



Ciencia Florestal



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ABSTRACT

Blastopsylla occidentalis Taylor, 1985 (Hemiptera: Aphalaridae) is a psyllid which host is the eucalyptus, originally from Australia, which was introduced in Brazil in the mid-1990s. Little is known on the biology of this insect. Here, we examine the effect of temperature on its development and the reproduction under laboratory conditions. The experiments were carried out in 2018 and 2019, in climate chambers regulated at five temperatures (15, 20, 25, 30 and 35°C). The parameters observed at this stage of the experiment were: period of the egg development (number of days, from laying to hatching); hatching rate of immatures; development of immatures; adult emergency rate; longevity, the period (in day) of laying until the death of the first generation adults (F1). *Blastopsylla occidentalis* completed the life cycle at temperatures of 20, 25 and 30°C, with an average duration of 32.78, 27.76 and 28.44 days, respectively. In the F1 generation, no posture was observed at 30°C, while at 20 and 25°C, the average fertility was 19.04 and 26.40 eggs per female, respectively. The average duration of the egg incubation was 6.70 (20 °C) and 5.59 (25 °C) days. Immature *B. occidentalis* did not complete their development at 15 and 35°C and we conclude that these temperatures limit its development. The optimum temperature range for the development and the reproduction was between 20 and 25°C, with 25°C, being the temperature at which the insect had the lowest mortality in all stages, the shortest development time and the highest average number of eggs/days. It is hoped that these results will help to improve the management techniques for the control of the species.

Keyword: Eucalypt; Thermal requirements; Forest protection; Psylloidea

1. INTRODUCTION

The representativeness of Brazil in the international market of forest products grows every day. According to the Brazilian Tree Industry (IBÁ), Brazil is the second largest pulp producer in the world, with an extension of 7.83 million hectares of planted forest and with approximately 5.7 million hectares belongs to the eucalyptus cultivation (INDÚSTRIA BRASILEIRA DE ÁRVORES, 2019). native of australia and belonging to the myrtaceae family, eucalyptus is standing out in Brazil for its adaptability to the tropical climate, which allows continuous growth associated to the rapid accumulation of biomass (LONGUE-JÚNIOR; COLODETTE, 2013).

With the growing area planted with eucalyptus in the country, reports of exotic pests has increased considerably in recent decades (SCHÜHLI et al., 2016). In between pests introduced in the mid-1990s and early 2000s, insects of the Psylloidea superfamily stand out, as they are found widely

distributed in eucalyptus plantations, causing damage (BURCKHARDT; OUVARD, 2012; QUEIROZ *et al.*, 2018).

In Brazil, four species of psyllids of the family Aphalaridae were introduced: *Ctenarytaina eucalypti* Maskell, 1890; *Ctenarytaina spatulata* Taylor, 1997; *Blastopsylla occidentalis* Taylor, 1985 and *Glycaspis brimbecombe* Moore, 1964 and the first collection of *Blastopsylla occidentalis* in the country occurred in 1997, in *Eucalyptus urophylla* plants and hybrids of *Eucalyptus urophylla* with *Eucalyptus grandis* (QUEIROZ *et al.*, 2018).

Adults of *Blastopsylla occidentalis* are small insects measuring between 1.5 to 2.0 mm, its wings have brown veins with the presence of a membrane gray, the yellowish coloration is predominant in the species, with differences in the hue, in which males are usually yellow and females are darker (BURCKHARDT; ELGUETA, 2000). Like other psyllids, the immatures and adults of *Blastopsylla occidentalis* feed on phloem sap.

In commercial nurseries, it can cause economic losses, since has a preference for younger shoots and leaves, which can affect growth of seedlings compromising productivity (QUEIROZ *et al.*, 2017). in plantations already established, with adult plants, the insect concentrates mainly on apical tips, causing stunting of the plants, distortion and stains in leaves and petioles, contributing to the loss of vigor throughout the plant attacked (SANTANA; BURCKHARDT, 2007; QUEIROZ; BURCKHARDT; MAJER, 2012). As for the attack symptoms, the most severe damage is evidenced when there is a high amount of immatures and adults in the apexes and younger leaves, regardless of the phase the plant is in (QUEIROZ *et al.*, 2017).

