



32 **1. Introduction**

33 Eucalyptus cultivation stands out in the Brazilian forestry sector due to its high  
34 economic viability and versatile applications (Barroso et al. 2004). Although the genus  
35 *Eucalyptus* comprises approximately around 730 species, only about 20 are  
36 commercially exploited, mainly to produce paper, pulp, timber, charcoal, essential oils,  
37 and biomass (Magaton et al. 2009; Penfold and Willis, 1954). Currently, eucalyptus  
38 plantations cover approximately 7.8 million hectares, accounting for 76% of all planted  
39 forests in the country (Ibá, 2024).

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40 The expansion of eucalyptus cultivation areas in Brazil has been accompanied by an  
41 increase in the occurrence of diseases and phytophagous insects, many of which are  
42 recognized as potential pests capable of causing serious damage to the crop (Pereira  
43 et al., 2001). The harm caused by these insect pests can compromise forest  
44 productivity directly in the field (Guo et al. 2023; Koghan, 1998). Among the main  
45 groups responsible for such losses are defoliating caterpillars, which directly impact  
46 plant development by causing partial or even complete defoliation of the canopy, an  
47 effect that can severely hinder the growth and yield of commercial eucalyptus  
48 plantations (Poderoso et al. 2013; Zanuncio et al. 2018).

49 Among the colonization caterpillars, a species that has recently gained attention is  
50 *Physocleora dukinfeldia* (Lepidoptera: Geometridae) (Warren, 1897). Originally  
51 recorded in Brazil in the municipality of Castro, Paraná, Brazil (Schaus, 1897), this  
52 species was only recognized as a pest in 2021, following an outbreak in eucalyptus  
53 plantations that resulted in severe defoliation and economic losses. Similar to other  
54 geometrid caterpillars known to affect forest crops, *P. dukinfeldia* has demonstrated  
55 the potential to become a significant threat to commercial eucalyptus production (Silva  
56 et al. 2023; Pereira, 2012).

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57 The emergence of *P. dukinfeldia* as an eucalyptus pest highlights a broader ecological  
58 process: the colonization of new host plants by phytophagous insects and frequently  
59 precedes the emergence of pest outbreaks in managed ecosystems (Massélière et  
60 al. 2017). When a native herbivore shifts to a commercially important plant species, it  
61 may find favorable conditions for development due to reduced interspecific  
62 competition, lack of natural enemies, or continuous food availability, factors often  
63 present in large-scale monocultures. However, successful establishment in a novel

64 host requires physiological and behavioral plasticity, which must be assessed through  
65 detailed biological studies (Agrawal, 2000; Schoonhoven et al. 2005).

66 Understanding the life cycle parameters of herbivorous insects on new hosts, including  
67 development time, survival rates, reproductive capacity, and feeding behavior, is  
68 essential to assess their establishment potential and predict future population  
69 dynamics (Gripenberg et al. 2010). Such data are crucial in forest production systems,  
70 where outbreaks of defoliators can lead to significant reductions in growth and wood  
71 yield. Moreover, early-stage studies on insect biology can reveal signs of host-use  
72 constraints or adaptive potential, guiding monitoring efforts and informing integrated  
73 pest management programs (Heidel-Fischer et al. 2019; Janz et al. 2006). In this  
74 context, the present study aimed to evaluate the biological performance of  
75 *P. dukinfeldia* on *E. urograndis* compared to its native hosts, *Schinus terebinthifolia*,  
76 in order to assess this pest's its potential for adaptation and establishment in  
77 commercial eucalyptus plantations.

