# Phenological and Physiological Changes in Adult Piezodorus guildinii (Hemiptera: Pentatomidae) Due to Variation in Photoperiod and Temperature

Authors: Zerbino, Maria S., Altier, Nora A., and Panizzi, Antônio R.

# PHENOLOGICAL AND PHYSIOLOGICAL CHANGES IN ADULT *PIEZODORUS GUILDINII* (HEMIPTERA: PENTATOMIDAE) DUE TO VARIATION IN PHOTOPERIOD AND TEMPERATURE

Maria S. Zerbino[1], Nora A. Altier[1] and Antônio R. Panizzi[2]

[1]INIA La Estanzuela. Ruta 50 km 11, CP 70000, Colonia, Uruguay

[2]EMBRAPA Trigo. Caixa Postal 451. 990012-970, Passo Fundo, RS, Brazil

*Corresponding author; E-mail: szerbino@inia.org.uy

## Abstract

The effect of photoperiod and temperature on *Piezodorus guildinii* (Westwood) adult body morphometry, color, lipid content, development of reproductive organs, and feeding activity at different ages was studied in the laboratory. Three different conditions were tested, each at 80 ± 10% RH: 14:10 h L:D at 25 °C, 10:14 h L:D at 25 °C, and 10:14 h L:D at 20 °C. Adults at 25 °C under 14 h photophase were larger and exhibited lower lipid content than those held at 20 °C under 10 h photophase. Highest percentages of females with clear pronotum band and connexivum were recorded at 10 h photophase regardless of the temperature. Development of the ovary was affected by both photoperiod and temperature. At 15, 30 and 45 days of age under 14 h photophase at 25 °C, the percentage of females with immature ovaries remained constant at 0%; in contrast, at 10 h (20 °C) this percentage declined from 60 to 33%. Testes size was affected by photoperiod and temperature; males at 14 h (25 °C) and at 10 h (20 °C) showed the largest and the smallest testes, respectively. The ectodermal sac condition was affected starting with males 15 days of age; the percentage of males with an expanded ectodermal sac was greater under the 14 h (25 °C) treatment than under the 10 h (20 °C) treatment. Feeding activity of adults under short photophase conditions (10 h) decreased. These laboratory results indicate that adults of *P. guildinii* maintained under a short (10 h) photophase at 20 °C enter into reproductive diapause, accumulate energetic reserves (lipids), show undeveloped reproductive structures and smaller size, clear coloration of the pronotum band and connexivum, and less feeding activity.

Key Words: *Piezodorus guildinii*, diapause, feeding activity, photoperiod, temperature

## Resumen

El objetivo de este estudio de laboratorio fue evaluar el efecto del fotoperiodo y la temperatura en: la morfometría, el color del cuerpo, contenido de lípidos, desarrollo de los órganos reproductivos y la actividad alimentaria de *Piezodorus guildinii* (Westwood). Fueron evaluadas tres condiciones diferentes (14:10 y 10:14 h L:O a 25 °C, y 10:14 h L:O a 20 °C), a 80 ± 10% HR. Los adultos en 14 h de fotofase (25 °C), fueron más grandes y tuvieron menor contenido de lipidos que en 10 h (20 °C). La mayor proporción de hembras con banda del pronoto y conectivo claro fue registrada en 10 h de fotofase (20 y 25 °C). La condición del ovario fue afectada por el fotoperiodo y la temperatura. A los 15, 30 y 45 días de edad, en 14 h de fotofase (25 °C), 0% de las hembras exhibió el ovario inmaduro; por el contrario en 10 h (20 °C), durante ese período el porcentaje de hembras con ovario inmaduro disminuyó de 60 a 33%. El fotoperiodo y la temperatura afectaron el tamaño de los testículos; siendo mayor y menor a 14 h (25 °C) y a 10 h (20 °C), respectivamente. La condición del saco ectodérmico fue afectada a partir de los 15 días de edad; la proporción de machos con saco ectodérmico expandido fue mayor a 14 h (25 °C) que a 10 h (20 °C). La actividad alimentaria de los adultos disminuyó en condiciones de fotofase corta (10 h). Los resultados obtenidos indican que los adultos de *P. guildinii* en condiciones de 10 h de fotofase y 20 °C ingresan en diapausa reproductiva, acumulan reservas energéticas (lípidos), tienen estructuras reproductivas inmaduras, cuerpo más pequeño, banda del pronoto y conectivo de coloración clara, y actividad alimentaria menor.

Palabras Clave: *Piezodorus guildinii*, diapausa, actividad alimentaria, fotoperiodo, temperatura

*Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) (common names: Neotropical green stink bug and small green stink bug in South America, and red-banded stink bug in the USA) is a Neotropical stink bug species that ranges from Argentina to the southern United States (Panizzi & Slansky 1985). It is an important pest of soybean in the Southern Cone of South America. It is the most abundant stink bug in Uruguay and Argentina, causing the largest economic loss in soybean (Zerbino 2010). It is an economically important pest of soybean production in the southern United States (Baur & Baldwin 2006; Kamminga et al. 2012).