The presence of *Blastopsylla occidentalis* is already reported in several Brazilian states, mainly in those that have large-scale commercial plantations and in regions with severe water deficit at some time of the year (QUEIROZ; BURCKHARDT; MAJER, 2012; SALIBA *et al.*, 2019). Several authors mention that in addition to the availability of food, temperature may also affect the bioecology of psyllids, as well as

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as well as other insects of the Hemiptera order, thus, it may influence the development time and how its population dynamics will be (OLIVEIRA *et al.*, 2010; QUEIROZ *et al.*, 2010; FONSECA *et al.*, 2016).

Phytosanitary problems in eucalyptus plantations are reasons for great concern for the forest sector, and to solve these problems, it is necessary to adoption of measures that could increase production costs, negatively affecting the profitability of the final product (VAN LIEROP *et al.*, 2015). It is estimated that an extension than one million hectares of planted forests in South America were atacadas por insetos-praga (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2015).

Unlike other insect pests associated with eucalyptus, *Blastopsylla occidentalis* has scarcity of information related to its biology. being this the first study of its nature in order to fill this demand. Therefore, the The objective of this study was to know the effect of temperature on the development and reproduction of *Blastopsylla occidentalis* under laboratory conditions.

2 MATERIAL AND METHODS

2.1 Study area

The research was carried out at the Laboratory of Entomology of the Biological Collection of Southern Amazon – ABAM of the Federal University of Mato Grosso, Sinop campus (11°51'51.4"S 55°28'58.4"W). To carry out the experiments, couples of *Blastopsylla occidentalis* were collected from eucalyptus trees planted at Fazenda Cunhatay (11°45'19.4"S; 55°23'14.1" W), located in the municipality of Sinop, Mato Grosso, and also in rearing insects carried out in a greenhouse. All insects were kept at room temperature until the beginning of the experiments.

2.2 Management and definition of eucalyptus clone for biological study

Eucalyptus clonal seedlings, approximately 90 days old, from hybrids *Eucalyptus grandis* x *Eucalyptus urophylla* (H-13); *Eucalyptus urophylla* x *Eucalyptus grandis* (I-144); *Eucalyptus urophylla* x *Eucalyptus camaldulensis* (VM-01), were planted in 180 ml disposable cups. Fortnightly, they received fertilization with solution nutritious (QUEIROZ *et al.*, 2020) and were isolated for 40 days for planting fixation, until they were infested in screened cages measuring 80 x 80 x 80 cm.

To select the clone, five infestations were carried out, and in each one, fifteen couples of *Blastopsylla occidentalis* were isolated in three cages that had ten seedlings of each clone, for a period of 24 hours for mating and posture of females. After this period, the couples were removed with assistance of the entomological aspirator and released in a greenhouse, for maintenance insect breeding. Among the three clones evaluated, VM-01 was selected, considering that in this clone the largest number of postures was observed.

