Effect of Photoperiod and Temperature on Nymphal Development and Adult Reproduction of Piezodorus guildinii (Heteroptera: Pentatomidae)

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EFFECT OF PHOTOPERIOD AND TEMPERATURE ON NYMPHAL DEVELOPMENT AND ADULT REPRODUCTION OF *PIEZODORUS GUILDINII* (HETEROPTERA: PENTATOMIDAE)

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ABSTRACT

The effect of photoperiod and temperature on the biology of nymphs and adults of *Piezodorus guildinii* (Westwood) was studied in the laboratory. Four different conditions were tested (14:10, 12:12 and 10:14 h L:D at 25 °C, and 10:14 h L:D at 20 °C), at 80 ± 10% RH. The shortest nymph development time was recorded at 14:10 h L:D (25 °C) (21.5 days) and the longest at 10:14 h L:D (20 °C) (42 days). The highest nymph mortality rate was recorded at 10:14 h L:D (20 °C) and the lowest at 14:10 h L:D (25 °C) (84.5 vs. 24.2%). Newly emerged females reared at 14:10 h L:D (25 °C) were heavier than those of the remaining treatments. Fresh body weight gain (mg) occurred only during the 1st week of adult life. Adult survivorship was highest at 10:14 h L:D (20 °C) and lowest at 14:10 h L:D (25 °C). Total longevity was shortest when adults were held at 14:10 h L:D (25 °C) and longest at 10:14 h L:D (20 °C) (38.6 vs. 98.8 days). The maximum percentage of ovipositing females occurred at 14:10 h L:D (25 °C) and the minimum at 10:14 h L:D (20 °C). Females maintained at 14:10 h L:D (25 °C) and 12:12 h L:D (25 °C) produced similar and greater number of egg masses than females at 10:14 h L:D (25 °C) and 10:14 h L:D (20 °C). The number of eggs/female was the greatest at 14:10 h L:D (25 °C) and the lowest at 10:14 h L:D (20 °C) (196.2 vs. 21.7 eggs/♀). Egg viability was similar under different photophases at 25 °C, while significantly reduced at 10:14 h L:D (20 °C) (54 vs. 4.1%). The longest egg incubation period was recorded at 10:14 h L:D (20 °C) and the shortest at 14:10 h L:D (25 °C) (7 vs. 4.1 days). These laboratory results suggest that *P. guildinii* does not reproduce during the time its preferred host soybean is unavailable at latitude 30°-35° S, which corresponds approximately to the conditions tested at 10:14 h L:D (20 °C).

Key Words: *Piezodorus guildinii*, biology, photoperiod, temperature

RESUMEN

Este trabajo tuvo como objetivo evaluar el efecto del fotoperiodo y la temperatura en la biología de ninfas y adultos de *Piezodorus guildinii* (Westwood). Fueron consideradas cuatro condiciones (14:10, 12:12, 10:14 h L:D a 25 °C, y 10:14 h L:D a 20 °C), manteniendo la humedad relativa a 80 ± 10%. El estado de ninfa tuvo una menor duración a 14:10 h L:D y 25 °C (21.5 días), mientras que la mayor duración se registró a 10:14 h L:D y 20 °C (42 días). La mayor y menor mortalidad de ninfas fue registrada en 10:14 h L:D a 20 °C y 14:10 h L:D a 25 °C (73.8 y 23.4% respectivamente). El peso promedio de las hembras adultas recién emergidas registrado en el tratamiento 14:10 h L:D a 25 °C fue significativamente superior a los correspondientes en los restantes tratamientos, los cuales fueron similares entre sí. No fueron detectadas diferencias en la ganancia de peso de los adultos. Los menores valores de longevidad de adultos se registraron a 14:10 h L:D a 25 °C, mientras que los mayores se registraron a 10:14 h L:D y 20 °C (38.6 vs. 98.8 días). El comportamiento reproductivo de las hembras varió con las condiciones evaluadas. El máximo porcentaje de oviposición se registró a 14:10 h L:D y 25 °C, y el mínimo a 10:14 h L:D y 20 °C (84.5 vs. 24.2%). El número de posturas sólo fue afectado por el fotoperiodo; las hembras criadas a 14:10 h L:D y 12:12 h L:D a 25 °C realizaron más posturas que en las condiciones de 10:14 h L:D a 25 y 20 °C. El número de huevos por hembra varió con el fotoperiodo y la temperatura; el mayor valor se registró a 14:10 h L:D y 25 °C y el menor a 10:14 h L:D y 20 °C (196.2 vs. 21.7 huevos/♀). El porcentaje de huevos eclosionados fue similar en los diferentes fotoperiodos a 25 °C, y significativamente superior al de 10:14 h L:D y 20 °C. El período de incubación fue más largo a 10:14 h L:D y 20 °C, y más corto a 14:10 h L:D y 25 °C (7 vs. 4.1 días). Los resultados obtenidos indican que *P. guildinii* no se reproduce en la entrezafra del cultivo de soja entre S 30°-35° de latitud, que se corresponde aproximadamente con las condiciones de 10:14 h L:D y 20 °C evaluadas en este estudio.

Palabras Clave: *Piezodorus guildinii*, biología, fotoperiodo, temperatura

Insects have developed various physiological adaptation strategies to survive adverse environments. Diapause is an important adaptive mechanism for dormancy during periods of unfavorable conditions (Tauber et al. 1986). The most conspicuous aspect of reproductive diapause in females is a halt in the process of oogenesis. Environmental factors, especially photoperiod and temperature, influence insect biology and behavior, and can be considered the main abiotic factors regulating diapause (Ali & Ewiess 1977; Ichimori et al. 1990). Temperature acts both in isolation and in conjunction with other cues. Thermoperiod fluctuates between years, which make it a less important seasonal indicator than photoperiod. However, temperature acts to modify or reinforce the effects of photoperiod (Leather et al. 1993).

