LIFE AND FERTILITY TABLES OF *Pineus boerneri* ANNAND (HEMIPTERA: ADELGIDAE) ON *Pinus* spp. (PINACEAE)

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Abstract

Biological parameters of *Pineus boerneri* were evaluated in laboratory using seedlings of *Pinus taeda* and *Pinus elliottii*. The nymphs were observed along their development. The total life cycle was not significantly different on *P. taeda* and *P. elliottii*, being, respectively, 69 and 65 days. The same trend was observed for mean fecundity, being about 40 eggs/female on *P. taeda* and 53 on *P. elliottii*. However, when daily mean fecundity was considered, the difference was significant, being 1.3 eggs/female/day on *P. taeda* and 1.8 on *P. elliottii*. The nymphal mortality was greater on *P. taeda* (50%) than on *P. elliottii* (28%). These results reflected on life expectancy which was higher on *P. elliottii* for the nymphal stage, but for the adult stage there was no significant difference between both host plants. The intrinsic rate of natural increase and the finite rate of increase were similar, indicating that *P. boerneri* shows the same reproductive capacity on both hosts, despite the net reproductive rate being much greater on *P. elliottii* (23.8 eggs/female) than on *P. taeda* (15.4). The highest nymphal survival and reproductive rates on *P. elliottii* indicate that this host plant is more suitable for the development of *P. boerneri*.
Keywords: Insect biology; insect-plant interaction; pine wooly aphid.

Resumo

Tabelas de vida e de fertilidade de Pineus boerneri Annand (Hemiptera: Adelgidae) em Pinus spp. (Pinaceae). Parâmetros biológicos de *Pineus boerneri* foram avaliados em laboratório em mudas de *Pinus taeda* e *Pinus elliottii*. As ninfas foram acompanhadas durante seu desenvolvimento. O período total do ciclo de vida não diferiu significativamente nas duas espécies, respectivamente 69 dias em *P. taeda* e 65 em *P. elliottii*. O mesmo ocorreu com a fecundidade média, com 40 ovos/fêmea em *P. taeda* e 53 em *P. elliottii*. Porém, considerando a fecundidade média diária, houve diferença significativa com 1,3 ovos/fêmea/dia em *P. taeda* e 1,8 ovos/fêmea/dia em *P. elliottii*. A taxa de mortalidade ninfal foi mais alta em *P. taeda* (50%) do que em *P. elliottii* (28%). Esse padrão refletiu-se na esperança de vida que foi maior em *P. elliottii* no estágio ninfal, mas semelhante no estágio adulto nas duas plantas hospedeiras. A capacidade inata da espécie para aumentar em número e a razão finita de aumento foram semelhantes, indicando a mesma capacidade reprodutiva nos dois hospedeiros. A taxa líquida de reprodução foi significativamente maior em *P. elliottii* (23,8 ovos/fêmea) que em *P. taeda* (15,4). As maiores taxas de sobrevivência ninfal e reprodutiva em *P. elliottii* evidenciam que esta planta é mais propícia para o desenvolvimento de *P. boerneri*.
Palavras-chave: Biologia do inseto; interação inseto-planta; pulgão-lanígero-do-pinus.

INTRODUCTION

The pine woolly aphid, *Pineus boerneri* Annand, 1928 (Hemiptera: Adelgidae), was first recorded in southern Brazil in 2000, in areas of *Pinus* plantation (Pinaceae) spreading rapidly to São Paulo and Minas Gerais (CARDOSO, 2007; IEDE *et al*., 2007; OLIVEIRA *et al*., 2008, LAZZARI *et al*., 2015). *Pineus boerneri* is an adelgid species spread over North America, South America, Africa, Asia and Oceania, infesting more than 40 species of *Pinus*, its exclusive host (MCCLURE, 1984; BLACKMAN; EASTOP, 1994; PETRO; MADOFFE, 2011; WYLIE; SPEIGHT, 2012; RODAS *et al.*, 2015). In in East Asia, possibly area of its origin, *P. boerneri* occurs in low populations on *Pinus thumbergii* Parlatore, 1868, not causing any economic damage (MCCLURE, 1990). However, in areas such as the United States, Australia and some countries of Africa, *P. boerneri* causes considerable damage on commercial plantations of *Pinus* (TANTON; ALDER, 1977; MAILU, 1980; MCCLURE, 1984, 1989).