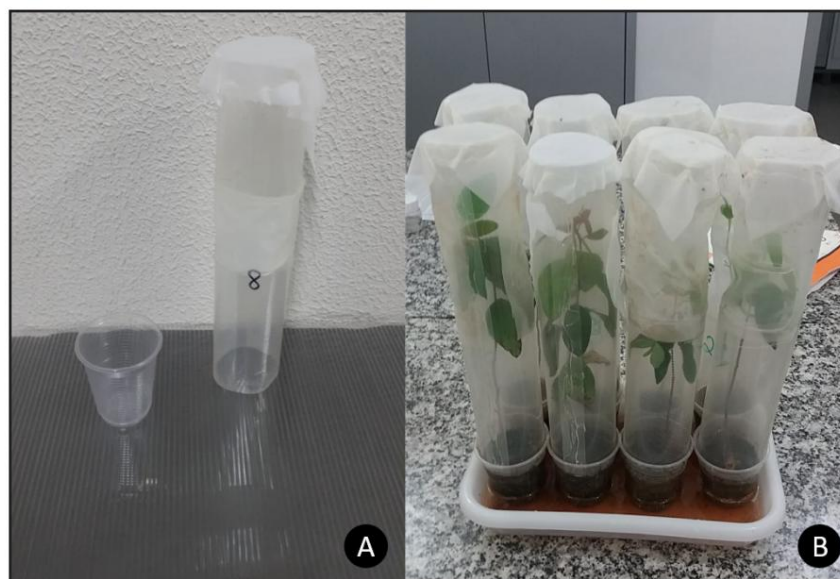
2.3 Biological study in the laboratory

To monitor the development cycle, fourteen infestations (1 infestation = 30 isolated seedlings). After the infestation has taken place, the seedlings were evaluated, and the number of eggs present was quantified, then These were isolated individually in cylindrical cages made of transparent polypropylene measuring 30 x 20 cm with an opening at the bottom, which was attached to the glass that contained the seedling, and a "voile" type screen was attached to the top for ventilation (Figure 1), and placed in climatized chambers like *Biochemical Oxygen Demand* (BOD), regulated at temperatures of 15, 20, 25, 30 and 35°C, photoperiod of 12/12 hours and 70% relative humidity.

The parameters observed daily at this stage of the experiment were:
incubation: period of development of the eggs, i.e. number of days, since the posture until the hatching of the immatures; hatching rate of immatures: percentage

$[(\text{number of immatures}) / (\text{number of eggs}) \times 100]$; immature development: period (in day), from hatching to adult emergence; percentage of mortality immature that did not reach the adult stage; adult emergency rate: percentage $[(\text{number of adults}) / (\text{number of immatures}) \times 100]$; longevity: number of days of Survival in the adult phase and complete cycle: period (in days) of laying until death of adults.

Figura 1 – Experimento com *Blastopsylla occidentalis* Taylor, 1985 (Hemiptera: Aphalaridae); A: Model of used cages; B: Eucalyptus seedlings infested with insect eggs



Source: Authors (2020)

To obtain the F1 generation, we waited 24 to 48 hours after the emergence of the adults to remove the insects from the plants with the entomological aspirator, and with the aid of a stereoscopic microscope, sexing and separation of the couples was carried out. In a new cage, with infestation-free eucalyptus seedlings, the release of the couples, in their respective temperatures, which daily were observed, until the emergence of the first posture and evaluation of the reproductive parameter (number of eggs/day per female), hatching rate and duration of the period of incubation of F1 immatures.

2.4 Statistical analysis

For all temperatures studied, each egg was considered a repetition observed from *Blastopsylla occidentalis*, so the number of repetitions was different between temperatures. The data obtained in each observed parameter (period of egg incubation, immature development, adult longevity, total cycle and incubation period of the F1 generation), were submitted to tests of normality and homoscedasticity and after verifying these assumptions, the analysis was performed of variance (ANOVA) and means compared by the Scott-Knott test at 5% of probability, with the aid of the Sisvar statistical program (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

The results indicated that the temperatures of 20 and 25°C were suitable for the development of the psyllid, while the temperatures of 15 and 35°C were limiting, as there was no emergence of adults (Table 1). Laws and Belovsky (2010) mentioned that the physiological, behavioral and developmental processes of insects are directly affected by temperature, since they have limited capacity of thermoregulation.