Studies that associate photoperiod and temperature with reproductive diapause have been conducted with several species of pentatomids (Saulich & Musolin 2012). Albuquerque (1993) observed under laboratory conditions that diapause was induced in *Oebalus poecilus* (Dallas) under short days. Hodek & Hodková (1993) established that photoperiod is an important factor for the diapause regulation of *Dolycoris baccarum* (L.). Adults of *Aelia fieberi* (Scott) were induced to diapause under short photophases (Nakamura & Numata 1997). Mourão & Panizzi (2002) observed that *Euschistus heros* (F.) underwent reproductive diapause, induced by a photophase of 12 h or less. Chocorosqui & Panizzi (2003) determined that *Dichelops melacanthus* (Dallas) presented reproductive oligopause under laboratory conditions, induced by 11 and 12 h light and characterized by the occurrence of periodic feeding, even under typical winter photophases. *Nezara viridula* (L.) responds to photoperiod not only qualitatively (diapause versus reproduction), but also quantitatively: photophase duration results in a graded response in the beginning of copulation and oviposition (Musolin & Numata 2003).

Piezodorus guildinii (Westwood) (common names: Neotropical green stink bug and small green stink bug in South America; red-banded stink bug in the USA) is a Neotropical stink bug species ranging from Argentina to the southern United States (Panizzi & Slansky 1985a). It is an important pest of soybean in the Southern Cone of South America and the most abundant stink bug in Uruguay and Argentina, being more common than the cosmopolitan southern green stink bug, *N. viridula*. In this geographic region, *P. guildinii* causes the largest economical losses to soybean crops (Zerbino 2010). In the United States it has been reported since the early 1900's (Kirkaldy 1909), and since then it has been reported in several states including South Carolina, Florida, Georgia, and New Mexico (McPherson & McPherson 2000). Several authors had previously reported the red-banded stink bug on soybean in the United States (references in Panizzi and Slansky 1985a; Panizzi & Slansky 1985b, c; McPherson et al. 1993), though it was not considered an economic pest. However, during the last decade it reached threshold levels and required insecticide sprays in southern Louisiana soybean crops (Baur & Baldwin 2006). More recently, it was considered a top soybean pest in southern USA (Kamminga et al. 2012). Feeding on a wide range of cultivated and non-cultivated plant hosts, with a particular fondness for legumes, the red-banded stink bug is capable of causing severe economic damage in soybean, alfalfa, and other bean crops (Panizzi & Slansky 1985a).

A considerable amount of work has been done on this species in Brazil (references in Panizzi et al. 2000, and in McPherson & McPherson 2000). Although several authors have studied its biology (Fraga & Ochoa 1972; Panizzi & Smith 1977; Panizzi et al. 1980; Costa & Link 1982; Panizzi & Slansky 1985a, b; Panizzi 1992; Cividanes & Parra 1994; Serra & La Porta 2001; Arroyo & Kamawura 2003; Massoni & Frana 2007), there is limited available information about the ecology of this insect. An understanding of the biotic and abiotic factors involved in the seasonality of this bug is essential to implement holistic and environmentally compatible soybean pest management programs. This laboratory study was carried out to evaluate the influence of photoperiod and temperature on nymph development, adult longevity, weight gain, and reproductive performance of *P. guildinii.*

Material and Methods

Insects, Origin and Routine Maintenance

Adults of *P. guildinii* were collected in 2011 at INIA La Estanzuela, Colonia, Uruguay (S 34° 20' W 57° 41'). They were taken to the laboratory and 20 pairs were placed in a clear plastic box (25 × 20 × 20 cm) to obtain eggs (Silva & Panizzi 2008). They were fed with green bean pods *Phaseolus vulgaris* (L.), dry soybean seeds *Glycine max* (L.) Merrill, and raw shelled peanuts *Arachis hypogaea* (L.). Insects were maintained at 25 ± 1 °C, 80 ± 10% RH, and 14 h photophase. Food was replaced on alternate days and distilled water was supplied every day by using moistened cotton in a plastic container (1.0 cm diameter). Egg masses were removed daily, mixed to avoid genetic effect, and divided into four groups in order to assign the treatments. To evaluate the effect of photoperiod and temperature on egg, nymph and adult biology, four different conditions (treatments) were tested: 14:10, 12:12, and 10:14 h L:D at 25 ± 1 °C, and 10:14 h L:D at 20 ± 1 °C. A photoperiod of 14:10 h L:D at 25 °C represents the approximate conditions recorded at the summer solstice, while a photoperiod of 10:14 h L:D at 20 °C represents

the approximate conditions recorded at the winter solstice at a range of latitude between 30° to 35° S.

Egg and Nymph Biology

Eggs were held in Petri dishes (9.0 × 1.5 cm) lined with moistened filter paper and maintained at the four conditions of photoperiod and temperature (treatments) described. Relative humidity was kept constant at 80 ± 10%. Chamber temperatures were monitored daily using HOBO® data loggers, model U23-001 (Onset Computer, Pocasset, Massachusetts) at 1h intervals. During the 1st instar, nymphs were maintained only with distilled water. On the first day of the 2nd stadium, nymphs from each treatment were individualized in plastic boxes (11.0 × 11.0 × 3.5 cm) lined with moistened filter paper. They were fed the same food as adults and distilled water was supplied as described above. Nymphs were maintained at the same conditions tested for the egg stage. Instar change and mortality were recorded daily. Upon emergence, adults were separated by sex and weighed on an electronic scale (OHAUS Pioneer™) (to the nearest 0.1 mg).