The taxonomic status of *P. boerneri* is not well defined and might have been described under different names in several parts of the world: *Pineus laevis* (Maskell) in New Zealand (EASTOP, 1966); *P. laevis* in Australia and *Pineus pini* Gmelin in Australia and Africa (EASTOP, 1966; BLACKMAN; EASTOP, 1994).

Tanton and Alder (1977) treat *P. boerneri* as *P. laevis,* forming the complex *laevis/pini*. Blackman and Eastop, (1994), however, treat *P. laevis* as a synonym of *Pineu pini* (Gmelin). According to these authors, the species described by Maskel, 1895 could be *P. pini*, which might occur simultaneously with *P. boerneri* in Australia and New Zealand, being confused due to morphological similarities.

The parthenogenetic oviparous female is the only known adult form in most parts of the world; it is apterous, sessile, and stays over the egg mass covered with wax threads secreted by glands in the head and thorax (MCCLURE, 1989; BLACKMAN; EASTOP, 1994; LAZZARI; CARDOSO, 2011; RODAS *et al.*, 2015). McClure (1989) registered four nymphal instars for *P. boerneri* in the United States, while Yassen and Ghani (1971) found only three instars in Pakistan. First instar nymphs are crawlers and responsible for dispersion and choice of the feeding sites on the host plant (BLACKMAN; EASTOP, 1994).

Climatic conditions and host plant characteristics, besides the impact of natural enemies and genetic determinations are the main factors affecting the development of insect populations. In Pakistan, Yassen and Ghani (1971) recorded three or four annual generations of *P. boerneri* and observed that climatic conditions influence egg incubation, pre-reproductive and oviposition periods, as well as fecundity. Latent periods were observed for 1st instar nymphs during the winter. In the United States, McClure (1989) detected variations in the duration of development along the seasons of the year.

Mailu *et al.* (1980) evaluated the biology of *P. boerneri* in field conditions in Kenya, and found a population mortality of 94% during lifetime, at egg stage (72.5%), nymphs (18.6%) and adults (2.9%). They attributed the high mortality rates to factors such as infertility, predation, low dispersion capacity, desiccation, excess of rain, starvation, and excess of resin in the host plant.

The process of choosing a host plant by an aphid is quite complex, involving a great variety of factors such as age, physiology and architecture of the host plant, which affect the amount/availability of nutrients (SMITH, 2005; LAZZARI; ZONTA-DE-CARVALHO, 2009). In addition, the plant may have chemical and physical defenses that block the settlement of the insect (arrestants) or disrupt the feeding process (SMITH, 2005; NISHIDA, 2014).

Due to lack of biological data of *P. boerneri* and its relation to host plants in Brazil, this research aims to determine biological parameters and life and fertility tables of this potential pest on commercial species of *Pinus*.

MATERIAL AND METHODS

For this study, 58 seedlings of *Pinus taeda* L. and 42 of *Pinus elliottii* Engelm., approximately five months old, were cultivated in plastic tubes in the laboratory of the Department of Zoology at the Federal University of Paraná (UFPR). The seedlings were infested with *P. boerneri* and placed randomly in trays with water on a shelf near a window to receive natural light during daytime. Temperature averaged 21±2 °C and relative air humidity 53±7%.

To start the experiments, eggs were obtained from adult females on infested seedlings. The area around each female was circled with a permanent marker and cleared up to facilitate removal of the eggs every 24 hours. Females were not removed because once they establish on a feeding site, they insert their stylets into the plant tissues and may break if removed. Each seedling was infested with 10 eggs and observed daily until emergence of the nymphs. From the second day on, the plants were examined under a magnifying glass. The site of establishment of each nymph was marked to facilitate the observation of the tiny individuals.