Current knowledge about the ecology of *P. guildinii* is limited. Some information is available on the effect of photoperiod and temperature on nymph and adult biology (Zerbino et al. 2013). Recently, Saulich & Musolin (2012) reviewed winter diapause in the seasonal cycle of stink bugs from the temperate zone. However, for Neotropical stink bug species, little is known about their ability to enter reproductive diapause and pass through adverse environmental periods.

The great majority of stink bugs enter diapause during the winter as adults (Saulich & Musolin 2012). Accumulation of energetic reserves, undeveloped reproductive structures, and changes in size, shape and coloration body, are among the most conspicuous alterations that occur among pentatomids during winter diapause (McPherson 1974; Ali & Ewiess 1977; Ito 1985; Alburquerque 1993; Hodek & Hodková 1993; Kobayashi & Numata 1995; Nakamura & Numata 1997; Musolin & Numata 2003; Mourão & Panizzi 2002; Chocorosqui & Panizzi 2003; Niva & Takeda 2003). In climates with moderate winters, adults of some heteropterans continue feeding (Shearer & Jones 1996; Mourão & Panizzi 2002; Chocorosqui & Panizzi 2003; Saulich & Musolin 2012).

Environmental factors, especially photoperiod and temperature, are the main abiotic factors regulating diapause (Ali & Ewiess 1977; Ichimori et al. 1990). Studies with different species of pentatomids that evaluated the effect of photoperiod on reproductive diapause, showed that it is induced by a 12-hour or less photophase (Ali & Ewiess 1977; Alburquerque 1993; Hodek & Hodková 1993; Kobayashi & Numata 1995; Nakamura & Numata 1997; Musolin & Numata 2003; Mourão & Panizzi 2002; Chocorosqui & Panizzi 2003; Niva & Takeda 2003). In some species, the photoperiodic response of diapause induction within a range of temperatures is thermostable (Musolin & Numata 2003); however, in other species temperature also plays a role in triggering diapause, modifying the photoperiodic effect (Numata et al. 1993; Hodek & Hodková 1993; Kobayashi & Numata 1995; Niva & Takeda 2003; Saulich & Musolin 2012). The main function of temperature is to shape the optimal timing of diapause during the season. In warm years winter diapause shifts to later dates, whereas in cold years an earlier induction of diapause takes place. The interaction of photoperiod and temperature creates a reliable ecological mechanism to regulate the timely onset of diapause (Saulich & Musolin 2012).

In this laboratory study we evaluated the influence of photoperiod and temperature on phenological and physiological changes, and on the feeding activity, of *P. guildinii*.

## MATERIAL AND METHODS

Three different treatments were applied to evaluate the effect of photoperiod and temperature on adult body morphometry, color, lipid content, development of reproductive organs, and on feeding activity of *P. guildinii*: 14:10 h L:D at 25 ± 1 °C , 10:14 h L:D at 25 ± 1 °C, and 10:14 h L:D at 20 ± 1 °C. The photophase of 14 h at 25 °C represents the conditions recorded at the beginning of the summer, while the photophase of 10 h at 20 °C represents the conditions recorded at the beginning of the winter at the site were the studied was conducted (latitude S 30-35°).

### Insects, Origin and Rearing Procedure

Adults of *P. guildinii* ($n$ = 400) were collected during summer 2012 at INIA La Estanzuela, Colonia, Uruguay (S 34° 20' W 57° 41'). They were taken to the laboratory and 20 pairs were placed into each of 20 clear plastic boxes (25 × 20 × 20 cm) to obtain eggs (Silva & Panizzi 2008). They were fed green bean pods (*Phaseolus vulgaris* L.), dry soybean seeds (*Glycine max* (L.) Merrill) and raw shelled peanuts (*Arachis hypogaea* L.), all equally available. Insects were maintained at 25 ± 1 °C, 80 ± 10% RH, and 14:10 h L:D. Food was replaced every other day and distilled water was supplied every day by moistening cotton in a plastic container (1.0 cm diam).

Egg masses were removed, mixed to avoid genetic effects, and divided into three groups. Each group was taken to a different growth chamber in order to assign the photoperiod/temperature treatments. Insects were kept at the same photophase and temperature conditions from egg to adult. During the 1st instar, nymphs were maintained only with distilled water. On the first day of the 2nd instar, groups of 10 nymphs were placed in a clear plastic box (11.0 × 11.0 × 3.5 cm) lined with filter paper. They were supplied with the same food of adults, described above. Food was renewed every other day and distilled water was supplied every day. Relative humidity was kept constant at 80 ± 10%. Chamber temperatures were monitored every day using HOBO loggers, model U23-001 (Onset Computer, Pocasset, Massachusetts).

Seasonal Morph and Lipid Content

At adult emergence, females and males were paired for each treatment. Each pair was placed into a clear plastic box lined with moistened filter paper and fed the same food offered to the nymphs. They were supplied daily with distilled water and food was renewed every other day. On days 1, 15, 30, and 45 of adult life, ca. 30 pairs from each treatment were killed by freezing at -20 °C. After adults were killed, digital pictures of each adult were taken, and measurements of the body were made using the software Image Pro-Express 5.1 (MediaCybernetics, Silver Spring, Maryland). The variables evaluated were: body length, pronotum width, and length of lateral margin of the pronotum. Females were also characterized by the color of the pronotum band and the connexivum. The color of the pronotum band varied from cream to purple. Females were typified as clear type when the color of the pronotum band was either cream, yellow or orange, and as dark type when the color was pink or purple. The color of the connexivum was recorded as clear when it was whitish-pink or as dark when it was blackish-pink.