## 78 **2. Materials and Methods**

### 79 **2.1 *Physocleora dukinfeldia* rearing**

80 *P. dukinfeldia* individuals were initially collected in a commercial eucalyptus  
81 area (Sylvamo do Brasil Ltda®), in Guatapará, SP, Brazil. Eggs and larvae were  
82 maintained in plastic containers (500 mL) covered with organza mesh, under controlled  
83 conditions (25 ± 2°C, 70 ± 10% relative humidity, and a 12-hour photoperiod). Larvae  
84 were fed with pure eucalyptus leaves (*Eucalyptus urophylla*), obtained from healthy  
85 plants. The pupae were kept in containers with soil substrate until complete  
86 sclerotization. Then, they were transferred to containers lined with moistened filter  
87 paper. Adults were kept in polyvinyl chloride (PVC) mesh cages, where a 20% honey  
88 solution and brown paper were available for adult feeding and female oviposition,  
89 respectively. Papers containing eggs were used to start new rearing cycles.

### 90 **2.2 Life cycle assessment**

91 The biology of *P. dukinfeldia* was assessed on two host plants, *Eucalyptus urograndis*  
92 (hybrid eucalyptus) and *Schinus terebinthifolia* (Brazilian peppertree), to better  
93 understand the developmental cycle of this defoliator species. The tree species used  
94 in the experiment were obtained from the Department of Crop Protection, São Paulo  
95 State University, Botucatu, SP, Brazil.

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96 Neonate larvae were individually placed in plastic containers (500 mL) containing  
97 leaves from each host plant. The leaves were inserted into Eppendorf tubes filled with  
98 water to maintain leaf turgor. Each container was considered an experimental unit, with  
99 100 replicates per tree species, in a completely randomized design. The experiment  
100 was carried out in a climate-controlled room ( $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity, and  
101 a 12-hour photoperiod).

102 The insects were inspected evaluated daily for to measure larval head capsule width,  
103 pupal weight (24 hours post-pupation), dates of pupation and imago emergence, and  
104 viability in individual periods of development. Based on these records, the following  
105 parameters were determined: the following parameters: duration of each larval instar,  
106 total larval period, duration of prepupal and pupal stages, and total development time  
107 (larva-adult); head capsule width, total larval period, larval and pupal viability (%),  
108 duration of prepupal and pupal stages, pupal weight (24 hours pos-pupation),  
109 morphological characteristics of the pupae, pupal viability (%), and total development  
110 time (larva-adult).

111 Head capsules were measured using an ocular micrometer attached to a  
112 stereomicroscope (Leica®), with 20 replicates. Pupal weights were obtained with an  
113 analytical balance (Shimadzu®, model ATY224), and pupal length and width were  
114 measured with a caliper. During inspectionevaluations, excrements were as removed,  
115 and consumed leaves were replaced.

### 116 2.3 Leaf consumption

117 During the biological performance assay, consumed leaves of *E. urograndis* were  
118 photographed for subsequent assessment of leaf area consumption. In parallel, an  
119 independent assay was conducted using *E. urophylla* leaves to compare foliar  
120 consumption between this species and the eucalyptus hybrid (*E. urograndis*). The  
121 remaining leaf area after feeding was measured using ImageJ software (Ferreira *et al.*  
122 2022). Leaves of *S. terebinthifolia* were not included in this analysis due to their  
123 morphology, which prevented accurate measurement of leaf area.

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### 124 2.4 Statistical analysis

125 The data were subjected to analysis of variance, and the F-test was used to detect  
126 significant effects. The assumption of residual normality was assessed by the Shapiro-

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127 Wilk test, and the homogeneity of variances was verified using Levene's test (PROC  
128 UNIVARIATE, SAS). When significant differences among treatments were detected,  
129 the means were compared by the Tukey's test at a 5% significance level, using the  
130 PROC MIXED procedure (Sas, 2011).

### 131 **3. Results**

#### 132 **3.1 Life cycle determination**

133 The mean duration of the first instar *larvae* was 4.90 days on *E. urograndis* and 5.58  
134 days on *S. terebinthifolia*, with no significant difference between host plants ( $F = 2.68$ ;  
135  $df = 1$ ;  $P = 0.1054$ ; Table 1). Similarly, development periods for the second and third  
136 instars showed no significant variation between host species (2<sup>nd</sup> instar:  $F = 1.31$ ;  $df =$   
137 1;  $P = 0.2559$ ; 3<sup>rd</sup> instar:  $F = 2.18$ ;  $df = 1$ ;  $P = 0.1441$ ). Larvae fed with the eucalyptus  
138 hybrid completed the second and third instars in 4.38 and 4.97 days, respectively, while  
139 those reared on *S. terebinthifolia* completed the same stages in 4.00 and 4.20 days.