Biological parameters evaluated for *P. boerneri* were: incubation period and egg viability, number of nymphal instars based on body size and casted exuviae, duration of nymphal development. For the adult females, were evaluated: longevity, daily fecundity and mean number of eggs per female (total fecundity). The data obtained for both experiments were tabulated, transformed into $\sqrt{x + 0,5}$, and the variance was analyzed by ANOVA. The averages were compared by the Tukey test at 5% probability. Age-specific life table was calculated in software *Tabvida* (PENTEADO, 2007). The egg viability data were not included in the age-specific life and fertility table, because the 1st instar nymphs disperse quickly and could not be counted. As a result, egg viability was considered to be 100%.

To construct the age-specific life table, the following parameters were determined: survivor's' percentage (Lx); number of dead individuals (dx); age population structure (Ex); life expectation (ex); and death probability at age x (100qx), where: Ex = [Lx + (Lx+1)]/2; ex = Tx/Lx; 100qx = (dx/Lx). 100.

To construct the fertility table, the values of age interval (x), specific fertility (mx) and survival probability (lx) were used for calculating the net reproductive rate (R0), the time interval between each generation (T), the intrinsic rate of natural increase (rm), the finite rate of increase (λ), and the time for the

population to double in number (DT), where: R0 = Σ (mxlx); T = (Σ mx.lx.x)/(Σ mx.lx); rm = logeR0/T = ln R0/T; λ = erm; TD = ln(2)/rm.

After tabulation of the data, the main indexes were represented graphically using Statistica version 5.5. Life expectation data were compared by G test at 5% probability. Variances of the indexes obtained from the fertility table were calculated using "Jackknife" and estimation (MANLY, 2006), and the averages compared by T test at 5% probability.

RESULTS

On *P. taeda*, a total of 447 eggs hatched (85.5%), from 522 eggs, on 48 seedlings out of 58 infested. However, only 65 nymphs (12.5%) established successfully and 52 were followed up along their development. In the experiment with *P. elliottii*, from the 364 eggs of *P. boerneri* placed on the seedlings, 289 hatched (79%) with only 66 nymphs (18%) establishing successfully and 46 followed up along their development. The mean egg incubation period was significantly shorter for *P. elliottii* in comparison to *P. taeda* (Table 1). The species presented four nymphal instars; only one single specimen underwent three instars.

Adult females stay on the top of the eggs, involving them with a great amount of wax threads, forming a woolly protection. The mean total number of eggs/female was 39.8 on *P. taeda* and 52.9 on *P. elliottii*, without significant difference among both host plants (Table 1). McClure (1984) registered similar number of eggs (45 eggs/female) for this same adelgid species on *P. resinosa* in the United States.

Table 1. Mean values and standard error for development parameters of *Pineus boerneri* (confidence interval at 95%) reared on two host plants, under laboratory conditions, in Curitiba, PR; 21±2 °C and relative air humidity of 53±7%.

Tabela 1. Desenvolvimento de *Pineus boerneri* (média ±erro padrão e intervalo de confiança a 95%) em duas espécies de plantas hospedeiras, em condições de laboratório, Curitiba, PR; 21±2 °C e umidade relativa do ar de 53±7%.

