Thermal requirements and estimate number of generations of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) in different *Eucalyptus* plantations regions

Pereira, FF.[a], *Zanuncio, JC.*[b], *Oliveira, HN.*[c*], *Grance, ELV.*[a], *Pastori, PL.*[a] *and Gava-Oliveira, MD.*[a]

[a]Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados – UFGD, Rod. Dourados-Itahum, Km 12, CP 241, CEP 79804-970, Dourados, MS, Brazil

[b]Departamento de Biologia Animal, Universidade Federal de Viçosa – UFV, CEP 36570-000, Viçosa, MG, Brazil

[c]Embrapa Agropecuária Oeste, BR 163, Km 253,6, CP 661, CEP 79804-970, Dourados, MS, Brazil

*e-mail: harley@cpao.embrapa.br

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Abstract

To use *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) in a biological control programme of *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae), it is necessary to study thermal requirements, because temperature can affect the metabolism and bioecological aspects. The objective was to determine the thermal requirements and estimate the number of generations of *P. elaeisis* in different *Eucalyptus* plantations regions. After 24 hours in contact with the parasitoid, the pupae was placed in 16, 19, 22, 25, 28 and 31 °C, 70 ± 10% of relative humidity and 14 hours of photophase. The duration of the life cycle of *P. elaeisis* was reduced with the increase in the temperature. At 31 °C the parasitoid could not finish the cycle in *T. arnobia* pupae. The emergence of *P. elaeisis* was not affected by the temperature, except at 31 °C. The number of individuals was between six and 1238 per pupae, being higher at 16 °C. The thermal threshold of development (Tb) and the thermal constant (K) of this parasitoid were 3.92 °C and 478.85 degree-days (GD), respectively, allowing for the completion of 14.98 generations per year in Linhares, Espírito Santo State, 13.87 in Pompéu and 11.75 in Viçosa, Minas Gerais State and 14.10 in Dourados, Mato Grosso do Sul State.

Keywords: biological control, parasitoids, *Thyrinteina arnobia*, thershold temperature.

Exigências térmicas e estimativa do número de gerações de *Palmistichus elaeisis* (Hymenoptera: Eulophidae) em diferentes regiões com plantios de *Eucalyptus*

Resumo

Para o uso de *Palmistichus elaeisis* Delvare e LaSalle, 1993 (Hymenoptera: Eulophidae) no controle biológico de *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) em eucalipto, é necessário estudar suas exigências térmicas, pois a temperatura pode afetar o metabolismo e aspectos bioecológicos dos insetos. Objetivou-se assim, determinar as exigências térmicas e o número de gerações de *P. elaeisis* em diferentes regiões com florestas de *Eucalyptus*. Permitiu-se o parasitismo por 24 horas e, após esse período, as pupas foram transferidas para câmaras climatizadas a 16, 19, 22, 25, 28 e 31 °C, 70 ± 10% de umidade relativa e fotofase de 14 horas. Verificou-se que o aumento da temperatura reduziu a duração média do ciclo de vida de *P. elaeisis*, sendo que a 31 °C o parasitoide não concluiu seu ciclo de vida em pupas de *T. arnobia*. A progênie do parasitoide por pupa variou de 6 a 1238 indivíduos e foi maior a 16 °C. A temperatura base (Tb) e constante térmica (K) desse parasitoide foram de 3,92 °C e 478,85 graus-dia, respectivamente. O número estimado de gerações anuais de *P. elaeisis*, em pupas de *T. arnobia*, para os municípios de Linhares, ES, Pompéu e Viçosa, MG e Dourados, MS foi de 14,98; 13,87; 11,75; e 14,10, respectivamente.

Palavras-chave: controle biológico, parasitoides, *Thyrinteina arnobia*, temperatura base.