140 The development time for the fourth instar differed significantly between host plants ( $F$   
141 = 15.56;  $df = 1$ ;  $P = 0.0002$ ), being longer on *E. urograndis* (5.86 days) compared to  
142 *S. terebinthifolia* (4.21 days). In contrast, no significant differences were observed for  
143 the fifth and sixth instars (5<sup>th</sup> instar:  $F = 0.70$ ;  $df = 1$ ;  $P = 0.4054$ ; 6<sup>th</sup> instar:  $F = 3.92$ ;  $df$   
144 = 1;  $P = 0.0524$ ). In the fifth instar, larvae fed with the hybrid eucalyptus and  
145 *S. terebinthifolia* exhibited mean durations of 4.97 and 5.35 days, respectively. In the  
146 sixth instar, the mean durations were 11.43 days for *E. urograndis* and 9.22 days for  
147 *S. terebinthifolia*.

148 Considering the total larval period, larvae fed with the hybrid eucalyptus exhibited a  
149 significantly longer duration (35.07 days) compared to those fed with *S. terebinthifolia*  
150 (31.56 days) ( $F = 5.93$ ;  $df = 1$ ;  $P = 0.0180$ ). The durations of the prepupal and pupal  
151 stages did not differ significantly between hosts (prepupa:  $F = 3.28$ ;  $df = 1$ ;  $P = 0.0755$ ;  
152 pupa:  $F = 0.96$ ;  $df = 1$ ;  $P = 0.3313$ ). The longest larva-adult development cycle was  
153 recorded on *E. urograndis* (52.00 days), which differed significantly from that observed  
154 on *S. terebinthifolia* (48.13 days) ( $F = 6.18$ ;  $df = 1$ ;  $P = 0.0160$ ).

#### 155 **3.2 Cephalic capsule measurements**

156 The *mean head capsule width* of *P. dukinfieldia* larvae differed significantly between  
157 host plants during the second instar ( $F = 5.73$ ;  $df = 1$ ;  $P = 0.0217$ ; Table 2), with values

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The only significant differences are... at this instar, on this host plant...

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Was the difference between male and female development, instar number?

158 of 0.28 mm on *E. urograndis* and 0.26 mm on *S. terebinthifolia*. However, no significant  
159 differences were observed for the third ( $F = 3.81$ ;  $df = 1$ ;  $P = 0.0583$ ), fourth ( $F = 1.42$ ;  
160  $df = 1$ ;  $P = 0.2408$ ), fifth ( $F = 3.66$ ;  $df = 1$ ;  $P = 0.0633$ ), and sixth instars ( $F = 3.84$ ;  $df =$   
161  $1$ ;  $P = 0.0574$ ).

### 162 **3.3 Larval and pupal viability**

163 Larval viability of *P. dukinfeldia* was low on both host plants, with 28.00% on  
164 *E. urograndis* and 32.00% on *S. terebinthifolia*, with no statistical difference between  
165 treatments ( $F = 0.38$ ;  $df = 1$ ;  $P = 0.5395$ ; Figure 1). However, pupal viability was  
166 significantly lower in larvae fed on *E. urograndis* (85.71%) compared to those reared  
167 on *S. terebinthifolia* (100.00%) ( $F = 5.16$ ;  $df = 1$ ;  $P = 0.0269$ ).