Egg and instar duration, total development time and nymph mortality were calculated for each treatment, and weight was measured at adult emergence. Treatments were set up in a completely randomized design. Each nymph that reached the adult stage was considered a replicate ($n > 30$). The experiment was repeated twice.

Adult Biology

Egg masses were obtained as described above, and maintained under the four conditions of photoperiod and temperature (treatments). Relative humidity was kept constant (80 ± 10%). Chamber temperatures were monitored every day using HOBO® data loggers, model U23-001 at 1 h intervals. 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 plastic box (11.0 × 11.0 × 3.5 cm) lined with filter paper. They were supplied with the food described above. Nymphs were reared under the same photoperiod as that of the egg stage. Food was renewed on alternate days and distilled water was supplied daily. At adult emergence, more than 30 pairs were formed for each treatment. Each pair was held in a plastic box lined with moistened filter paper, and fed the same food as nymphs. They were supplied distilled water on a daily basis and food was renewed on alternate days. Fresh body weight (mg) for both sexes was taken at the moment of emergence and once a week thereafter until mortality, using an electronic scale (OHAUS Pioneer™) (to the nearest 0.1 mg). Males and females that died were not replaced.

The reproductive performance was evaluated using the following parameters: percentage of females ovipositing, preoviposition and oviposition period, number of egg masses/female, number of eggs/female, and egg viability. Survivorship and longevity of males and females were also evaluated. Treatments were set up in a completely randomized design, and each pair ($n > 30$) was considered a replicate. The experiment was replicated twice.

Statistical Analyses

Egg and instar duration and development time (in days) were analyzed with the generalized linear model with the Poisson distribution and logarithmic function (PROC GENMOD, SAS Institute, version 9.2), as they are discrete variables and an association between means and variance was detected. The percentages of nymph survivorship in each instar and the total mortality were analyzed using generalized linear model with distribution binomial (PROC GENMOD, SAS Institute, version 9.2). In both cases, results are presented as the likelihood ratio statistics of the Chi-square distribution.

Weight at adult emergence was analyzed with the general linear model (PROC GLM, SAS Institute, version 9.2), due to the fact that the variance was homogeneous and there was no association between means and variance. Means were compared using Tukey-Kramer honestly significant (HSD) test for significance ($P \leq 0.05$). The differences between male and female fresh body weight were compared using Student's *t*-test ($P \leq 0.05$).

Data of adult survivorship over the time of the four treatments were analyzed by simple linear regression. To comply with the assumptions of linear regression, the data were transformed to LN (x +1). Slope coefficients were compared using Student's *t*-test ($P \leq 0.05$).

The percentage of females ovipositing was analyzed using generalized linear model with distribution binomial and the link function logit (PROC GENMOD, SAS Institute, version 9.2). The results are presented as the likelihood ratio statistics of the Chi-square distribution. Preoviposition and oviposition period, number of egg masses and eggs per female, and longevity were analyzed with the generalized linear model with the Poisson distribution and logarithmic function (PROC GENMOD, SAS Institute, version 9.2), as they are discrete variables and a strong association between means and variance was detected.

The results are presented as the likelihood ratio statistics of the Chi-square distribution.

Egg viability was analyzed with the general linear model (PROC GLM, SAS Institute, version 9.2), due to the fact that the variance was homogeneous and there was no association between means and variance. Means were compared using Tukey-Kramer honestly significant (HSD) test for significance ($P \leq 0.05$).

Data regarding change in fresh body weight were analyzed using the mixed model (Proc MIXED, SAS Institute, version 9.2), with initial body weight measured at day one as a covariate and the subsequent weights as repeated measures in the analysis. The covariance structure that fitted the data was ANTE (1). The model statement included the interaction term (gain*treat) to test for heterogeneity of slopes.

Results and Discussion

Because results from the two experiments were similar, data were combined in the final statistical analyses.

Eggs

There was a highly significant effect (χ^2 = 23.39; df = 3; $P < 0.0001$) of treatments on the incubation period. The results indicated that there was a negative correlation between photophase duration and temperature with the incubation period. Eggs held at 14:10 h L:D (25 °C) had the shortest incubation period (4.1 days), while those held at 10:14 h L:D (20 °C) had the longest (7.0 days) (Table 1). Mourão & Panizzi (2000) working with *E. heros*, at 25 °C and under different photoperiods observed that the incubation period was longer under short photophase (10 h) in comparison with long photophase (14 h). In turn, Ali & Ewiess (1977) for *N. viridula* and Albuquerque (1993) for *O. poecilus* found no effect of the photoperiod on the incubation period of eggs. The results obtained in this study and those obtained by the aforementioned authors may indicate that the embryos of different species during egg incubation have different sensitivity to photoperiod. Moreover, the time required to hatch increased significantly when the temperature decreased from 25 to 20 °C under short photoperiod conditions (10:14 h L:D).

Cividanes & Parra (1994) reported that *P. guildinii* eggs hatched faster as temperature increased from 20 to 26 °C; however, they did not find differences within the range of 26 to 28 °C. Values obtained in this study under the longest photophase at 25 °C (4.1 days) were similar with those reported by Cividanes & Parra (1994) at 26 °C (4.2 days). The results obtained by these authors at 14 h L and 20 °C (9 days) were slightly higher

Table 1. Mean (±SEM) development time, survivorship and mortality of *Piezodorus guildinii* eggs and nymphs at different photoperiods and temperatures under laboratory conditions.