Adult pairs were divided into two groups. In the first group, lipid content was measured using the method of Panizzi & Hirose (1995): insects were held in an oven (60 °C) for about 48 h, and then dry weight (DW) was recorded to the nearest 0.01 mg using an electronic scale. For lipid extraction a Twisselman extractor (IVA Co., Argentina) with 6 extraction tubes was used. Insects were individually identified with a unique number and placed in a cotton cloth bag (3.0 × 4.0 cm). Six bags were conditioned in each extraction tube (7.5 × 3.0 cm); 250 mL of the extraction solvent (hexane) was added to a volumetric flask and placed in the Twisselman extractor. The equipment was heated to approximately 120 °C, after which the extraction process was continued for 2 h. After that, each adult was placed back into the oven (60 °C) for 15 h and then weighed once more to obtain its weight without lipid (TW). The following formula was applied to obtain percentage of stored lipids: % lipid = [(DW-TW) /DW] × 100.

Adults associated with the second group were dissected and the developmental stage of each adult's reproductive organs was evaluated. Ethyl alcohol (70%) was used to clean the abdominal cavity of the insects to allow the best view of the reproductive organs. Females were ranked according to Mourão & Panizzi (2002) in one of three categories: immature = ovaries with no differentiation or no development, and no vitellary constriction; intermediate = ovaries with differentiation with visible chambers and vitellary constrictions with oocytes; and mature = ovaries totally developed with oocytes well-developed. For males the condition of the ectodermal sac (collapsed or expanded) was evaluated according to Esquivel (2009). In addition, testes were extracted from the abdominal cavity and measured using the software Image Pro-Express 5.1. They were ranked as developed (values falling within the range of the highest mean ± one SEM and higher), and immature (values falling within the range of the lowest mean ± one SEM).

Feeding Activity

On the first day of the emergence, ca. 76 adults from each treatment were transferred individually into a clear plastic box (11.0 × 11.0 × 3.5 cm) lined with moistened filter paper, recording the gender of each adult. The feeding activity of adults was measured at days 10, 20, 30 and 40 of adult life. Prior to each evaluation they were fasted for 48 h in the presence of water, and then an immature soybean pod (R 5.5) was offered for 24 h. During the first seven days of adult life, and between each feeding activity evaluation, adults were allowed to feed on green bean pods, dry soybean seeds, and raw shelled peanuts, all equally available. Food was replaced every other day and distilled water was supplied every day by moistening cotton in a plastic container (1.0 cm diam).

After 24 h of feeding activity, the immature soybean pods were taken out and immersed in an acid fuchsin solution (1g of fuchsin + 2 ml of alcohol + 300 mL of water) for 1 h to stain the stylet sheaths. After that, the pods were washed with running tap water and the number of stylet sheaths was enumerated under magnification with a stereomicroscope (Bowling 1980).

Statistical Analyses

Each experiment was run twice, and photoperiod and temperature conditions were randomly re-assigned subsequent runs. The experimental design was factorial split plots in randomized blocks, with a different number of subsamples per treatment because of mortality. The whole plots were the treatments, and the split plots where the eight combinations given by gender and age. Morphometric data (body length, pronotum width, and length of the lateral margin of the pronotum), lipid content, and testes size were analyzed with general linear mixed model (PROC MIXED, SAS Institute 2008). Means were compared by contrasts ($Pr > |t| < 0.05$).

The percentages of females that displayed the pronotum band and connexivum type clear or dark were analyzed using the generalized linear model with the binomial distribution (PROC GENMOD, SAS Institute 2008). The model considered the main effects of treatment, age and age*treatment interaction. The percentages of the females with different ovarian developmental

stages were also analyzed using this generalized linear model. Data corresponding to 1 day-old adults were analyzed using the binomial distribution and the link function logit because at this age only intermediate and immature stages were recorded. Data obtained at 15, 30, and 45 days were analyzed using a multinomial distribution and the link function cumlogit. Each age was analyzed individually. The percentages of males with different developmental stage of testes and different conditions of the ectodermal sac were analyzed using the generalized linear model with binomial distribution and the link function logit. Each age was analyzed individually. The results are presented as the likelihood ratio statistics of the Chi-square distribution.

Feeding activity data were analyzed using the generalized linear mixed model (PROC GLIMMIX, SAS Institute, version 9.2) with the Poisson distribution and logarithmic function, with the number of stylet sheaths measured at day 10 of adulthood as a covariate and the subsequent assessments at day 20, 30 and 40 as repeated measures in the analysis. The covariance structure that fitted the data was AR (1). The model statement included the interaction term (age*treatment) to test for heterogeneity of slopes. Means of treatments were compared by contrasts (Pr>|t|< 0.05).