168 The morphological characteristics of *P. dukinfeldia* pupae, including weight, length, and  
169 width, showed no statistically significant differences between host plants for either sex.  
170 Among males, mean values were similar between treatments with *E. urograndis*  
171 (0.0713 mg; 10.90 mm in length; 3.32 mm in width) and *S. terebinthifolia* (0.0722 mg;  
172 11.09 mm in length; 3.37 mm in width) (weight:  $F = 0.04$ ;  $df = 1$ ;  $P = 0.8527$ ; length:  $F$   
173 = 0.40;  $df = 1$ ;  $P = 0.5301$ ; width:  $F = 0.18$ ;  $df = 1$ ;  $P = 0.6724$ ; Table 2). Similarly, female  
174 pupae exhibited comparable measurements across host plants (weight: 0.0747 mg in  
175 *E. urograndis* and 0.0863 mg in *S. terebinthifolia*; length: 11.57 mm and 11.7 mm;  
176 width: 3.44 mm and 3.56 mm, respectively) (weight:  $F = 3.40$ ;  $df = 1$ ;  $P = 0.0755$ ; length:  
177  $F = 0.84$ ;  $df = 1$ ;  $P = 0.3668$ ; width:  $F = 1.91$ ;  $df = 1$ ;  $P = 0.1772$ ).

### 178 **3.4 Foliar consumption**

179 No significant differences were observed for total leaf consumption among the  
180 eucalyptus species evaluated in this study ( $F = 0.01$ ;  $df = 1$ ;  $P = 0.9514$ ; Figure 2). The  
181 consumption of *P. dukinfeldia* was 0.7309 cm<sup>2</sup>/larva and 0.7173 cm<sup>2</sup>/larva in *E.*  
182 *urophylla* and *E. urograndis*, respectively.

### 183 **4. Discussion**

184 The results demonstrated that *P. dukinfeldia* is capable of completing its life cycle on  
185 *E. urograndis*, although with a significantly prolonged larval development time and life  
186 cycle (larva-adult) compared to its native host, *S. terebinthifolia*. The observed  
187 extension, particularly during the fourth instar and the total larval period, suggests that  
188 eucalyptus may impose physiological or nutritional challenges to the development of

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189 this species, which is consistent with patterns reported for other insect herbivores  
190 (Campos et al. 2022).

191 The increase in developmental time from the fourth instar onward may indicate a  
192 delayed adaptive response to secondary compounds present in *E. urograndis*, such  
193 as monoterpenes and sesquiterpenes, which are commonly associated with the  
194 chemical defense of *Eucalyptus* species (Araújo et al. 2010; Cheng et al. 2009;  
195 Elangovan and Poonam, 2023). However, the ability to complete development on *E.*  
196 *urograndis* demonstrates that *P. dukinfeldia* has the potential to establish and adapt to  
197 this crop, which justifies special attention in monitoring its occurrence in commercial  
198 eucalyptus plantations. Furthermore, the longer life cycle observed on the eucalyptus  
199 hybrid (52 days) is consistent with that of other defoliator species, such as *Thyrinteina*  
200 *arnobia* (Stoll, 1782) and *Iridopsis panopla* Prout, 1932, whose developmental duration  
201 may also range from 40 to 60 days depending on environmental conditions and host  
202 plant (Jesus et al. 2015; Oliveira et al. 2005; Santos et al. 1996).

203 The mean head capsule width of *P. dukinfeldia* increased progressively across the six  
204 instars on both host plant species, reflecting the typical larval growth pattern observed  
205 in Lepidoptera. The consistent increment in head capsule width throughout the instars  
206 indicates a stable growth rate, possibly associated with the larva's physiological  
207 adaptation to the host plant (Calvo and Molina, 2008; Castañeda-Vildózola et al. 2016;  
208 Panzavolta, 2007).