Hodkinson (2009) mentions that when temperatures are not in the ideal range for psyllid development, may result in slower development and high mortality rate. A fact that is observed at the lowest temperature of this study (15°C), in which the mean duration of the incubation period was 18.30 ± 0.16 days, this being the longest period for the hatching of immatures and limiting for the development of *Blastopsylla occidentalis*. Wilcken *et al.* (2015) observed that the temperature of 18°C is limiting for the development of *Glycaspis brimblecombei*, thus being higher than that found for *Blastopsylla occidentalis*.

The hatching rate of immatures at 15°C was 35% (Table 1), being limiting for insect development. Santa-Cecília *et al.* (2011) reported that the temperature

was a limiting factor in their studies and that high mortality at 15°C can be attributed to the reduction in the metabolism of individuals, which results in greater time for development in the immature stages. Queiroz *et al.* (2020) cite that under greenhouse conditions, in Colombo - Paraná, the psyllid *Blastopsylla occidentalis* does not tolerate very low temperatures.

Table 1 - Number of eggs, mean \pm standard error and period variation interval incubation and hatching rate, number and mortality rate of immatures, total and rate of adults of *Blastopsylla occidentalis* Taylor, 1985 (Hemiptera: Aphalaridae) at five temperatures in BOD. Sinop - MT, Brazil

Temperature (°C)	Eggs	Incubation			Immature		Adults	
		Average \pm	Interval	Outbreak	Number	Mortality (%)	Number	Emergency (%)
		EP (days)	of variation	(%)				
15	259	18,30 \pm 0,16 d	14 – 20	35	90	100	0	0
20	362	7,42 \pm 0,06 b	6 – 9	67	243	53	114	47
25	400	5,95 \pm 0,04 a	5 – 8	91	362	34	238	65
30	420	8,03 \pm 0,05 c	6 – 11	79	331	69	101	31
35	236	7,49 \pm 0,16 b	6 – 10	25	59	100	0	0

Source: Authors (2020)

Where: Means followed by the same letter in the column do not differ significantly by the Scott-Knott test at 5% probability.

At the highest temperature of the study (35°C) the lowest hatching rate was obtained of immatures (25%) and the incubation period had an average duration of 7.49 (Table 1). Schowalter (2016) mentions that insects when exposed to temperatures above the ideal increase the metabolic cost resulting in low nutrition and reducing the survival of individuals. Colonies of *Blastopsylla occidentalis* remained in full development under greenhouse conditions, where temperatures maximum reached 50°C (QUEIROZ *et al.*, 2020). It is noteworthy that under the conditions

of this experiment the temperatures were constant throughout the period and, in the works by Queiroz *et al.* (2020), temperatures fluctuated between days and nights, and the maximums and minimums did not last for many hours.

Therefore, it is inferred that the results presented here for the insect in laboratory conditions are different from those observed in a greenhouse, in which the thermal amplitude oscillates between the day and night periods, thus being able to favor the development not only of this psyllid, but also of other insects (SANTA-CECÍLIA *et al.*, 2011; COLINET *et al.*, 2015).

Analyzing the results of the incubation period, the temperatures 20°C and 35°C presented a similar average (Table 1), therefore, they did not differ statistically, while the other temperatures differed from each other, by analyzing the means of the Scott-Knott test. At 20°C, the mean duration of the incubation period was 7.42, presenting a hatching rate of 67%, obtaining 243 immatures.

The temperature of 25°C was the one that presented the shortest time for hatching of the immature (5.95 days). While, for 30°C, the mean was 8.03 ± 0.05 days (means referring to all immatures that hatched, including those that did not develop to adulthood). Corroborating the results of the present study, Rodrigues (2004) mentions that temperatures close to 25°C are within the optimum threshold for the development of the vast majority of insects, since they are in these conditions of temperature development is more satisfactory.

Individuals who completed the developmental cycle at 20, 25, and 30°C presented the averages of the incubation period of 6.97, 5.80 and 7.40 days, respectively. The shortest duration of immature development period was in 25°C with 13 days, and maximum of 26 days at 20°C (Table 2). The full cycle of *Blastopsylla occidentalis* at 30°C was 28.44 days, with a total of 101 adults emerged. Second Souza and Kirst (2010), immature insects may have their development reduced or accelerated depending on the temperature condition to which it is submitted and the results presented here corroborate this statement.