1. Introduction

The lepidopterous defoliators species are dangerous and harmful pests, presenting frequent outbreaks and damage to the eucalyptus forest (Zanuncio et al., 2009). *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) also known as the "brown eucalyptus caterpillar", is the main Brazilian defoliator pest being present in most of the areas planted with eucalyptus (Peres Filho and Berti Filho, 2003). Anjos et al. (1987) record that in field surveys, it was found that the total defoliation of eucalyptus trees by *T. arnobia* reduced the average volume of wood in 60% and caused 6% of tree mortality.

Chemical control might prove to be efficient; however, it presents a series of limitations such as difficulty in applying insecticides, reduction of the population of its natural enemy, intoxication and environmental contamination, increased costs and induction of resistant insects. The parasitoids are natural enemies of major importance for the stability balance of the eucalyptus agroecosystem. These parasitoid assemblages are characterised by their high diversity and potential in the biological control of lepidopterous defoliators (Oliveira et al., 2003; Pereira et al., 2008).

Palmistichus elaeisis Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) is a pupal parasitoid that is effective in the control of certain serious lepidopterous pests of eucalyptus (Pereira et al., 2009a, c). They have been reported occurring in pupae of *Eupseudosoma involuta* Sepp, 1852 (Lepidoptera: Arctiidae), *Euselasia eucerus* (Dalman, 1823) (Lepidoptera: Riodinidae) and *Sabulodes* sp. (Lepidoptera: Geometridae) (Bittencourt and Berti Filho, 2004b), *Dirphia moderata* Walker, 1855 (Lepidoptera: Saturniidae), *Halysidota pearsoni* Watson, 1980 (Lepidoptera: Arctiidae), *T. arnobia* and *T. leucoceraea* Rindge, 1961 (Pereira et al., 2008) all of them, pests of eucalyptus crops.

However, an important aspect in biological control efficacy is temperature requirement. Through mathematical models it is possible to forecast pest occurrence as well their biological control agent occurrence (Higley et al., 1986). Knowing the parasitoid temperature requirements, it is possible to control laboratorial livestock of this insect aiming for later release (Bueno et al., 2008).

The thermal requirement for *P. elaeisis* on *T. arnobia* pupae was studied under laboratory conditions, showing that the development time from egg to adult tends to decrease when the temperature increases (Bittencourt and Berti Filho, 2004b). Lower development time was also observed for other parasitoids when the temperature is above or below the optimum temperatures (Pratissoli et al., 2006; Pastori et al., 2008). Nevertheless, Oliveira et al. (2000) showed that the same specie of *Trichogramma maxacalli* Voegelé and Pointel, 1980 (Hymenoptera: Trichogrammatidae), collected in different regions, can show distinct behaviour and biological aspects, and Borba et al. (2006) and Pratissoli et al. (2006), reported differences between the thermal requirements from the same strain of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae).

To obtain efficient biocontrol of pests, a species and/or strain with better adaptation to the climatic conditions of the region should be selected (Borba et al., 2006). Therefore, the aim of this research was to define the reproduction and thermal requirements of a population of *P. elaeisis* collected in Minas Gerais state on *T. arnobia* pupae, and estimate the number of generations for producing and/or potential to produce eucalyptus.

2. Materials and Methods

2.1. Laboratory establishment of T. arnobia

Eggs of this species were obtained at the Laboratory of Biological Control of Insects and the Animal Biology Department of the Federal University of Viçosa (UFV), Minas Gerais state and after hatching, larvae of Lepidoptera were placed in bags of organza fabric (0.70 × 0.40 cm), involving branches of eucalyptus plants and removed to other branches, every three days. Pupae were collected from those branches, sexed, separated into pairs and placed in plastic pots (500 mL) with a plastic lid with holes in the middle, which was sealed with a fine mesh screen, from the organza fabric type. Strips of paper stuck to the cover were placed in pots for ovipositioning in a room heated to 25 ± 2 °C, 70 ± 10% of relative humidity and 14 hours of photophase.

2.2. Rearing of P. elaeisis

Adults of parasitoids were kept in glass tubes (2.5 diameter × 17.0 cm), labelled, covered with organza fabric type, containing in its interior, plastic containers (3.0 × 4.0 cm) with an aqueous solution of honey at 10%. To keep the progeny, pupae of *T. arnobia* of 24 to 72 hours were exposed to parasitism for 24 hours at a temperature of 25 ± 2 °C, 70 ± 10% of relative humidity and 14 hours of photophase.