Conditions	Stadium duration (days)							Total development time (1^{st}–5^{th} instars) (days)		% Total mortality
	Eggs	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}		Female	Male	
10:14 L:D 20 °C	7.0 ± 0.2 a[1]	4.6 ± 0.2 a [149][2]	9.3 ± 0.3 a [83]	8.7 ± 0.5 a [64]	8.0 ± 0.3 a [48]	11.7 ± 0.4 a [39]		41.9 ± 0.9 a	42.2 ± 1.2 a	73.8 a
10:14 L:D 25 °C	5.8 ± 0.1 b	3.4 ± 0.1 b [75]	5.5 ± 0.2 b [68]	4.8 ± 0.2 b [57]	4.9 ± 0.2 b [50]	6.3 ± 0.2 b [37]		24.5 ± 0.5 b	25.1 ± 0.4 b	50.7 b
12:12 L:D 25 °C	5.4 ± 0.1 b	2.8 ± 0.1 b [55]	5.3 ± 0.2 b [46]	4.6 ± 0.2 b [41]	4.5 ± 0.2 bc [37]	6.7 ± 0.2 b [32]		24.5 ± 0.5 b	23.9 ± 0.4 bc	40.0 b
14:10 L:D 25 °C	4.1 ± 0.1 c	3.1 ± 0.2 b [47]	5.3 ± 0.4 b [44]	4.7 ± 0.5 b [42]	3.7 ± 0.3 c [39]	5.1 ± 0.4 c [36]		21.2 ± 0.8 b	22.0 ± 0.5 c	23.4 c

[1]Means in each column followed by the same letters are not significantly different ($P < 0.05$) based on likelihood ratio.
[2]Number of nymphs that complete each instar is given in brackets.

than the corresponding results of this study at 10 h L and 20 °C (7 days). This difference could be attributed to the fact that the bugs of both studies were collected from two regions with different climatic regimes, subtropical and temperate (23° 18' and 34° 20' S, respectively). The temperature threshold for development and/or the degree day required to complete development may vary with the latitude as result of local adaptation to climatic conditions (Stacey & Fellowes 2002). Species with a lower threshold temperature for development have a greater rate of development at low temperatures (Trudgill et al. 2005). As in this study, several authors working with different species of pentatomids, found that when temperature decreased egg development time increased. Ali & Ewiess (1977) for *N. viridula* found that the incubation period was significantly longer at 20 °C than at 25 °C, under short photophase (10 h). Chocorosqui & Panizzi (2002) reported that no nymphs of *D. melacanthus* hatched when eggs were incubated at a temperature lower than 20 °C under long photophase (14 h).

Nymphs

Nymph Survivorship and Total Mortality. There were significant effects of the treatments on nymph survivorship between 2nd-4th instars (2nd $\chi^2 = 51.25$, df = 3; $P < 0.0001$, 3rd $\chi^2 = 9.3$; df = 3; $P = 0.0256$, 4th $\chi^2 = 9.25$; df = 3; $P = 0.0261$), whereas for the 5th instar there was not ($\chi^2 = 4.61$; df = 3; P = 0.2027). The greatest differences in nymph survivorship among treatments occurred in the 2nd instar, with the temperature decrease under short photophase having a more conspicuous negative effect on survivorship than the decrease in photophase at 25 °C (Fig. 1). According to the likelihood ratio statistics of the Chi-square

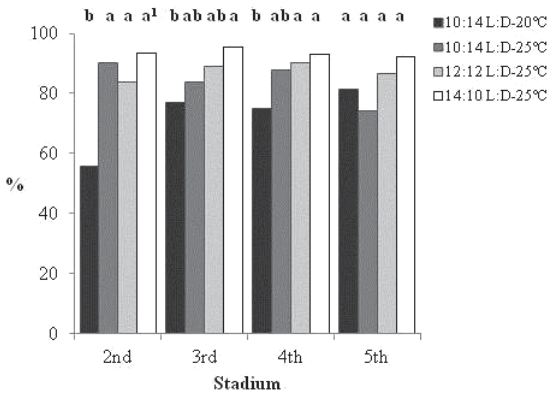

Fig. 1. Survivorship of *Piezodorus guildinii* nymphs at different instars under variable conditions of light and temperature regimes in the laboratory. [1]Means in each instar followed by the same letters are not significantly different ($P < 0.05$) based on likelihood ratio.

distribution, the lowest value of nymph survivorship was obtained under the photoperiod of 10:14 h L:D (20 °C) (55.7%), whereas in the other treatments nymph survivorship, which varied from 83.6 to 93.6% (Fig. 1). During the 3rd to 5th instars under 10:14 h L:D (20 °C), nymph survivorship was greater than that recorded for the 2nd instar. For the rest of the treatments nymph survivorship for instars 3rd to 5th was similar to that of the 2nd instar. Results obtained suggest that the 2nd instar is very sensitive to the decrease of temperature under short photophase. Panizzi & Smith (1977) also recorded high mortality in 2nd instar, but they explained that the high mortality appeared to be caused by nymphs falling onto their back and being unable to right themselves. *Dichelops melacantus* showed the same sensibility when was reared under long photoperiod and 20 °C (Chocorosqui & Panizzi 2002). In the 3rd and 4th instars the nymph survivorships recorded under short photophase (10 h) at 20 °C were lower than those recorded under long photophase (14 h) at 25 °C (Fig. 1).