## RESULTS AND DISCUSSION

### Seasonal Morph and Lipid Content

Size and Coloration of the Body. Treatments (photophase/temperature) had a significant effect on body length ($F_{2,2} = 61.7$, $P =0.01$), pronotum width ($F_{2,2} = 44.2$, $P =0.02$), and lateral margin length of the pronotum ($F_{2,2} = 29.1$, $P =0.03$). The analyses also indicated that morphometric variables were significantly different for genders (body length: $F_{1,683} = 394.7$, $P < 0.0001$; pronotum width: $F_{1,684} = 314.1$, $P < 0.0001$; lateral margin length of the pronotum: $F_{1,684} = 156.9$, $P < 0.0001$).

Photophase and temperature had a similar effect on both sexes for each variable; the interaction treatment*gender was not significant (body length: $F_{2,683} = 1.5$, $P = 0.21$; pronotum width: $F_{2,684} = 2.63$, $P = 0.07$; lateral margin length of the pronotum: $F_{2,684} = 1.4$, $P = 0.26$). There was a significant effect of age on body length ($F_{3,683} = 21.9$, $P < 0.0001$), on pronotum width ($F_{3,684} = 13.2$, $P < 0.0001$), and on the lateral margin length of the pronotum ($F_{3,684} = 12.6$, $P < 0.0001$); although this was not expected and biologically not explained, that may be due to the group assignment. Adult insects do not grow, and the size of an adult insect is determined by the size of the last instar (Nijhout 2003). However, for these variables, the effects of the treatment were similar at the different ages; the interaction treatment*age was not significant (body length: $F_{6,683} = 1.5$, $P= 0.18$; pronotum width: $F_{6,684} = 1.4$, $P =0.22$; lateral margin length of the pronotum: $F_{6,683} = 0.9$, $P =0.51$). The interaction treatment*age*gender was not significant for all variables (body length: $F_{6,683} = 0.5$, $P = 0.80$; pronotum width: $F_{6,684} = 0.5$, $P = 0.84$; lateral margin length of the pronotum: $F_{6,684} = 0.4$, $P = 0.86$).

Results of the morphometric evaluation indicated that body size of *P. guildinii* adults was under photoperiod control but not temperature, at least under the short photoperiod treatment. Values of body length, pronotum width and lateral margin length of the pronotum recorded at 14 h of photophase at 25 °C were significantly greater than those recorded under short (10 h) photophase at 25 and 20 °C, which were similar. Females were larger than males (Table 1).

A review of nine studies on the effects of temperature on body size of heteropterans concluded that in 78% of the cases decreases in temperature led to smaller size (Atkinson 1994). Few studies were found that assessed the effects of abiotic factors on the size or shape of pentatomids. In species with a shoulder spine [such as *Euschistus servus* (Say), *E. heros* (Fabr.), *Oebalus poecilus* (Dallas), and *D. melacanthus* (Dallas)], the size and shape of the spine were affected by photoperiod

TABLE 1. SIZE OF ADULTS (MM) OF *PIEZODORUS GUILDINII* (MEAN ± SEM) AT THREE SETS OF PHOTOPHASE AND TEMPERATURE CONDITIONS IN THE LABORATORY.

| Conditions/ gender | Mean ± SEM (mm)[1] | | | |
| --- | --- | --- | --- | --- |
| | Body length | Pronotum width | Lateral margin pronotum length | Testes length |
| 10h, 20 °C | 9.52 ± 0.04 b [227] | 5.03 ± 0.01 b [227] | 1.96 ± 0.01 b [227] | 1.37 ± 0.02 c [75] |
| 10h, 25 °C | 9.50 ± 0.04 b [240] | 5.01 ± 0.01 b [240] | 1.96 ± 0.01 b [240] | 1.50 ± 0.02 b [75] |
| 14h, 25 °C | 9.83 ± 0.04 a [245] | 5.17 ± 0.01 a [245] | 2.04 ± 0.01 a [245] | 1.71 ± 0.02 a [71] |
| Females | 9.88 ± 0.04 A [360] | 5.20 ± 0.01 A [360] | 2.04 ± 0.01 A [360] | |
| Males | 9.36 ± 0.04 B [352] | 4.94 ± 0.01 B [352] | 1.94 ± 0.01 B [352] | |

[1]Means followed by the same lowercase letter or uppercase letter in each column are not significantly different (Pr > _t_ < 0.05). Consult text for ANOVA details.

(McPherson 1974; Alburquerque 1993; Mourão & Panizzi 2002; Chocorosqui & Panizzi 2003). Niva & Takeda (2003) showed for *Halyomorpha halys* (Stål) that under a short photophase the head and pronotum widths were significantly reduced. Nakamura (2002) reported that *Dolycoris baccarum* (L.) adults held at 25 °C under long photophase conditions (16:8 h L:D) were larger than those raised under short photophase (12:12 h L:D).

Body size is one of the most important life-history traits because it is correlated with numerous physiological and fitness traits including fecundity and survivorship (Danks 1987; Honĕk 1993; Nylin & Gotthard 1998). The results reported by Zerbino et al. (2013) suggest that body size of *P. guildinii* is associated with reproductive performance, survivorship, and growth rate.