The development of immatures was 21.5 days (20°C), this being the longest average duration of the period. For the total cycle of *Blastopsylla occidentalis* in this average temperature was 32.78 days, values lower than those found for *Ctenarytaina spatulata*, which averaged 44.89 days (total cycle) (QUEIROZ; ZANOL, 2006). Fonseca *et al.* (2016) reported that the effect of temperature can vary between species and highlighted that lower temperatures generally influence the duration of biological development.

Table 2 - Number of individuals observed (N), mean duration \pm standard error of incubation and immature development periods, adult longevity and rate of mortality after 24 hours of emergence of adults, total cycle and their respective ranges of variation (days) for the biological development of *Blastopsylla occidentalis* Taylor, 1985 (Hemiptera: Aphalaridae), em cinco temperaturas. Sinop - MT Brazil

Temperature (°C)	N	Incubation (days)	Development immature (days)	Longevity	Cycle total	Mortality in 24 hours after emergence (%)
20	114	6,97 \pm 0,06 b (6 – 8)	21,57 \pm 0,16 c (17 – 26)	4,22 \pm 0,15 b (1 – 6)	32,78 \pm 0,21 c (24 – 37)	13,15
25	238	5,80 \pm 0,11 a (5 – 8)	15,77 \pm 0,11 a (13 – 22)	6,08 \pm 0,09 c (1 – 8)	27,76 \pm 0,10 a (24 – 32)	0,84
30	101	7,40 \pm 0,07 c (6 – 8)	17,93 \pm 0,18 b (14 – 23)	3,03 \pm 0,09 a (1 – 4)	28,44 \pm 0,18 b (23 – 33)	9,90

Source: Authors (2020)

Where: Means followed by the same letter in the column do not differ significantly by the Scott-Knott test at 5% probability.

It was observed that the best temperature for incubation of eggs and development of immature *Blastopsylla occidentalis* was 25°C. In this way, the immature completed their development in less time (5.80 and 15.77 days), with

complete cycle average of 27.7 days. Introducing the longevity of adults prolonged compared to 20 and 30°C (Table 2). Corroborating the information, Hodkinson (2009) mentions that the development of most psyllid species is directly linked to the temperature, and to have a good development successful it is also necessary that there is a synchronism between these individuals and the host plant. Rodrigues (2004) mentions that at temperatures close to 25°C insects have the fastest development, which was evidenced in the data biologicals presented here.

The statistical analysis performed for the biological data resulted in a difference significant in all observed parameters (Table 2). For the three temperatures, that the complete cycle was observed, adult mortality in the first 24 emergency hours was relatively low, with rates of: 13.15% (20°C); 0.84% (25°C) and 9.90% (30°C). Thus confirming the temperature of 25°C as ideal for the rearing of the insect under laboratory conditions.

At temperatures 20, 25 and 30°C, the reproductive capacity of 38, 66 and 32 females, respectively (Table 3). At 30°C there was development of insect, however, at this temperature, no postures were observed, this feat also was observed for *Glycaspis brimblecombei* (FIRMINO, 2004). At 30°C the longevity of the adult was reduced and this data corroborates the work of Barbosa *et al.* (2019), in which reported that temperatures above 30°C can contribute to the reduction fertility and survival. When studying *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) Hall, Wenninger and Hentz (2011) observed that the thresholds lower and upper estimated for oviposition are 16.0°C and 41.6°C, respectively.