2.3. Temperature effect on the development of P. elaeisis in T. arnobia pupae

Twenty-four-hour-old pupae of *T. arnobia* were sexed, weighed [(10 females) (716.80 ± 21.25 mg) and (10 males) 242.77 ± 9.19 mg)] and individualised in glass tubes (14 × 2.2 cm) with 72-hours-old females of *P. elaeisis* (Pereira et al., 2009a) and closed with cotton for 24 hours. Based on preliminary tests, we used six females of *P. elaeisis* per male pupae of *T. arnobia*, and 15 females per female pupae (Pereira et al., 2010). At the end of that period, the females were removed manually and the tubes transferred to climatic chambers at temperatures of 16, 19, 22, 25, 28 and 31 °C, 70 ± 10% of relative humidity and 14 hours of photophase. The duration of the period between egg to adult, daily, always at the same time, and the percentage of emergence and sex ratio [SR= number of females/ (number of females + males] were evaluated. The sex of the parasitoids was determined by morphological characteristics of their antenna and abdomen (Delvare and LaSalle, 1993).

2.4. Statistical analyses

The experimental design was completely randomised, with six treatments (temperatures) and 20 replications (10 male and 10 female pupae). The data were subjected to analysis of variance and when significant at 5% probability, regression analysis was performed. The values of percentage of parasitism and emergence of *P. elaeisis* were subjected to analysis of generalised linear models with binomial distribution ($p \leq 0.05$) with R Statistical System (Ihaka and Gentleman, 1996). The threshold temperature (Tb) and thermal constant (K) were calculated by the hyperbole method (Haddad et al., 1999) based on the duration of the cycle (egg-adult) of *P. elaeisis*. The annual number of generations of parasitoids was predicted for Pompéu and Viçosa in Minas Gerais state, Linhares in Espírito Santo state and Dourados in Mato Grosso do Sul state with the Equation 1:

$$NG = \{T\,(Tm - Tb) / K\} \quad (1)$$

in which K = Thermal Constant, Tm = average temperature of each studied site, Tb = threshold temperature and T = time taken in days, based on the normal thermal of these eucalyptus sites from 1973 to 1990 provided by INMET ("Instituto Nacional de Meteorologia") and the "Serviço de Monitoramento Agrometereológico" of "Embrapa Agropecuária Oeste". These areas were selected because they are producing and/or have the potential to produce eucalyptus in the states of Espírito Santo, Minas Gerais and Mato Grosso do Sul.

3. Results

The average duration of the cycle (egg-adult) of *P. elaeisis* in pupae of *T. arnobia* decreased when the temperature raised and ranged from 39.92 to 21 days to 16 and 28 °C, respectively ($R^2_{Treat} = 0.9094$; $F = 602.0025$; $P = 0.0001$; $gl_{erro} = 75$) (Figure 1). At 31 °C, *P. elaeisis* could not complete its life cycle in pupae of *T. arnobia* and no emergence was observed, but 100% of these pupae had pre-pupae of *P. elaeisis* (136.83 ± 22.88).

The percentage emergence of *P. elaeisis* in pupae of *T. arnobia* was not influenced by the temperature (89.47% at 19 °C and 100% elsewhere) ($\chi^2 = 1.2929$, $p = 0.2555$), except at 31 °C.

The progeny of *P. elaeisis* by *T. arnobia* pupae ranged from six to 1238 individuals being higher at 16 °C ($R^2_{Treat} = 0.7991$, $F = 4.1979$, $p = 0.0085$; $gl_{erro} = 75$) (Figure 2).

The sex ratio of *P. elaeisis* in pupae of *T. arnobia* was similar at different temperatures, with averages of 0.90, 0.92, 0.84, 0.94 and 0.91 to 16, 19, 22, 25 and 28 °C, respectively ($p \geq 0.05$).