The analyses of the data on the color of the pronotum band of females indicated that there was a significant effect of the interaction treatment*age ($\chi^2$ = 26.99; df = 6; $P$ < 0.0001). At 25 °C, the percentage of females with a clear pronotum band was higher under short (10 h) compared to long (14 h) photophase, particularly at day 1. From day 15 on, the percentages obtained under short photophase at different temperatures (25 and 20 °C) were similar and higher than those recorded under long photophase (25 °C) (Fig. 1A). While the percentages observed over time did not vary significantly under different photophases at 25 °C, they decreased significantly under short photophase (20 °C) between ages 1 to 15 from 100 to 63%, and, thereafter, values were similar (Fig. 1A).

The analyses of the percentages of females with different connexivum colors indicated a significant effect of the interaction treatment*age ($\chi^2$ = 35.45; df = 6; $P$ < 0.0001). The percentage of females with a clear connexivum generally followed what was observed for the pronotum band color pattern. At day 1, the percentage of females with a clear connexivum was significantly higher under short (10 h) photophase at 20 °C compared to the other treatments. From day 15 on, the percentages with clear connexivum under short photophase were similar at 25 and 20 °C and higher than those recorded under long (14 h) photophase at 25 °C (Fig. 1B). While the percentages of females with a clear connexivum did not vary significantly over time under different photophases at 25 °C, under short photophase at 20 °C significant differences were found over time (Fig. 1B).

Results obtained for the color of the pronotum band and connexivum indicate that these color variables were a consequence of both photophase and adult age. Abdominal coloration of the pentatomid *D. melacanthus* was found to be related to photoperiod and age (Chocorosqui & Panizzi 2003). In contrast, Mourão & Panizzi (2002) reported that although *E. heros* adults
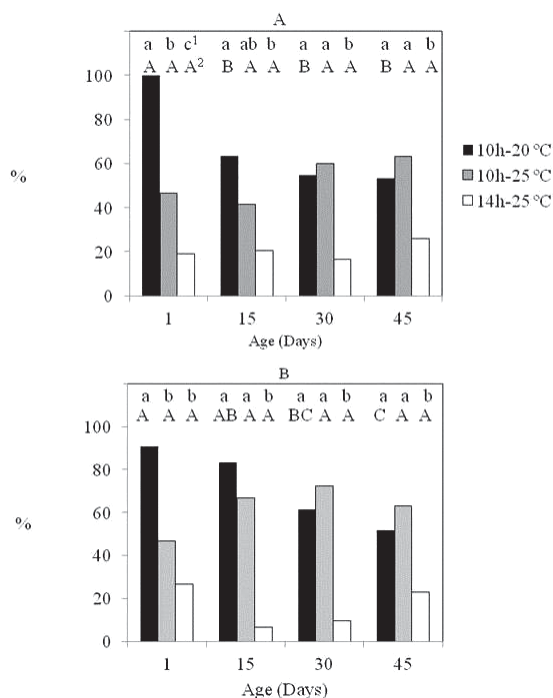


Fig. 1. Percentage of *Piezodorus guildinii* adult females at different ages with a clear type pronotum band (A) and clear type connexivum (B), maintained under 3 sets of photophase and temperature conditions in the laboratory. [1]Means followed by the same letter within each age are not significantly different *(p < 0.05)* based on the likelihood ratio. [2]Means followed by the same uppercase letters between ages do not differ significantly (*p* < 0.05 based on likelihood ratio.

with two different colors were observed, color was not related to variation in the photophase. Changes in body coloration during the season are typical of many true bugs, and are often controlled by photophase and usually related to diapause (Saulich & Musolin 2012). Musolin & Numata (2003) and Musolin et al. (2007) stated that the color changes are regulated by day length and associated with the physiological state of the individuals.

Several works with different species of pentatomids established that color changes were related to diapause, as in the case of *E. servus* (Say), *E. tristigmus tristigmus* (Say), *N. viridula*, *Oebalus ypsilongriseus* (DeGeer), *Plautia stali* (Scott), and *Thyanta calceata* (Say) (references in Saulich & Musolin 2012). In general, the main trend in seasonal coloration change is the prevalence of brown coloration or dull texture of the integument in overwintering insects. This makes them less conspicuous, providing them passive protection from predators (Saulich & Musolin 2012).

Lipid Content. The analyses of the data on the body lipid content of *P. guildinii* adults indicated that the effects of treatment ($F_{2, 2}$ = 3.4, $P$ = 0.23), gender ($F_{1, 355}$ = 2.8, $P$ = 0.09), and the interactions gender*age ($F_{3, 355}$ = 0.23, $P$ = 0.87) and treatment*age*gender ($F_{6, 355}$ = 1.3, $P$ = 0.24) were not statistically significant, while the interaction treatment*age had a significant effect ($F_{6, 355}$ = 2.3, $P$ = 0.035).