Total nymph mortality (instars 2-5) was affected by photoperiod and temperature ($\chi^2 = 46.99$; df = 3; $P < 0.0001$) (Table 1). Total nymph mortality increased when photophase decreased at 25 °C; the value recorded at 14:10 h L:D (23.4%) was lower than those recorded at 12:12 and 10:14 h L:D (40 and 50.7%, respectively), which were similar. However, differences between treatments under the short photophase (10 h) at different temperatures were greater; when temperature decreased from 25 to 20 °C, total nymph mortality increased significantly from 50.7 to 73.8%. (Table 1). Cividanes & Parra (1994) did not find differences in nymph mortality of *P. guildinii* when reared at 25 °C and 20 °C under long photoperiod conditions (14:10 h L:D). These results suggest that nymphs of this insect have low adaptability to temperatures below 20 °C under short photophase. Therefore, in temperate regions, nymphs are unlikely to survive during mid-autumn and winter. Similar results were obtained by Ali & Ewiess (1977), who established that photoperiod did not exert a conspicuous effect on the mortality of nymphs of *N. viridula* at the same temperature, and that the nymph mortality at 20 °C was higher than at 25 °C.

Other species of pentatomids also showed variation in total nymph mortality induced by the length of the photophase. Mourão & Panizzi (2000) for *E. heros* observed higher mortality under short photophase in comparison to long photophase (56.7 vs. 28.3%). Chocorosqui & Panizzi (2003) for *D. melacanthus* recorded the highest total nymph mortality (60%) under short photophase at 25 °C. They also found almost 100% mortality when nymphs were reared at 20 °C under long photophase (Chocorosqui & Panizzi 2002).

Nymph Development Time. There were highly significant effects of treatments on nymph development time (1st instar χ^2 = 18.33; df = 3; P = 0.0004) (2nd χ^2 = 66.70, 3rd χ^2 = 66.49, 4th χ^2 = 58.57, and 5th χ^2= 115.97; df = 3; $P < 0.0001$). During the first three instars, no differences were recorded in the development rate under different photoperiod at 25 °C. The length of photophase only affected development of 4th and 5th instars, which were completed faster under long photophase. Similar results were obtained by Chocorosqui & Panizzi (2002) for *D. melacanthus*. In contrast, Mourão & Panizzi (2000) reported that 2nd and 3rd instar nymphs of *E. heros* exposed to short photophase needed more time to complete their development.

For both genders, the mean number of days to complete the nymphal stage varied with the environmental conditions used (females χ^2 = 156.24, males χ^2 = 155.34; df = 3; $P < 0.0001$)(Table 1). The decrease of photophase did not exert an important effect in total nymph development time, for the three photoperiods at 25 °C; total development time of females was similar and shorter than that recorded at 10:14 h L:D (20 °C). For males total nymph development time at 25 °C was shorter at 14:10 h L:D than at 10:14 h L:D (Table 1). Albuquerque (1993) reported that the length of photophase at 25 °C did not significantly affect the nymph development time of *O. poecilus*. In contrast, nymph development time of *N. viridula*, *E. heros* and *D. melacanthus* was reduced significantly when photophase increased (Ali & Ewiess 1977; Mourão & Panizzi 2000; Chocorosqui & Panizzi 2003). The differences found by different authors on the effect of photoperiod on nymph development of different species of pentatomids indicate that sensitivity to day length is variable and may appear at different stages of development (Saulich & Musolin 2012). This illustrates the importance of performing studies locally in order to determine the influence of the abiotic factors on the insect biology and behavior (Ali & Ewiess 1977).

The decrease in temperature significantly increased the effect of the short photophase on nymph development time (Table 1). Both genders reared at 10:14 h L:D required 40% more time to complete the nymphal stage at 20 °C than at 25 °C. The values of the total nymph development time determined by Cividanes & Parra (1994) for *P. guildinii* reared at 14:10 h L:D (26 °C) (20.2 days) and 20 °C (44.2 days) were similar to those obtained in this study for 14:10 h L:D (25 °C) (21.2 days) and 10:14 h L:D (20 °C) (41.9 days). This similarity may suggest that the temperature exerts a more conspicuous effect than photoperiod in total nymph development time.

Chocorosqui & Panizzi (2003) also detected effects of temperature on the nymph development time of *D. melacanthus*, stating that values tended to be longer when temperature decreased and no nymphs developed when temperature was lower than 20 °C. Ali & Ewiess (1977), working with a range of temperature between 20 and 30 °C, determined that the rate of nymph development time of *N. viridula* decreased as the temperature increased.

Fresh Body Weight at Adult Emergence. The fresh body weight of females varied with the conditions tested ($F_{3,151}$ = 10.43; $P < 0.0001$). Photoperiod affected fresh body weight of newly emerged females. They were heaviest when reared under long photophase (14 h) (25 °C), probably because nymphs reared under these conditions fed more than those that developed under short photophase (Table 2). The fresh body weight of males was not affected by photoperiod or temperature ($F_{3,143}$ = 2.2; P = 0.0908). Females were heavier than males in all conditions tested (Table 2).

Ali & Ewiess (1977) did not find great variation in body weight for newly emerged *N. viridula* when nymphs were reared under different photophases, but they reported that adults were heavier at 20 °C than at 25 °C. Other authors studying other species of pentatomids obtained different results. Mourão & Panizzi (2000) for *E. heros*, and Chocorosqui & Panizzi (2003) for *D. melacanthus* established that the weight of females tended to be higher with long photophase, although no statistically significant differences were recorded.