The longevity of females of *P. elaeisis* emerged from *T. arnobia* pupae showed an increase up to 22 °C and decreased from this temperature ($R^2_{Treat} = 0.9695$; $F = 26.0722$; $p = 0.0001$; $gl_{erro} = 99$) (Figure 3). The longevity of males of this parasitoid was not influenced by temperatures ($p \geq 0.05$). The males lived less than females and they copulate immediately, after its emergence.

The thermal requirements for the immature stage of *P. elaeisis* in *T. arnobia* pupae was based on the model $Y = (1 / D) = -0.008178 + 0.02088 \times (R^2 = 94.40)$ whose values for threshold temperature (Tb) and thermal constant (K) were 3.92 °C and 478.85 degree-days (GD), respectively (Figure 4).

Because of the threshold temperature and thermal requirements for *T. arnobia* (Peres Filho and Berti Filho, 2003) and *P. elaeisis* estimated this research, it was possible

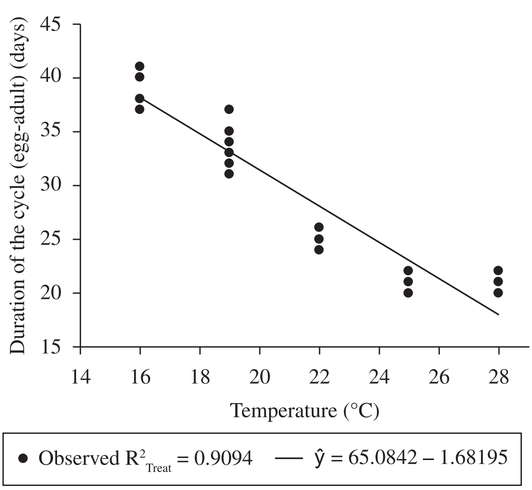

Figure 1. Duration of the cycle (egg-adult) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) per pupa of *Thyrinteina arnobia* (Lepidoptera: Geometridae) at different temperatures. 70 ± 10% of relative humidity and 14 hours of photophase ($F = 602.0025$; $p < 0.0001$).

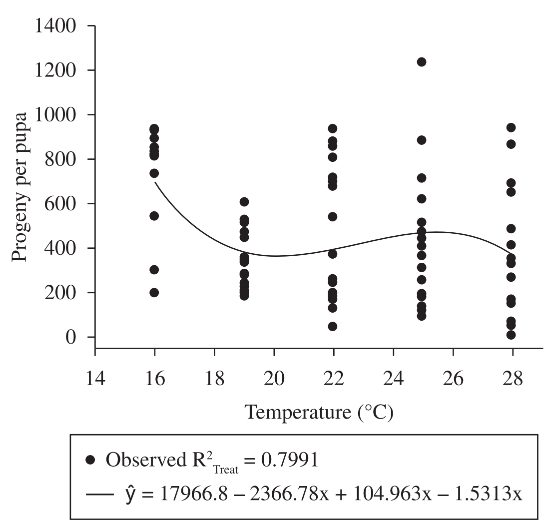

Figure 2. Progeny of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) per pupa of *Thyrinteina arnobia* (Lepidoptera: Geometridae) at different temperatures. 70 ± 10% of relative humidity and 14 hours of photophase ($F = 4.1979$; $p = 0.0085$).

to verify the number of generations per year based on the average temperature of 20 years in Pompéu, Minas Gerais state, and it was found that *P. elaeisis* can have 13.87 generations per year, while *T. arnobia* did not exceed the 5.83 annual generations, showing that the population of *P. elaeisis* will be faster than that of *T. arnobia* (Figure 5). For all the studied regions that produce eucalyptus, it was found that the annual number of generations of *P. elaeisis* was higher than that of the *T. arnobia* (Figure 6).