Parameters	*P. taeda* (n = 52)	*P. elliottii* (n = 46)
Eggs (days)	7.5 ± 0.1 b (7.2 – 7.8)	6.2 ± 0.1 a (5.9 – 6.5)
1st instar (days)	8.9 ± 0.2 a (8.5 – 9.3)	11.0 ± 0.3 b (10.5 – 11.63)
2nd instar (days)	5.8 ± 0.2 a (5.3 – 6.3)	5.8 ± 0.2 a (5.3 – 6.2)
3rd instar (days)	6.7 ± 0.9 a (4.9 – 8.4)	5.4 ± 0.3 a (4.8 – 6.0)
4th instar (days)	5.1 ± 0.5 a (4.1 – 6.2)	5.1 ± 0.2 a (4.5 – 5.6)
Total nymphal period (days)*	24.5 ± 0.8 a (22.9 – 26.0)	27.3 ± 0.7 b (25.9 – 28.7)
Adult longevity (days)	37.4 ± 4.6 a (27.94 – 46.82)	31.7 ± 3.4 a (24.8 – 38.6)
Total life cycle (days)*	69.1 ± 4.5 a (59.9 – 78.3)	65.1 ± 3.5 a (57.9 – 72.4)
Oviposition period (days)	32.2 ± 4.4 a (23.0 – 41.3)	27.2 ± 3.1 a (20.8 – 33.6)
Fecundity (eggs/female)	39.8 ± 6.5 a (26.4 – 53.3)	52.9 ± 8.3 a (35.9 – 69.9)
Daily fecundity	1.3 ± 0.1 a (1.1 – 1.6)	1.8 ± 0.1 b (1.5 – 2.1)

* The values of the total nymphal period and life cycle were calculated as an average of the complete data set, not the sum of the mean values. ** Different letters indicate significant differences on the line by the Tukey test (p<0,05%).

The life cycle of *P. boerneri* extended for approximately 69 days on *P. taeda* and 65 days on *P. elliottii*, without significant difference between the two hosts (Table 1). Mailu *et al.* (1980), recorded a life cycle of 75 days, under field conditions, with a temperature ranging from 19 °C to 24 °C. McClure (1989) observed a longer cycle, of 80 to 168 days, depending on the observed generation.

On *P. taeda*, the mortality rates (dx) of 1st and 2nd instar nymphs of *P. boerneri* was observed between the 18th and 21st day of life (three dead individuals) and between the 21st and 24th day (six dead), representing death probabilities of 6% and 13.3%, respectively for the periods. High mortality rates were observed also in the 4th instar and among teneral adults, between the 36th and 39th day (5 individuals – death probability of 15% for the period). There was 50% mortality during the nymphal stage, being 15.5% in the 1st instar, 13.5% in the 2nd, 7.5% in the 3rd and 13.5% in the 4th instar.

On *P. elliottii,* the mortality rates (dx) of 1st and 2nd instar nymphs also occurred between the 18th and the 21st day and between the 21st and the 24th, respectively, with three deaths in each period. The death probability in both ages was 6.8% for the first and 7.3% for the second period. Again, it was observed a high

mortality rate for 4th instar nymphs and teneral adults, with death probability of 13.3%. There was 28% mortality in the nymphal stage, being 6.4% in the 1st instar, 8.7% in the 2nd, 4.2% in 3rd, and 8.7% in the 4th instar, similar to what was found for *P. boerneri* on *P. taeda*.