Adults

Adult Survivorship and Longevity. Adult survivorship gradually decreased over time in all treatments. The effects of the treatments were different on both genders (Fig. 2). The decrease of survivorship of females under different photophases at 25 °C was similar and higher than the decrease recorded under short photophase (10 h) (20 °C) (Table 3). In contrast, the decrease of survivorship of males was affected by photoperiod and temperature. The slope obtained under long

TABLE 2. MEAN (± SEM) FRESH BODY WEIGHT AT ADULT EMERGENCE OF *PIEZODORUS GUILDINII* AT DIFFERENT PHOTOPERIODS AND TEMPERATURES UNDER LABORATORY CONDITIONS.

	Fresh body weight (mg)	
Conditions	Female	Male
10:14 L:D 20 °C	49.84 ± 0.69 bA[1]	43.71 ± 0.54 aB
10:14 L:D 25 °C	51.55 ± 1.07 bA	45.34 ± 0.96 aB
12:12 L:D 25 °C	49.93 ± 0.72 bA	46.37 ± 0.66 aB
14:10 L:D 25 °C	56.70 ± 1.37 aA	46.68 ± 1.68 aB

[1]Means followed by the same lowercase letters in each column and uppercase letters in each row do not differ significantly ($P < 0.05$) using Tukey's test and t test, respectively.

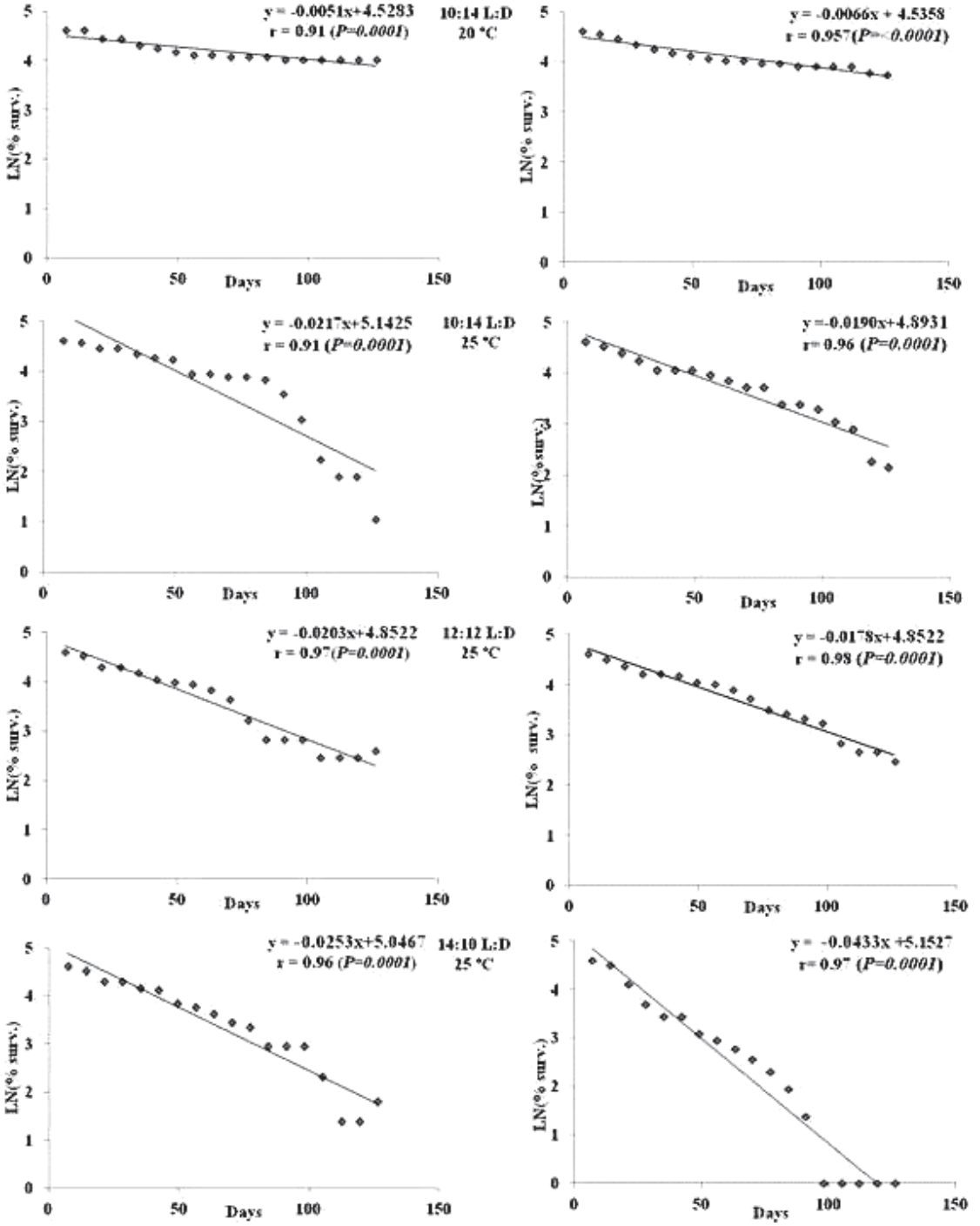

Fig. 2. Survivorship up to 126 days of adult *Piezodorus guildinii*, expressed as logarithm (LN) (%+1). Females right figures; males left figures.

photophase (14 h) (25 °C) was higher than those obtained at 12 h and 10 h (25 °C), whereas under short photophase (10 h) the decrease of survivorship was higher at 25 °C than at 20 °C (Table 3).

