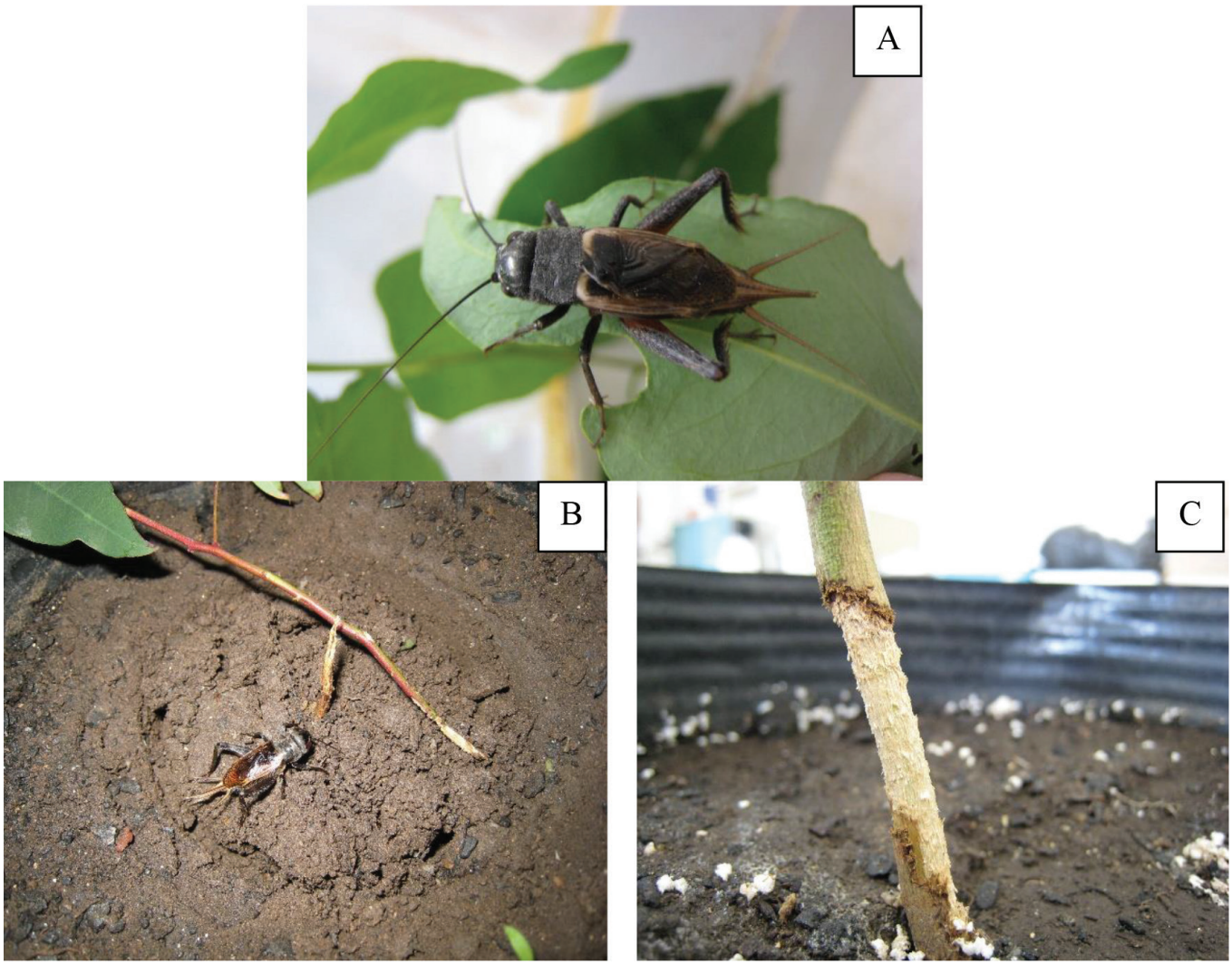

Fig. 2. *Gryllus assimilis* (Orthoptera: Gryllidae). **A**. Adult; **B, C**. Damage to *Eucalyptus* sp. (Myrtaceae).

abdomen for the male to introduce his copulatory organ into her genital opening, remaining in this position for less than a minute. Noises caused by equipment and people in the laboratory caused the female to dismount from the male several times with both returning to the mating position. Courtship lasted for up to one hour and the copulation period lasted about eight minutes. The spermatophore was observed at the insertion of the genital opening of copulated *G. assimilis* females. The calling sound emitted by individuals alone in the laboratory rearing container was continuous, strident, and often attracted sexually receptive females.

Sound production by resting *G. assimilis* males is a common behavior of *Gryllus* spp. The sound is produced by a structure called the pars stridens composed of small teeth on the ventral region of the right tegmen that are scraped by a "scraper" on the border of the left tegmen (portion of the anal edge), similar to a "washboard". These teeth generally present a triangular, uniform, and sloping morphology and gradually decrease in size at both ends (David et al. 2003). The sound may be indicative of cricket species, but *G. assimilis* individuals emitted different sounds in the field in North Carolina, USA (Fulton 1932). After the emission of territorial marking sounds, disputes between *Gryllus* spp. males and females are aggressive, but females do not necessarily mate with the winning male (Loranger and Bertram 2016). Vigorous males of *Gryllus* spp. win more competitions with rivals for territory and mating (Bertram and Rook 2012). The *G. assimilis* courting sound has two components: "chirps" and "ticks". "Chirps" are groups of low amplitude and frequency sound pulses and "ticks" are single pulses of greater amplitude with a high dominant frequency. Although variable, a cricket sound consists of five "chirps" followed by two "ticks" (Vedenina and Pollack 2012). The *Gryllus* spp. courtship sound forms the basis for sexual choice (Rebar et al. 2009). The calling sound is less complex with only one element, while the courtship call has two which differ in time and frequency (Elsner and Popov 1978). The sound of *G. assimilis* has been registered in oscillograms by Weissman et al. (2009), Shestakov and Vedenina (2012), and Pacheco et al. (2013).

Host plants in the field.—Eucalyptus spp. seedlings were more damaged by *G. assimilis* nymphs and adults after the manual removal of weeds (Fig. 2). This suggests that the weeds are preferred food for this insect. In this study, damage by *G. assimilis* was mainly observed on rass-jack weeds, *Bidens pilosa* (Asteraceae), native to the Americas (Rejmánek et al. 2017); lilac tassel flower, *Emilia sonchifolia* (Asteraceae), native to Asia (Sheikh and Dixit 2017); *Solanum* sp. (Solanaceae); and amaranth, *Amaranthus hybridus* (Amaranthaceae), native to North America

(Iamonico and El Mokni 2017). *G. assimilis* fed on weeds of *M. sativa*; Kentucky bluegrass, *Poa pratensis*; bindweed species, *Convolvulus* (Convolvulaceae); crabgrass, *Syntherisma sanguinale*; Dulac and Bermuda grass, *Capriola dactylon* (Poaceae) as well as decomposing bodies of its own species and animal carcasses in areas near Manhattan, Kansas, USA (Ackert and Wadley 1921), confirming the polyphagous habits of this insect.

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