The life expectancy (e_x), which relates the survival rate to the number of survivors, was higher on *P. elliottii*, 17.89, against 16.48 on *P. taeda*. This index presented the same pattern on both species of *Pinus*, being relatively high in the beginning and decreasing until the end of the evaluation period (Figures 1-2). The G test, applied to compare the two life tables, did not show significant differences in the parameters of survival of *P. boerneri* on both host plants, despite the higher mortality rate of nymphs on *P. elliottii*.
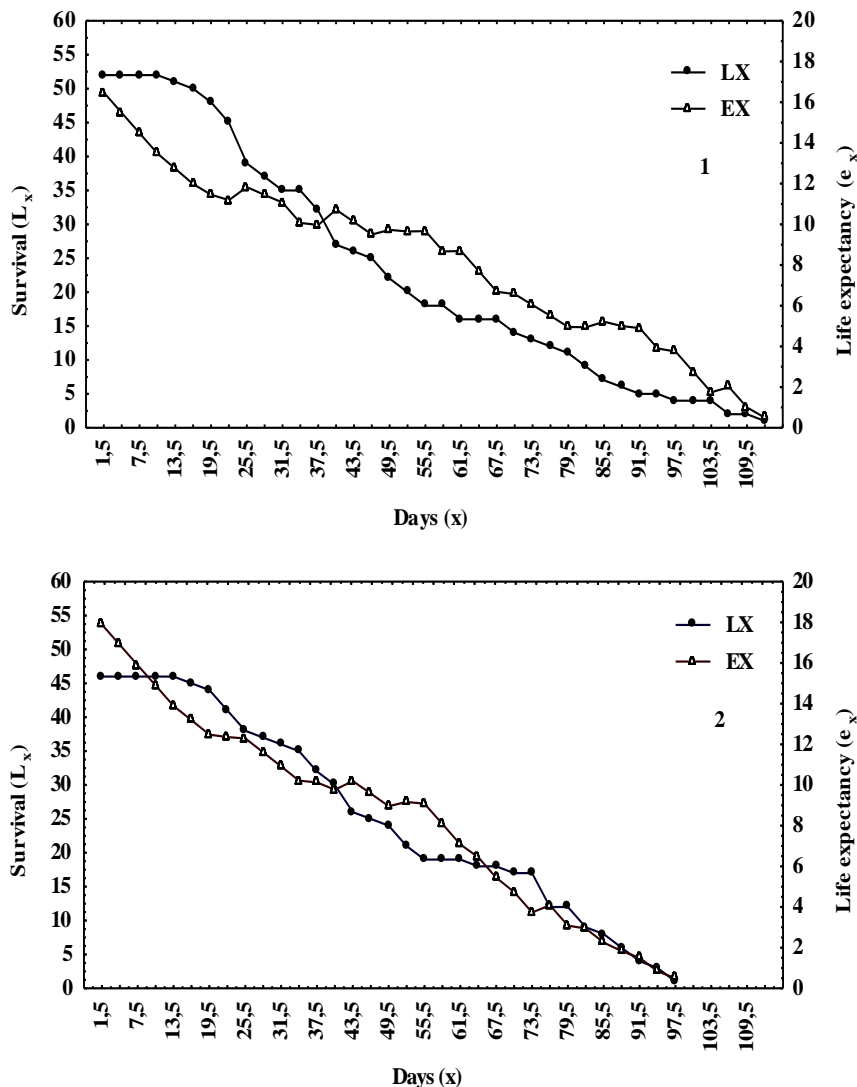

Figures 1-2. Number of survivors (L_x) and life expectancy (e_x) of *Pineus boerneri* reared on *P. taeda* (1) and *P. elliottii* (2) under laboratory conditions, in Curitiba, PR; 21±2 °C and relative air humidity of 53±7%.

Figuras 1-2. Número de sobreviventes (Lx) e esperança de vida (ex) de *Pineus boerneri* criados em *P. taeda* (1) e *P. elliottii* (2) em condições de laboratório, Curitiba, PR; 21±2 °C e umidade relativa do ar de 53±7%.

The mean pre-reproductive and post-reproductive periods of *P. boerneri* females on *P. taeda* were1.2 days and 3.5 days, respectively. On *P. elliottii*, these were 1.5 days and 2.6 days, respectively. On *P. taeda*, adult females entered the reproductive period by the 24th day until the 108th day of life (Figures 3-4). The highest fecundity rate (m_x) of 3.6 eggs/female occurred between the 39th to the 41st day of life. On *P. elliottii*, females entered the reproductive period by the 27th day until the 93th day, with a higher m_x, but not differing statistically

from the other host plant. The fecundity rate m_x was observed to occur much later on *P. elliottii*, between the 54th to the 57th day, with the production of 6.58 eggs/female (Figures 3-4). The mean total number of eggs/female was 39.8 on *P. taeda* and 52.9 on *P. elliottii*, without significant difference among both host plants (Table 1).