209 The low survival rate during the larval stage may reflect the species' intrinsic sensitivity  
210 to host plant defenses (Araújo et al. 2010; Carneiro et al. 2024). However, nearly one-  
211 third of the larvae successfully completed development, demonstrating the pest's  
212 potential to establish in commercial eucalyptus plantations. Pupal viability was  
213 significantly lower in larvae fed on *E. urograndis*, suggesting that sublethal effects of  
214 feeding on the alternative host may accumulate and impact the final transition to  
215 adulthood. This reduction in performance is characteristic of early stages of host range  
216 expansion, during which the pest is still undergoing adaptation to the novel host  
217 (Agrawal, 2003). Over time, *P. dukinfeldia* populations may experience selection and  
218 adapt to eucalyptus, potentially increasing host-use efficiency and reducing observed  
219 mortality rates.

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220 Despite the variations in development time and viability rates observed between host  
221 plants, *P. dukinfeldia* maintained a stable phenotypic pattern during the pupal stage.  
222 The absence of morphometric differences also suggests that although *E. urograndis*  
223 may pose challenges during larval development, the surviving individuals do not exhibit  
224 visible impairments in final growth. This pattern is common in cases of compensatory  
225 ontogenetic selection, ~~in where~~ individuals with greater ability to utilize the host  
226 offset early developmental deficits with more efficient growth rates during the later  
227 stages (Maino and Kearney, 2015). Furthermore, pupal size is directly related to the  
228 reproductive potential of adults, especially females, with direct implications for  
229 fecundity and population viability (Lee et al. 2023; Santos et al. 2023).

230 From a nutritional standpoint, both eucalyptus species tested in this study provide  
231 similar conditions for leaf consumption by the pest. *P. dukinfeldia* appears to feed with  
232 comparable efficiency across different eucalyptus species, reinforcing its potential  
233 generalist behavior regarding host plant use. Although individual foliar consumption  
234 was relatively low in this study (less than 1 cm<sup>2</sup> throughout development), this may be  
235 offset by population outbreaks if environmental conditions favor pest density increases.  
236 Therefore, even species with low individual consumption can cause significant damage  
237 at the population level, particularly during the early stages of infestation and in the  
238 absence of effective management strategies (Speight et al. 2008).

## 239 **5. Conclusion**

240 The emergence of *P. dukinfeldia* as a defoliator of *E. urograndis* highlights the need for  
241 proactive biological studies on newly observed pest species in forest plantations.  
242 Although this insect exhibited extended development times and reduced viability on  
243 eucalyptus compared to its native host, *S. terebinthifolia*, it was capable of completing  
244 its life cycle, indicating its potential to establish and adapt to commercial eucalyptus  
245 systems. This pioneering study provides essential baseline data on the biology,  
246 development, and feeding behavior of *P. dukinfeldia* under controlled conditions. The  
247 results underscore the pest's capacity to exploit eucalyptus as a viable host, even in  
248 the early stages of host adaptation. Given that individual foliar consumption was  
249 relatively low, but survival to adulthood was achievable, future outbreaks cannot be  
250 ruled out. The data generated here serve as a valuable reference for integrated pest

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251 management strategies and reinforce the importance of continuous monitoring and  
252 research in the face of emerging defoliators in commercial plantations.

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**Table 1 – Means *lengthnumber* (± SE) of 1st, 2nd, 3rd, 4th, 5th, and 6th instar periods, larval period, prepupal period, pupal period and larva-adult development cycle of *Physocleora dukinfieldia* in two forest species.**

Species	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	6 <sup>th</sup> instar	Larval period
<i>Eucaliptus urograndis</i>	4.90 ± 0.17	4.38 ± 0.16	4.97 ± 0.26	5.86 ± 0.23 a	4.97 ± 0.17	11.43 ± 0.39	35.07 ± 0.54 a
<i>Schinus terebinthifolia</i>	5.58 ± 0.21	4.00 ± 0.14	4.20 ± 0.19	4.21 ± 0.11 b	5.35 ± 0.21	9.22 ± 0.45	31.56 ± 0.56 b
<i>P</i>	0.1054	0.2559	0.1441	0.0002	0.4054	0.0524	0.0180
<b>Prepupal period</b>			<b>Pupal period</b>			<b>Larva-adult period</b>	
<i>Eucaliptus urograndis</i>	2.33 ± 0.12		15.04 ± 0.20			52.00 ± 0.53 a	
<i>Schinus terebinthifolia</i>	1.88 ± 0.06		14.38 ± 0.28			48.13 ± 0.60 b	
<i>P</i>	0.0755		0.3313			0.0160	

<sup>1</sup>Means followed by the same lowercase letter per column do not differ from each other by the LS-Means adjusted by Tukey's test (*P* ≤ 0.05).