Total mean longevity was affected by the photoperiod and the temperature, with a similar response for both genders (females $\chi^2 = 21.73$, males $\chi^2 = 29.69$; df = 3; $P < 0.0001$) (Table 3). Adults

TABLE 3. SLOPE (B) OF DE SIMPLE LINEAR REGRESSION OF SURVIVORSHIP AND MEAN (± SEM) LONGEVITY OF *PIEZODORUS GUILDINII* ADULTS AT DIFFERENT PHOTOPERIODS AND TEMPERATURES UNDER LABORATORY CONDITIONS.

	b LN (% Survivorship+1)[1]		Longevity (days)[2]	
Conditions	Female	Male	Female	Male
10:14 L:D 20 °C	0.0051 ± 0.0012 b	0.0067 ± 0.0010 c	107.4 ± 12.9 a [33][3]	94.1 ± 11.9 a [33]
10:14 L:D 25 °C	0.0217 ± 0.0049 a	0.0190 ± 0.0032 b	62.8 ± 6.13 b [33]	63.4 ± 7.1 b [35]
12:12 L:D 25 °C	0.0203 ± 0.0022 a	0.0178 ± 0.0017 b	50.8 ± 5.7 bc [37]	60.3 ± 6.3 b [37]
14:10 L:D 25 °C	0.0253 ± 0.0037 a	0.0433 ± 0.0053 a	46.1 ± 5.4 c [32]	31.2 ± 3.8 c [32]

[1]Slopes in each column followed by the same letters are not significantly different at $P < 0.05$ based on t test.
[2]Means in each column followed by the same letters are not significantly different ($P < 0.05$) based on likelihood ratio.
[3]Number of adults in brackets.

lived longer under short photoperiod conditions (10:14 h L:D) (20 °C) (females = 107.0 days; males 94.1 days); rather, adults reared with long photoperiod (14:10 h L:D) (25 °C) had the shortest lifespan (females = 46.1 days; males= 31.2 days) (Table 3). When adults of *P. guildinii* were reared in greenhouse in summer and winter in Curitiba - Brazil, Panizzi & Smith (1977) reported similar values of total longevity to those obtained in this study. Females held under summer and winter conditions lived 41.2 and 96.6 days, respectively; while males lived 34.0 and 112.5 days, respectively. In contrast, Cividanes & Parra (1994) found that under long photophase (14 h) the longevity of adults of *P. guildinii* at 20 °C was less than at 26 °C. The results obtained for longevity in this study and those reported by Panizzi & Smith (1977) and Cividanes & Parra (1994) may likely suggest the occurrence of an interaction in the response of this variable to both, photoperiod and temperature. The greater longevity under winter conditions (shorter photoperiod and lower temperature) may be due to a decrease in neuro-hormonally mediated metabolic activity (Tauber et al. 1986). Particularly, females spend less reserve in egg production by decreasing their reproductive activity (Slansky & Panizzi 1987).

Adult Reproduction. Photoperiod and temperature affected the percentage of ovipositing females ($\chi^2 = 27.19$; df = 3; $P < 0.0001$) (Table 4). Temperature decrease had a more conspicuous negative effect on the percentage of ovipositing females than the decrease in photophase at 25 °C. The maximum value was obtained at 14:10 h L:D (25 °C) (84.5%), and the minimum at 10:14 h L:D (20 °C) (24.2%). Similar values were recorded at 12:12 h L:D (25 °C) (61.5%) and 10:14 h L:D (25 °C) (62.1%), which were different from the other two treatments. Cividanes & Parra (1994) evaluating the percentage of ovipositing *P. guildinii* females under long photophase (14 h) at 20 °C, reported similar values (24%) than those obtained in this work under the same temperature and short photophase (10 h). These results suggest that the temperature has a more important role than photoperiod on percentage of females ovipositing.

Photoperiod and temperature affected the reproductive performance of females (Table 4). There were highly significant effects of treatments for preoviposition period, egg masses and number of eggs/female ($\chi^2 = 62.86$; $\chi^2 = 60.58$; $\chi^2 = 29.92$; df = 3; $P < 0.0001$), and viability ($F_{3, 77} = 14.99$; $P < 0.001$) (Table 4).

The preoviposition period was affected by photophase and temperature, the effect of temperature being greater under short photophase (Table 4). Minimum preoviposition values occurred when females were maintained at 14:10 h L:D (25 °C) (8.0 days), and the maximum value was obtained at 10:14 h L:D (20 °C) (50.1 days). No differences were recorded for female preoviposition period at 25 °C under 12:12 h L:D (11.3 days) and 10:14 h L:D (14.5 days) (Table 4).

Cividanes & Parra (1994) determined that the preoviposition period for *P. guildinii* females under long photophase (14 h) was longer at 20 °C (47 days) than at 26 °C (13 days). Hodek & Hodková (1993) for *D. baccarum* reported longer preoviposition period for bugs reared at shorter photophase. Musolin & Numata (2003) established that photoperiod had more pronounced effects on the preoviposition period at 20 °C.

Photoperiod and temperature had different effects on the egg masses and number of eggs/female. The number of egg masses was affected only by photoperiod. Females maintained under 14:10 h L:D (25 °C) and 12:12 h L:D (25 °C) produced a similar and greater number of egg masses (8.8 and 6.9, respectively) than females reared under 10:14 h L:D (25 and 20 °C), (3.6 and 3.1 egg masses, respectively) (Table 4). All treatments had a significantly different number of eggs/female, with the greatest value corresponding to 14:10 h L:D (25 °C) (196.2), and the lowest recorded under 10:14 h L:D (20 °C) (21.7) (Table 4). Cividanes & Parra (1994) observed under long photophase that temperature affected the number of eggs/female of *P. guildinii*, with means values significantly higher at 26 °C (310 eggs/female) than at 20 °C (28 eggs/female).