For adults 1 and 15 days old, there was no effect of treatment on the lipid content. At days 30 and 40 of adulthood, adults maintained under short (10 h) photophase at 20 °C had higher lipid content than those held under a 14 h photophase at 25 °C (Fig. 2). While the lipid contents recorded under short photophase at 20 and 25 °C were different for 30 day-old adults, at day 45 of adulthood the percent values were similar (Fig. 2). Results obtained indicated that lipid accumulation in *P. guildinii* was more pronounced for adults kept at a 10 h photophase at 20 °C; while for those kept at 14 h photophase at 25 °C the levels of lipid storage remained the same across all ages (Fig. 2).

Adult pentatomids have been shown to have larger amounts of body fat in winter than in summer including *N. viridula* and *E. heros* in Londrina, Brazil (Panizzi & Hirose 1995) and *N. viridula* in Hawaii (Jones & Westcott 2002). Other authors who studied the effect of photoperiod on the lipid content of adults of different species of pentatomids in the laboratory found that under short photophase, adults accumulated lipids (Niva & Takeda 2003; Chocorosqui & Panizzi

2003). Changes in length of photophase and temperature had the same effect on adult *P. guildinii* regardless of gender, similar to other pentatomids (Panizzi & Hirose 1995; Mourão & Panizzi 2002; Chocorosqui & Panizzi 2003).

Degree of Development of Reproductive Organs. The development of the ovaries was affected by the photophase and temperature. At day 1, no differences were detected between treatments ($\chi^2$ =5.31; df = 2; $P$=0.07), and almost no females showed ovarian development (Fig. 3). In contrast, at days 15, 30 and 45, there was a significant effect of the treatments (15 days $\chi^2$ = 24.6, 30 days $\chi^2$ = 42.40, 45 days $\chi^2$ = 33.90; df = 2; $P$ < 0.0001). From day 15 on, the percentages recorded under a long (14 h) photophase at 25 °C were different than those under a short (10 h) photophase at 20 °C (Fig. 3). For instance, under long (14 h) photophase at 25 °C the proportion of females with immature ovaries was 0% from day 15 to day 45, while under short (10 h) photophase and 20 °C during the same time-period, the percentage of females exhibiting immature ovaries declined from 60 to 33%.

The decrease of the photophase reduced the maturity of the ovaries. The photoperiodic response was influenced by the decrease of temperature as in others insect species (Danks 1987; Cho et al. 2008; Saulich & Musolin 2012). For adult females 15 to 30 days old, under short photophase, the percentages with immature ovaries were higher at 20 °C than at 25 °C. These results suggest that *P. guildinii* does not have thermostability of photoperiodic responses, such as *N. viridula* and *Arma custos* (F.) (Musolin 2012; Volkovich & Saulich 1994).

The slow decline in the percentage of females that contained undeveloped ovaries under a 10 h photophase at 20 °C, combined with the contin-
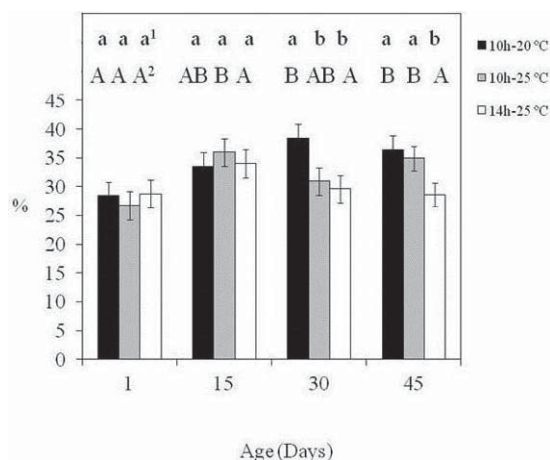


Fig. 2. Percentage of lipid content (X ± SEM) of *Piezodorus guildinii* adults at different ages, maintained under 3 sets of photophase and temperature conditions in the laboratory. [1]Means followed by the same lowercase letters within each age are not significantly different (Pr>|t|< 0.05). [2]Means followed by the same uppercase letters for the same treatment between ages are not significantly different (Pr>|t|< 0.05)
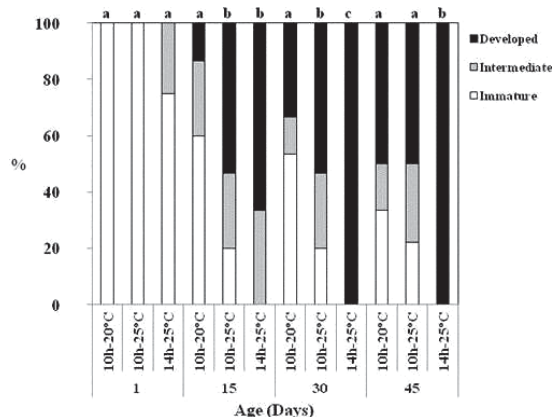


Fig. 3. Percentage of *Piezodorus guildinii* females with ovaries at different degrees of development at different ages, maintained under 3 sets of photophase and temperature conitions in the laboratory. Means followed by the same letters in each age do not differ significantly ($P$ < 0.05) based on likelihood ratio.