The survival rate (l_x) of *P. boerneri* was similar on both host plants, decreasing gradually along lifetime (Figures 3-4); however, nymphal survival (l_x) was lower on *P. taeda* than on *P. elliottii*.
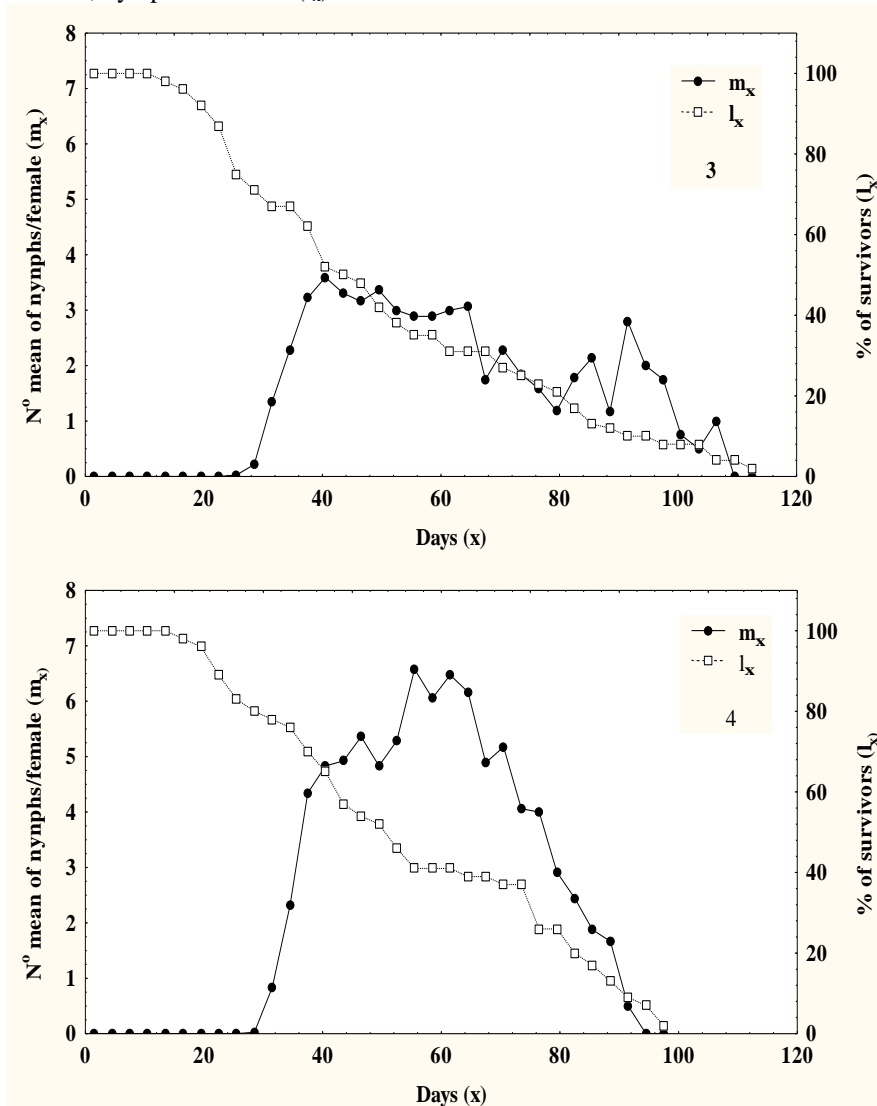
Figures 3-4. Mean number of nymphs produced by female (m_x) and survival percentage (l_x) of *Pineus boerneri* during their reproductive period, on *P. taeda* (2) and *P. elliottii* (3) under laboratory conditions, in Curitiba, PR; 21±2 °C and relative air humidity of 53±7%.

Figuras 3-4. Número médio de ninfas produzidas por fêmea e porcentagem de sobrevivência (lx) de de *Pineus boerneri* em todo o período reprodutivo, em *P. taeda* (2) e *P. elliottii* (3) sob condições de laboratório, em Curitiba, PR; 21±2 °C e umidade relativa do ar de 53±7%.