← **Отформатировано:** По ширине, интервал Перед: 12 пт, после: 0 пт, межстрочный, одинарный

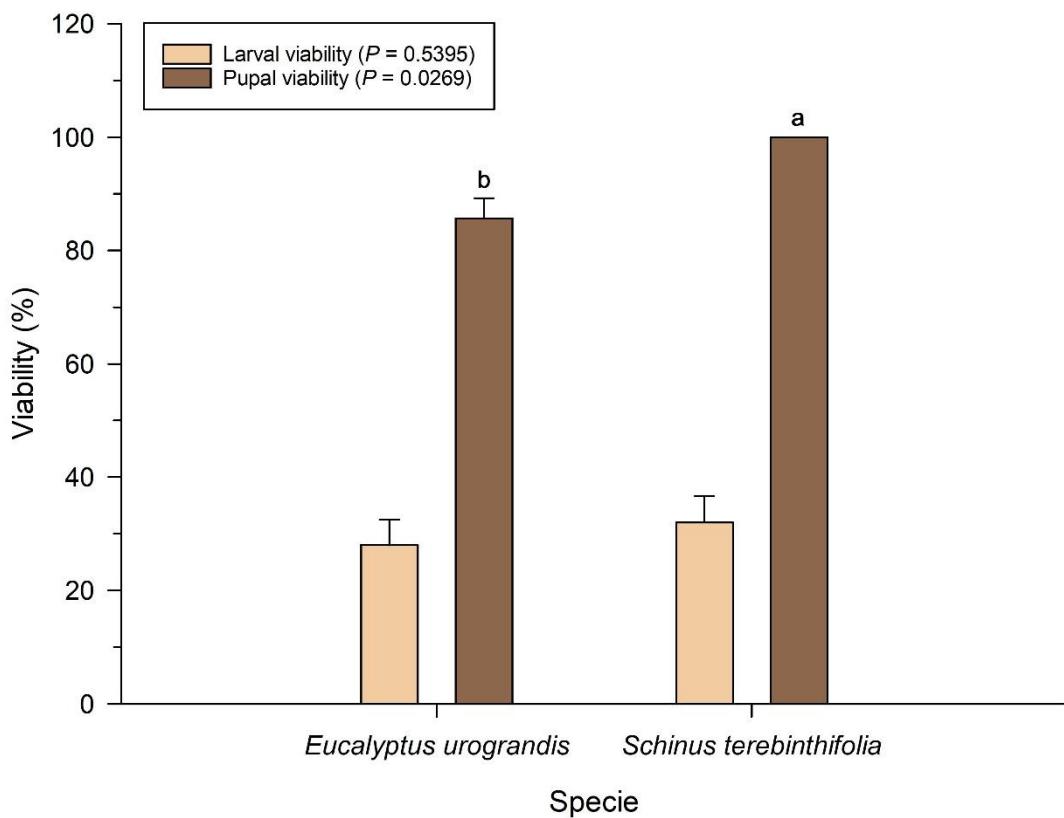
**Table 2 – Means (± SE) head capsule width (mm) of *Physocleora dukinfeldia* larvae fed on two forest tree species.**

Specie	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	6 <sup>th</sup> instar
<i>Eucaliptus urograndis</i>	0.28 ± 0.01 a	0.46 ± 0.01	0.74 ± 0.02	1.15 ± 0.03	1.65 ± 0.05
<i>Schinus terebinthifolia</i>	0.26 ± 0.00 b	0.43 ± 0.00	0.71 ± 0.01	1.07 ± 0.02	1.53 ± 0.03
<i>P</i>	0.0217	0.0583	0.2408	0.0633	0.0574