4. Discussion

This inverse relationship was found for *P. elaeisis* when reared in pupae of *Anticarsia gemmatalis* Hübner, 1818, *Heliothis virescens* (Fabr., 1781), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), *Diatraea saccharalis* (Fabr., 1794) (Lepidoptera: Crambidae) and *T. arnobia* (Bittencourt and Berti Filho, 2004a). Differences were also observed when comparing the cycle duration between these parasitoids and hosts at each temperature, indicating that both environmental factors and host species may interfere in the development of parasitoids (Bittencourt and Berti Filho, 2004a; Canto-Silva et al., 2005; Jacas et al., 2008; Golizadeh et al., 2008; Pandey and Tripathi, 2008; Pastori et al., 2008).

The mortality of parasitoids in a pre-pupae stage indicates that its upper thermal limit is below 31 °C in pupae of *T. arnobia*. At temperatures above 30 °C, *P. elaeisis* in pupae of five lepidopteran species stopped their development (Bittencourt and Berti-Filho, 2004b). At 16 until 28 °C, the emergence rates of *P. elaeisis* in

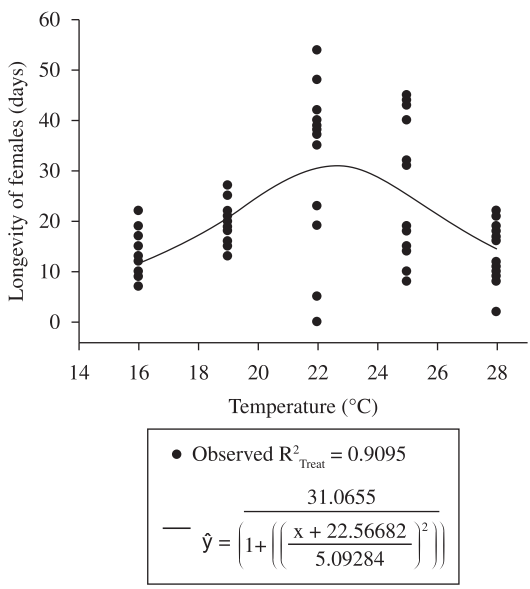

Figure 3. Longevity of females of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) emerged from pupa of *Thyrinteina arnobia* (Lepidoptera: Geometridae) at different temperatures. 70 ± 10% of relative humidity and 14 hours of photophase (F = 26.0722; p = 0.0001).

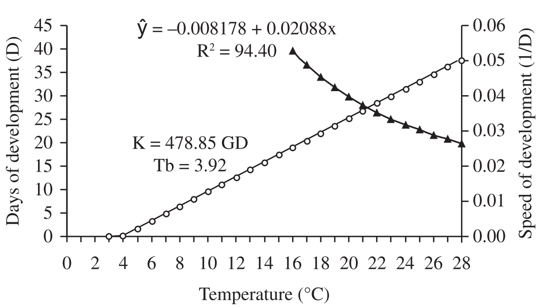

Figure 4. Duration (days) and speed of development of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) per pupa of *Thyrinteina arnobia* (Lepidoptera: Geometridae) at different temperatures. 70 ± 10% of relative humidity and 14 hours of photophase.

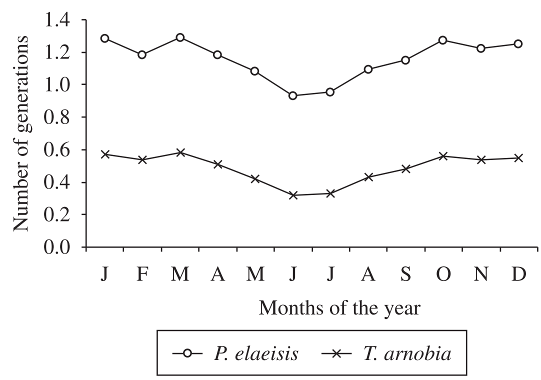

Figure 5. Estimated monthly number of generations of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) and *Thyrinteina arnobia* (Lepidoptera: Geometridae), for the municipality of Pompéu, Minas Gerais state, Brazil.