The intrinsic rate of natural increase (r_m) and the finite rate of increase (λ), was similar for both host plants (Table 2). The intrinsic rate of natural increase was 0,06 for the both plants species and the finite rate of increase was 1.06 eggs/day on *P. taeda* and 1.07 eggs/day on *P. elliottii*.

The net reproduction rate (R_0) was statistically different among the specimens of *P. boerneri* reared on the two host plants: 23.8 eggs/female on *P. elliottii* and 15.41 eggs/female on *P. taeda* (Table 2). The mean generation time (T) of *P. boerneri* was significantly smaller on *P. taeda* (45.1 days) than on *P. elliottii* (49.2 days). Consequently, the time necessary for the species to duplicate in number of individuals is smaller on *P. elliottii* (Table 2).

Table 2. Parameters of the life fertility table of *Pineus boerneri* (mean ± standard error) on two hosts, under laboratory conditions, in Curitiba, PR; ; 21±2 °C and relative air humidity of 53±7%. (x = 1 day).

Tabela 2. Parâmetros da tabela de fertilidade de vida para *Pineus boerneri* (estimativa + erro padrão) em dois hospedeiros, sob condições de laboratório em Curitiba, PR; 21±2 °C e umidade relativa do ar de 53±7%. (x = 1 dia).

	R_0	T	r_m	λ	DT
Pinus taeda	15.41 a (± 0.09)	45.09 a (± 0.04)	0.06 a (± 0.0001)	1.06 a (± 0.0001)	11.43 a (± 0.0001)
Pinus elliottii	23.8 b (± 0.09)	49.2 b (± 0.04)	0.06 a (± 0.0001)	1.07 a (± 0.0001)	10.76 b (± 0.01)

* R_0 = net reproduction rate; T = generation time; r_m = intrinsic rate of natural increase; λ = finite rate of increase; DT = doubling time.

** Different letters indicate significant differences in the columns by the Tukey test (p< 0,05%).

DISCUSSION

The mean egg incubation period was different between *P. elliottii* and *P. taeda*. The major causes of egg mortality to *P. boerneri* were desiccation, infertility, and eventual damage caused by handling due to their small size. Mailu *et al.* (1980), evaluating the biology for the specie in field conditions in Kenya, counted an average of 167 eggs/cm of the branch, but after two weeks only 46 1st instar nymphs were established (27.5%). The authors mentioned that the main mortality factors were egg infertility, desiccation, rain, predators, nymph dispersion, and other unknown causes.

The number of nymphal instars agree with the observations of McClure (1989) for *P. boerneri* on *Pinus resinosa* Aiton in the United States. Yassen and Ghani (1971) detected three instars for both *P. boerneri* and *Pineus* sp. on *Pinus roxburghii* Sargent, in Pakistan.

The development time of 1st instar nymphs was longer than that of the other instars. Also, it was longer on *P. elliottii* than on *P. taeda*. The reason of the prolonged period is because they take up to three days to find a place to settle down. Yassen and Ghani (1971) found a similar pattern for *P. boerneri* and *Pineus wallichianae* Yassen e Ghani, 1971, with the nymphs wandering for 2 to 3 days and 1 to 2 days, respectively, before establishing. On the other hand, McClure (1984) observed that 1st instar nymphs of *P. boerneri* and *Pineus coloradensis* Gillette, 1907 found a feeding site soon after hatching.

In general, as the nymphs start feeding at a certain site, they become sessile for the rest of their lives. McClure (1984) also observed such behavior for *P. boerneri* and *P. coloradensis*. However, we observed that when the nymphs were disturbed during handling they would abandon that feeding site and eventually die.