Temperature influenced the F1 generation of *Blastopsylla occidentalis*, consequently, females from temperatures 20 and 25°C had the same daily averages of 19.04 and 26.40 eggs, respectively. Since at 25°C, females oviposited up to 38 eggs per day, while at 20°C the maximum number of eggs was 29 (Table 3). *Glycaspis brimblecombei* had averages of 31.2 (22°C) and 31.7 (26°C), but although

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either at other temperatures, it is observed that at 25°C females of *Blastopsylla occidentalis* oviposited close to the average found at 26°C by Firmino (2004).

For *Ctenarytaina eucalypti*, females can oviposit from 20 to 100 eggs under conditions natural (QUEIROZ; BURCKHARDT; MAJER, 2012).

Table 3 - Number of females observed, total number of F1 eggs, average and variation of (eggs per day \pm SE), number and hatching rate (%) of immatures and mean duration ($X \pm$ SE) of incubation periods and variation intervals (days) of F1 immatures from populations of *Blastopsylla occidentalis* Taylor, 1985 (Hemiptera: Aphalaridae), em cinco temperaturas in Sinop - MT, Brazil

Temperature (°C)	females observed	Total of eggs F1	average of eggs F1 \pm EP (days)	Outbreak of immature F1 (%)	immature observed	Period incubation Media \pm EP (days)
20	38	476	19,04 \pm 0,91 b (11 – 29)	52	250	6,70 \pm 0,04 b (6 – 8)
25	66	660	26,40 \pm 1,33 a (14 – 38)	85	566	5,59 \pm 0,30 a (5 – 7)
30*	32	-	-	-	-	-

Source: Authors (2020)

In which: * there were no postures. Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test at 5% probability.

It was observed that there was no difference in the mean duration of the incubation period of the F1 generation when compared with the average duration of their progenitors, in this way the females at 20°C had an average of 6.97 while for F1 the average was 6.70 days, and at 25°C, the averages were 5.80 days for parent females and 5.59 for first-generation immatures. It is noteworthy that, for the first generation, both temperatures maintained the shorter duration for hatching than their progenitors, so the immatures hatched in at least 6 (20°C) and 5 days (25°C), but there was a reduction of 1 day at maximum duration for hatching at 25°C.

The results presented here corroborate the literature that relate mainly the temperature 25°C for the best development and reproduction of insects. It is expected that these results will serve to conduct studies for the application management techniques for eventual control of the species.

4 CONCLUSION

The optimal temperature range for the development and reproduction of *Blastopsylla occidentalis* was between 20 and 25°C, with 25°C being the temperature in that the insect showed lower mortality in all phases, shorter development and higher average number of eggs/day. At 30°C, although the insect develop until the adult stage, the females did not lay eggs and at 15°C and 35°C the insects have not completed their development.

It is recommended for the creation of *Blastopsylla occidentalis*, in the laboratory, temperatures between 20 and 25°C, which the insect has its best development from the incubation period to reproduction. What can help in future studies with the species, including the introduction of management techniques with predators.

THANKS

The first author is grateful for the support of the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financing Code 88882.459209/2019-01, for granting a master's scholarship. To Embrapa Florestas through Project nº 03.13.12.008.00.00. Paula Pucci and Jaqueline Cremonese for their collaboration in laboratory tests of psyllid creation at Embrapa Florestas. To Flora Sinop and to Cunhatay Farm for their collaboration.

TBSK, DLQ and MRB: developed the research project; TBSK: organized and performed the experiment; TBSK and EAA: analyzed the data and performed the tests statisticians; TBSK: wrote the article; MRB and DLQ: guided the research; MRB, DLQ and DB: reviewed for publication submission in the journal.

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How to cite this article

Kunast, TB; Queiroz, DL; Burckhardt, D.; Andrade, EA; Barreto, MR Effect of temperature on development and reproduction of *Blastopsylla occidentalis* (Hemiptera: Aphalaridae) under laboratory conditions. *Forest Science*, Santa Maria, v. 31, no. 1, p. 440-455, 2021. DOI 10.5902/1980509843846. Available at: <https://doi.org/10.5902/1980509843846>. Accessed on: xx abbreviated month 202x.