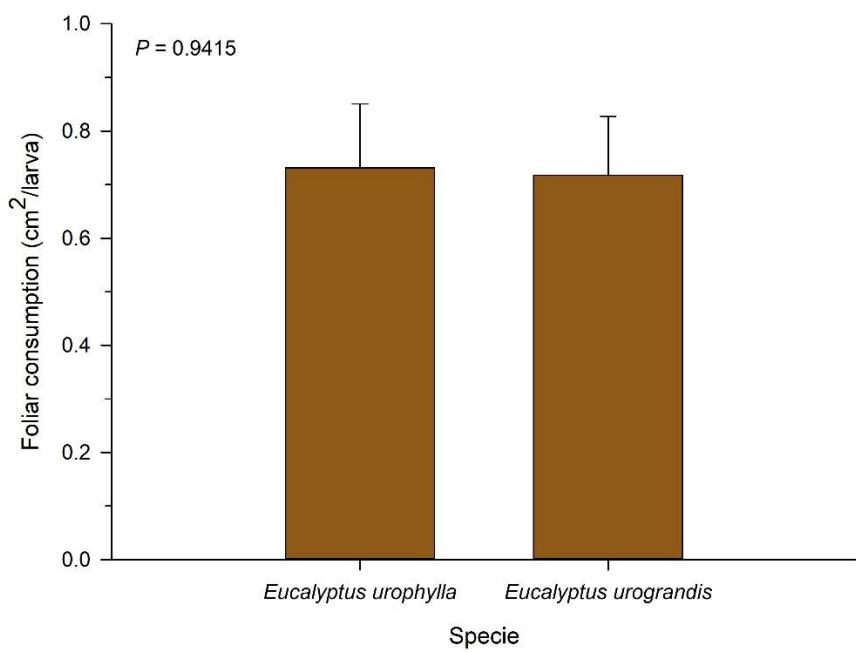
<sup>1</sup>Means followed by ~~the~~-different lowercase letters per column differ from each other by the LS-  
Means adjusted by Tukey's test (*P* ≤ 0.05).

**Table 3** – Mean  $\pm$  number ( $\pm$  SE) of weight, length, and width of male and female pupae of *Physocleora dukenfildea* from larvae fed with two forest species.

Species	Males		
	Weight (mg)	Length (mm)	Width (mm)
<i>Eucaliptus urograndis</i>	0.07 <del>43</del> $\pm$ 0.00	10.90 $\pm$ 0.25	3.32 $\pm$ 0.06
<i>Schinus terebinthifolia</i>	0.07 <del>22</del> $\pm$ 0.00	11.09 $\pm$ 0.13	3.37 $\pm$ 0.06
<i>P</i>	0.085 <del>27</del>	0.53 <del>04</del>	0.67 <del>24</del>
Females			
<i>Eucaliptus urograndis</i>	0.0747 $\pm$ 0.00	11.57 $\pm$ 0.12	3.44 $\pm$ 0.04
<i>Schinus terebinthifolia</i>	0.0863 $\pm$ 0.00	11.77 $\pm$ 0.15	3.56 $\pm$ 0.05
<i>P</i>	0.07 <del>655</del>	0.3 <del>7668</del>	0.18 <del>772</del>



**Figure 1** - Means ( $\pm$  SE) of larval and pupal viability of *Physocleora dukinfeldia* in forest species. Means followed by the same lowercase letter do not differ from each other by the LS-Means adjusted by Tukey's test ( $P \leq 0.05$ ).



**Figure 2** – Total leaf consumption ( $\pm$  SE) of *Physocleora dukinfeldia* larvae fed with leaves of forest species. Total leaf consumption considers the total consumed by the individual until its death or passage to the prepupal phase.