The mortality rates (dx) and the total mortality in the nymphal stage indicate that the nymphs of *P. boerneri* adapt better on *P. elliottii* than *P. taeda*. Mailu *et al.* (1980) determined the apparent mortality rate of 67% for nymphs of *P. pini* (=*P. boerneri*) in Kenya. This value was higher than ours, probably because they worked in field conditions, under the pressure of predators and no control of climatic factors.

The reproductive period and the total mean fecundity of *P. boerneri* on the two host plants was similar, although the daily mean fecundity was higher on *P. elliottii*. The pre-reproductive period of *P. boerneri* on *P. roxburghii*, Sarg. in Pakistan, ranged from few days to weeks, for different generations (YASSEN; GHANI 1971).

The intrinsic rate of natural increase (rm) is a measure of the multiplication rate of a species and depends on its intrinsic capacity of reproduction in a given environment. *P. boerneri* presented the same multiplication rate on both host plants, which suggests that the plants may not affect the reproductive capacity. Since this index was positive, we conclude that the populations are increasing and both host plants are equally favorable for the development of *P. boerneri*. Also, the finite rate of increase, which measures the number of individuals added to a population in a given period of time, was similar for both host plants and confirms the positive rm.

The net reproduction rate (R0) was different for the both plants species and reflects on the high nymphal mortality recorded on *P. taeda*, with only 50% of the nymphs reaching the adult stage. On *P. elliottii*, the mortality was even higher, 72%, which could explain why *P. boerneri* exhibits the same innate reproduction capacity on both host plants, but higher liquid reproduction rate on *P. elliottii*.

The net reproduction rate (R0) is the mean number of offspring produced per female in one generation; it takes in consideration the reproductive capacity and the individual survival and. This parameter was different among the specimens of *P. boerneri* reared on the two host plants and reflects on the high nymphal mortality recorded on *P. taeda*, with only 50% of the nymphs reaching the adult stage. On *P. elliottii*, the mortality was

even higher, 72%, which could explain why *P. boerneri* exhibits the same innate reproduction capacity on both host plants, but higher liquid reproduction rate on *P. elliottii*.

The mean generation time (T) shows that *P. boerneri* tends to increase its population more quickly when reared on *P. elliottii* than *P. taeda*. In fact, it was observed in laboratory that the seedlings of this plant species usually presented a larger number of colonies of *P. boerneri*.

It is known that fecundity rates and population growth of insects can be directly affected by chemical and morphological traits of their host plants, as well as their nutritional condition, such as the nitrogen level (LEATHER *et al.*, 2005; AQUEEL; LEATHER, 2011; AQUEEL *et al.*, 2014; NISHIDA, 2014). Leather *et al.* (2005) mention that host nutritional quality can affect the population dynamics of the aphid *Drepanosiphum platanoidis* (Schrank), mainly its growth rates and fertility. On the other hand, most insects have the capability of tolerating the toxins and allelochemicals produced as defense barriers by a host plant. The herbivore insect may exhibit particular behaviors and physiological and enzymatic counteraction mechanisms in order to survive (DESPRÉS *et al.* 2007). Although this research did not intend to investigate plant defense mechanisms, our results evidenced that *P. elliottii* was more favorable for pest population development, whereas *P. taeda* was more resistant against the development of *P. boerneri*.

Our study on the biology of *P. boerneri* reared on the most important commercial species of *Pinus* (*P. taeda* and *P. elliottii)* demonstrates the ability of this insect to reproduce and increase its population in few days, more successfully on *P. elliottii* and less on *P. taeda*. The information presented is fundamental for forestry management decisions, particularly in the selection/cultivation of more resistant species or varieties of pines in order to prevent the outbreak of pine wooly aphid populations and damage that might follow.

CONCLUSION

- The significantly greater reproductive rates and survival times of nymphs and adults of *P. boerneri* reared on *P. elliottii* indicates that this plant species is less resistant to infestation by pine wooly aphids.
- The life and survival tables confirm that *P. boerneri* is better adapted to *P. elliottii* than to *P.taeda*.

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