TABLE 4. REPRODUCTIVE PERFORMANCE OF FEMALE *PIEZODORUS GUILDINII* AT DIFFERENT PHOTOPERIODS AND TEMPERATURES UNDER LABORATORY CONDITIONS.

Conditions	Females ovipositing (%)	Preoviposition period (days)	Number/female		Egg hatchability (%)
			Egg masses	Eggs	
10:14 L:D 20 °C	24.2 c [33][3]	50.1 ± 22.0 a[1] [8]	3.1 ± 1.0 b [7]	21.7 ± 5.0 d [7]	4.1 ± 4.5 b[2] [7]
10:14 L:D 25 °C	62.1 b [30]	14.5 ± 2.2 b [16]	3.6 ± 0.6 b [16]	48.2 ± 7.9 c [16]	54.6 ± 9.1 a [19]
12:12 L:D 25 °C	61.5 b [39]	11.3 ± 1.4 b [19]	6.9 ± 0.9 a [25]	106.3 ± 29.5 b [22]	43.6 ± 5.3 a [24]
14:10 L:D 25 °C	84.5 a [33]	8.0 ± 0.4 c [26]	8.8 ± 1.4 a [27]	196.2 ± 34.7 a [27]	64.2 ± 3.7 a [27]

[1]Means in each column followed by the same letters are not significantly different ($P < 0.05$) based on likelihood ratio.
[2]Means followed by the same letters do not differ significantly ($P < 0.05$) using Tukey's test.
[3]Number of females in brackets.

Chocorosqui & Panizzi (2003) reported that females of *D. melacanthus* reared under long photoperiods (14:10 and 13:11 h L:D) had a better reproductive performance, high percentage of females ovipositing, more number of eggs masses and number of eggs, in comparison to females under short photoperiods (12:12 and 10:14 h L:D). Similar results were obtained by Mourão & Panizzi (2002), studying the biology of *E. heros*; they indicated that females reared under 10:14 h L:D produced infertile eggs. For another pentatomid, *D. baccarum*, Hodek & Hodková (1993) reported greater fecundity for bugs reared at longer than those reared at shorter photophase. However, Ali & Ewiess (1977) did not observe significant differences in the reproductive parameters of *N. viridula* at different photoperiods (10:14 h L:D and 14:10 h L:D).

Egg viability was not significantly affected by different photoperiod conditions at 25°C. However, under 10 h of photophase, it decreased significantly when temperature decreased from 25 °C to 20 °C (Table 4). On the other hand, under long photophase (14 h), Cividanes & Parra (1994) did not find an effect on egg viability of *P. guildinii* when temperatures decrease from 26 °C to 20 °C. The results of this study and those obtained by Cividanes & Parra (1994) may likely suggest the occurrence of an interaction in the response of egg viability with photoperiod and temperature.

Adult Body Weight Gain. Both males and females of *P. guildinii* gained weight during their first week of adult life (Fig. 3 A, B). However, changes in fresh body weight thereafter were similar for all tested conditions (interaction gain weight*treatment $F_{3, 949} = 1.48$; $P = 0.2180$). Females gained significantly more weight than males. Chocorosqui & Panizzi (2003) reported no significant differences in weight gain/ week for both sexes of *D. melacanthus* under different photoperiods at 25 °C, during a 4-wk evaluation period.

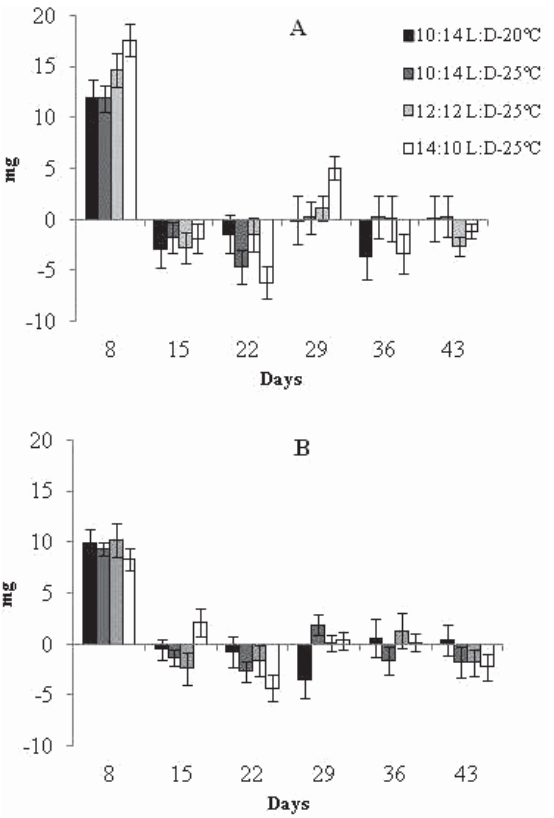

Fig. 3. Mean (+SEM) percentage change of fresh body weight during the first six weeks of adult life of *Piezodorus guildinii*. A) females; B) males.

CONCLUSIONS

Results obtained in this study indicate that photoperiod and temperature had different effects on the several variables evaluated. Differences, particularly for reproductive performance of females, demonstrate the importance and the role of temperature and photoperiod as environmental factors regulating the development and seasonality of *P. guildinii*. These laboratory results suggest that, in the geographic region between 30°-35° S latitude this insect may hibernate as

an adult and may not perform reproductive activity from mid-fall to mid-spring, when it preferred host soybean is unavailable, which corresponds approximately to the conditions tested at 10:14 h L:D and 20 °C.

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