ued occurrence of intermediate ovaries, suggest that the exposure to the short photoperiod and 20 °C delayed but did not prevent ovarian development, as determined by Spurgeon & Brent (2010) for *Lygus hesperus* (Knight). Saulich & Musolin (2009) established that in species that overwinter as adults, the absence of oviposition is caused by direct suppression of maturation and/or oviposition due to low temperature, rather than by induction of reproductive diapause. Zerbino et al. (2013), studying the reproductive performance of *P. guildnii* found that the preoviposition period was five times shorter under long (14 h) photophase at 25 °C compared to short (10 h) photophase at 20 °C; under long photophase at 25 °C *P. guildinii* had the highest percentages of ovipositing females, number of egg masses, and number of total eggs/female, while a decrease in temperature increased the negative effect of the short photophase – females under short photophase at 20 °C showed the lowest values for all variables, including percentage of egg hatchability (4%).

The percentage of females with undeveloped ovaries recorded in the present study was smaller than those obtained for other pentatomids. Higuchi (1994) working with *P. hybneri* reported that under a short photophase (≤ 12 h), more than 80% of 15 day-old females had immature ovaries. Mourão & Panizzi (2002) determined for *E. heros* that 90% of 30 day-old females had immature ovaries when were maintained under a short (10 h) photophase at 25 °C. Moreover, Chocorosqui & Panizzi (2003), studying *D. melacanthus*, found that under a short (11 h) photophase and 25 °C, 80% of 20 day-old females had immature ovaries. Musolin & Numata (2003) established that 60 day-old *N. viridula* females held under a short (10 h) photophase at 25 °C had 100% of undeveloped ovaries.

There was significant effect of the treatments ($F_{2,2}$ = 93.3, $P$ =0.01) and age ($F_{3,202}$ = 22.8, $P$ < 0.0001) on testes size; while the treatment*age interaction was not significant ($F_{6,202}$ = 1.9; $P$ = 0.08). The values of testes size for the three treatments were significantly different (Table 1). Males maintained under long (14 h) photophase

at 25 °C had the largest testes, and those held under short (10 h) photophase at 20 °C had the smallest. Testes size of males 1-day old were significantly smaller (1.38 ± 0.02 mm) than those at 15 (1.55 ± 0.02 mm), 30 (1.57 ± 0.02 mm), and 45 (1.60 ± 0.02 mm) days of adulthood, which were not different from each other.

Results showed that testes with a size between 1.21 to 1.54 mm were immature, and those ≥ 1.55 mm were developed. The analyses of the percentages of the testes ranking indicated that there was a significant effect of treatments of adults at the four ages (1 day $\chi^2$ = 9.7; df = 2; $P$ = 0.008, 15 days $\chi^2$ = 25.63; df = 2; $P$ < 0.0001, 30 days $\chi^2$ = 11.40; df = 2; $P$ = 0.003, 45 days $\chi^2$ = 13.89; df = 2; $P$ = 0.001). Regardless of age, the proportion of males with developed testes was significantly greater under long photophase at 25 °C than under short photophase at 20 °C (Fig. 4A).
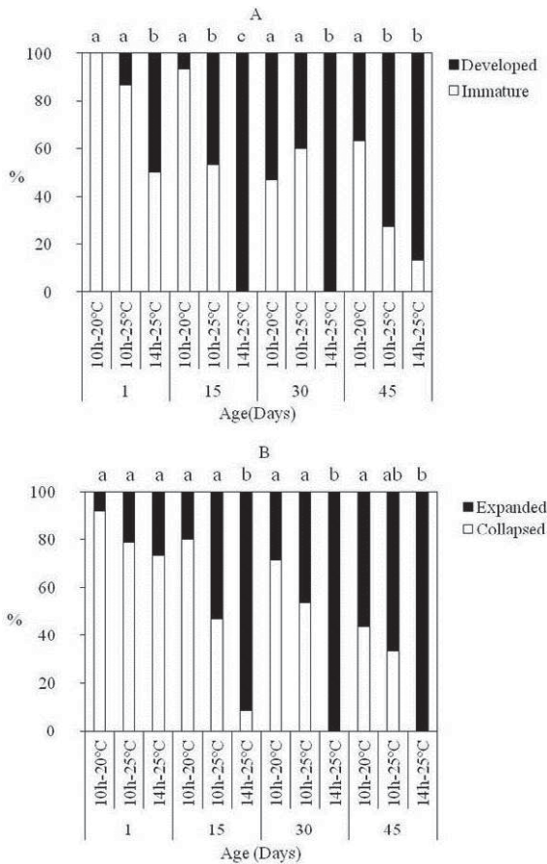


Fig. 4. Percentage of males of *Piezodorus guildinii* with different degree of development of the reproductive organs of males at different ages, maintained under 3 sets of photophase and temperature conitions in the laboratory; A) testes; B) ectodermal sac. Means followed by the same letters in each age do not differ significantly ($P$ < 0.05) based on likelihood ratio.