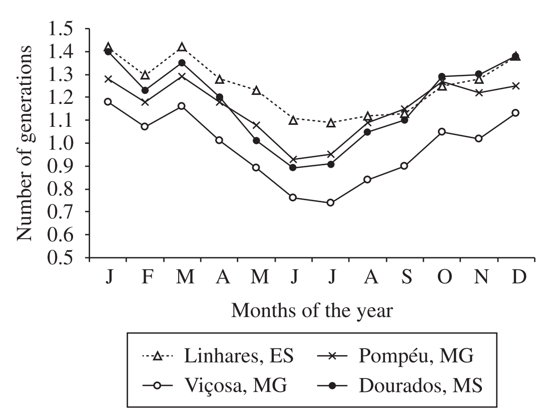

Figure 6. Estimated number of generations of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) in pupae of *Thyrinteina arnobia* (Lepidoptera: Geometridae) at different months of the year, for producing areas of eucalyptus in the states of Espírito Santo, Minas Gerais and Mato Grosso do Sul, Brazil.

T. arnobia pupae were high, showing that this parasitoid can be reared in this thermal band without compromising its development. This is important because it allows the assumption that *P. elaeisis* can parasite and develops pupae in this pest at sites that presented similar temperatures. Therefore, the release of parasitoids in the field should be performed by looking at the thermal limits of the natural enemy for the establishment and efficiency to be higher (Jacas et al., 2008; Pandey and Tripathi, 2008).

Although the number of individuals was higher in the temperature of 16 °C, the biological characteristics were considered satisfactory at all temperatures, except 31 °C. Most of *T. arnobia* pupae parasitised by this parasitoid to 16 °C were females that presented twice the pupae size than the male of this pest and had contributed to a higher progeny of *P. elaeisis* at this temperature.

Regarding the sex ratio, the large proportion of female parasitoids is an important feature, especially in mass rearing systems and in the selection of individuals to be released in the field, because of the predominance of female parasitoids in relation to males it may increase the number of individuals produced in the next generation (Uçkan and Gulel, 2002; Amalin et al., 2005; Pereira et al., 2009b).

The temperatures between 22 to 25 °C can be considered ideal for the survival and reproduction of adult females of the parasitoid and this temperature range coincides with the ideal for the development of *T. arnobia* (Peres Filho and Berti Filho, 2003). This implies that in the climatic conditions where *T. arnobia* reaches a potential maximum development, *P. elaeisis* can be used successfully for control. The temperature of 22 °C was also considered less limiting in the development of *P. elaeisis* in pupae of *D. saccharalis, A. gemmatalis, H. virescens, S. frugiperda* and *T. arnobia* (Bittencourt and Berti Filho, 2004b). This demonstrates that the biological control agent may express greater reproductive longevity in locations which have similar thermal conditions.

The thermal requirements allow the calculation of the time necessary to complete the development of the insect pests and parasitoids (Ferreira et al., 2003), enabling it to compare the results for the thermal requirements of *P. elaeisis* with the ones of *T. arnobia*, which were 11.91 °C and 637.479 GD (Peres Filho and Berti Filho, 2003). The largest number of generations of *P. elaeisis* compared with *T. arnobia* is even greater in the regions concerned, which is an important feature in a biological control programme.

However, the thermal requirements of this parasitoid can be affected according to the host, with the thermal constant from egg to adult, ranging from 353.1 to 407.7 degree-days and the lower thermal threshold between 5.0 and 7.5 °C, when reared in five different lepidopteran species (Bittencourt and Berti Filho, 2004b). Differences reported in different studies also indicate that the parasitoid species and/or strain and the host species can affect the thermal requirements of *Trichogramma* (Bueno et al., 2010)

In this study, the individuals of *P. elaeisis* used were collected in Viçosa, Minas Gerais state (Pereira et al., 2008), a district with an average annual temperature of 19.4 °C and so these individuals could be adapted to lower temperatures.

Palmistichus elaeisis showed satisfactory development in *T. arnobia* pupae, between 16 to 28 °C, and therefore, this parasitoid may be recommended to control this pest, especially in Linhares, Espirito Santo State, Pompeu and Viçosa in Minas Gerais state and in Dourados in Mato Grosso do Sul state.

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