TABLE 2. FEEDING ACTIVITY OF ADULTS OF *PIEZODORUS GUILDINII* DURING 24 H AT THREE SETS OF PHOTOPHASE AND TEMPERATURE CONDITIONS IN THE LABORATORY.

| Conditions | Number of stylet sheaths/adult/day[1] | |
| --- | --- | --- |
| | Slope LN(x) | (Mean ± SEM) |
| 10 h, 20 °C | -0.044 a | 1.43 ± 0.18 b [221][2] |
| 10 h, 25 °C | -0.035 a | 1.90 ± 0.20 b [263] |
| 14 h, 25 °C | -0.011 b | 2.77 ± 0.25 a [225] |

[1]Means followed by the same letters in each column are not significantly different (Pr > |t| < 0.05).
[2]Number of adults in brackets.

The decrease in photophase and temperature reduced the size of the testes; they occupied a small portion of the abdominal cavity, but otherwise their appearance was normal. Males of *N. viridula* held under short photophase also showed testes with normal appearance (Ali & Ewiess 1977; Esquivel 2009). In others pentatomids the difference between immature and developed testes is considerable, so no measurements are necessary to evaluate their condition. For instance, Mourão & Panizzi (2002) observed for *E. heros* that undeveloped testes were 1 mm or less in length; Chocorosqui & Panizzi (2003) for *D. melacanthus* established that immature males showed testes with normal appearance, but smaller size. In the last two studies, under short (< 12 h) photophase conditions at day 20, 90% of males had immature testes.

When measurement of the testes size is not feasible, it is useful to evaluate the condition of the ectodermal sac (Esquivel 2009; Spurgeon & Brent 2010). There was no significant difference among treatments with respect to the condition of the ectodermal sac at day 1 of adulthood ($\chi^2$ = 1.64; df = 2; $P$ = 0.44), but there were significant treatment effects at 15, 30 and 45 days of age (15 days $\chi^2$ = 16.61; df = 2; $P$ = 0.0002, 30 days $\chi^2$ = 14.34; df = 2; $P$ = 0.0008, 45 days $\chi^2$ = 6.44, df = 2; $P$ = 0.04). Across all ages, the proportion of males with an expanded ectodermal sac was greater under long (14 h) photophase at 25 °C than under short (10 h) photophase at 20 °C; no differences were detected between values recorded under a short photophase at the two temperatures (Fig. 4B). Similar results were obtained by Spurgeon & Brent (2010) for the heteropteran *L. hesperus*.

Results obtained in this study and those reported by Zerbino et al. (2013) indicate that *P. guildinii* increases the tendency to enter into a reproductive diapause with short day length (10 h) and low temperature (20 °C). Preliminary results (M. S. Zerbino, unpublished data) suggest that field-collected insects exhibit a greater and more consistent diapause response to short photophase and low temperature than do laboratory-reared insects. Similar results were reported by Spurgeon & Brent (2010) for *L. hesperus*.

Feeding Activity. The numbers of feeding sites (stylet sheaths) recorded on soybean pods varied according to the treatments at different ages (interaction of treatment*age $F_{3,478}$ = 11.89, $P$ = < 0 .0001), and to the genders ($F_{1,222}$ = 23.38, $P$ = < 0.0001). The interaction treatment*gender ($F_{2,222}$ = 2.48, $P$ = 0.09) was not statistically significant.

Results of analyzes indicate that feeding activity was under photoperiod control but not temperature at least under the short photoperiod treatment. The number of feeding sites recorded did not decline significantly over time when adults were hold under long (14 h) photophase and 25 °C (slope ≠ 0 Pr > |t| = 0.14). However, it decreased significantly when adults were maintained at 10 h photophase under different temperatures (25 and 20 °C) (slope ≠0 Pr>|t|= <0.0001, both treatments); the slopes been similar in these cases (Table 2). The mean number of stylet sheaths recorded under long photophase and 25 °C was significantly higher than those at short photophase treatments (20 and 25 °C), which did not differ (Table 2).

Few studies were found in the literature on the effect of photoperiod and temperature on feeding activity of pentatomids. Adults of *E. heros* increase their feeding activity with increasing photophase, and decrease it with age (Mourão & Panizzi 2002).These authors found that under short (≤ 12 h) photophase this decline occurred between the 1st and 2nd week of adult life, and under long (14 h) photophase it occurred between the 2nd and 3rd week of adult life. Niva & Takeda (2003) found that the 5th instar nymphs of *H. halys* showed less feeding activity under short (12 h) than under a long (16 h) photophase.

A greater number of feeding sites were associated with males than females regardless of age (2.6 vs 1.47 stylet sheaths/adult/day, respectively). The fact that *P. guildinii* males fed more than females is in accordance with previous data (Panizzi et al. 1995). In other pentatomids, such as *N. viridula*, *E. heros* and *Acrosternum hilare* (Say), both genders showed similar feeding activity (Bowling 1980; Simmons & Yeargan 1988; Panizzi et al. 1995).

## CONCLUSIONS

Results obtained in this study indicate that phenology and physiology of *P. guildinii* adults are affected by the length of photophase and temperature. Under short (10 h) photophase at 20 °C they display particular traits: accumulation of energy reserves (lipids); undeveloped reproductive organs; smaller body size; change in the coloration of the pronotum band and connexivum; and reduced feeding activity, all of which indicative of reproductive diapause.

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