Phytoparasitica (2022) 50:13–20
https://doi.org/10.1007/s12600-021-00933-9

ORIGINAL ARTICLE

Survival and performance of *Sarsina violascens* (Lepidoptera:Lymantriidae) larvae on *Eucalyptus* species and hybrids

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Received: 29 April 2020 / Accepted: 21 May 2021 / Published online: 18 June 2021

Abstract The defoliating caterpillar *Sarsina violascens* causes economic losses in Brazilian *Eucalyptus* crops. Information on the susceptibility of *Eucalyptus* species and hybrids to insect pests is scarce. As such, this study aimed to assess the development of *S. violascens* on *Eucalyptus* species and hybrids under laboratory conditions. Neonate caterpillars were reared on leaves of *Eucalyptus grandis*, *E. urophylla*, *E. camaldulensis* and *E. globulus*; and hybrids of *E. urophylla* x *E.urophylla*, *E. urophylla*×*E. grandis*, *E. grandis*×*E. camaldulensis* and *E. urophylla*×*E. camaldulensis*. Biological parameters such as instar and larval phase duration, pupal weight, adult longevity and fecundity, and eclosion rate were evaluated. *Eucalyptus globulus* is not an *S. violascens* host, leading to 100% larval mortality in the first instar. *Eucalyptus grandis* extended the larval phase of the insect and decreased larval survivorship and female pupal weight, resulting in high levels of deformity in adults and low egg eclosion, in addition to exhibiting significant antibiosis to *S. violascens*. *Eucalyptus camaldulensis* and VM-1 extended the larval period and reduced pupal weight. *Eucalyptus urophylla* was better suited to the development and reproduction of *S. violascens* than other *Eucalyptus* species and hybrids. *E. grandis* is a promising source of resistance to *S. violascens*. The use of resistant species and hybrids contributes to pest management, reducing the need for chemical control.

Keywords Purple moth · Defoliating caterpillars · Host-plant resistance · Antibiosis · Forest pest

Introduction

Eucalyptus (Myrtaceae) is one of the most planted genera in the global forest sector, mainly because of its species' rapid growth and adaptability to different environmental conditions (Campinhos, 1999; Wingfield et al., 2008). Commercial forest crops in Brazil occupy 7.83 million ha, providing pulp, timber, charcoal, rubber, resin, and livestock shading. *Eucalyptus* species and hybrids are the country's most cultivated trees, accounting for 75% of Brazilian

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forest crops and producing about 36.0 m^3/ha/year in 2018 (IBGE, 2017; IBA, 2019).

Caterpillars such as *Sarsina violascens* (Herrich-Schaeffer, 1856) (Lymantriidae), *Thyrinteina arnobia* (Stoll, 1782) (Geometridae), *Eupseudosoma aberrans* (Schaus, 1905) (Arctiidae), *Eupseudosoma involute* (Sepp, 1852) (Arctiidae) and *Sabulodes caberata* (Guenée, 1857) (Geometridae) (among other species of the families Geometridae, Arctiidae and Lymantriidae) are important pests in *Eucalyptus* plantations due to their severe defoliating capacity (Kowalczuck et al., 2012; Zanuncio et al., 1992b, 2006, 2016). Commonly known as the purple moth, *Sarsina violascens* is a native species from Argentina, Brazil, and Mexico (FAO, 2009). It feeds on several Myrtaceae species present in Brazilian vegetation and, with the introduction of *Eucalyptus*, expanded from these native species to *Eucalyptus* (Paine et al., 2011; Zanuncio et al., 2013). Leaf feeding by insects reduces wood production (Batista-Pereira et al., 2006).

Sarsina violascens is commonly associated with *Eucalyptus* (Zanuncio et al., 2006, 2018; Bernardi et al., 2011; Kowalczuck et al., 2012, Garlet et al., 2016, Ribeiro et al., 2016). The caterpillar attacks several species of *Eucalyptus* (*Eucalyptus grandis*, *E. saligna*, *E. camaldulensis*, *E. urophylla*, *E. cloeziana* and *E. nesophylla*) and *Corymbia citriodora* (Berti Filho, 1983; Zanuncio, 1993; Zanuncio et al., 1992a). Depending on the species of the *Eucalyptus* host, a *S. violascens* larvae can consume a leaf area of approximately 249.74 cm^2 (Zanuncio et al., 1992a) during its complete development. Management of defoliating caterpillars in *Eucalyptus* crops is achieved mainly with insecticides (Zanuncio et al., 1993; Guedes et al., 2000) and the use of beneficial insects such as parasitoids (Oliveira et al., 2008; Pereira et al., 2008) and predators (Soares et al., 2009). Resistant *Eucalyptus* species and hybrids are another alternative to control phytophagous insects, including borers (Hanks et al., 1991), the bronze bug (Soliman et al., 2012), red gum lerp psyllid (Pereira et al., 2013), and caterpillars such as *T. arnobia* (Jesus et al., 2015).

Antibiosis involves both chemical and morphological plant defenses that adversely affect insect biology (Smith, 2005), influencing the survival, development, and reproduction of insects and their progeny. Effects on insects include high mortality of early instars, decreased size and/or weight, longer immature phase, and reduced adult longevity and fecundity (Padmaja, 2016; Smith, 2005; Smith & Clement, 2012). The use of plant genotypes that exhibit this resistance mechanism contributes to limiting the development and size of subsequent insect populations (Baldin et al., 2019). Despite the large number of studies in the literature that investigate *Eucalyptus* resistance or tolerance of different phytophagous insects, the use of species or hybrids potentially resistant to *S. violascens* has been poorly studied.

Planting resistant *Eucalyptus* species and/or hybrids may be an effective strategy to control insect pests, thereby lowering wood production costs. The present study aimed to evaluate several biological parameters of *S. violascens* on four species and five hybrids of *Eucalyptus* and identify the possible expression of antibiosis as a resistance mechanism.

Materials and methods

The *Sarsina violascens* caterpillars used in this study originated from a colony reared on the leaves of *Eucalyptus* hybrid 519 (*E. urophylla* × *E. grandis*). To establish the insect colony, *S. violascens* caterpillars were collected from clonal *Eucalyptus* crops in Alagoinhas, Bahia state, Brazil, and kept in covered plastic containers (4.5 cm in diameter and 6 cm high, with a volume of 80 mL) until the pupal phase. After emergence, the adults were placed in cylindrical PVC cages, 10 cm in diameter and 20 cm high, sealed at the top and bottom with Petri dishes and lined internally with silk paper. The eggs were removed and maintained in plastic containers until the caterpillars hatched.

A non-choice test was performed using neonate *S. violascens* caterpillars subjected to nine treatments: four *Eucalyptus* species (*E. grandis*, *E. urophylla*, *E. camaldulensis* and *E. globulus*) and five hybrids (*E. urophylla* x *E.urophylla* (117–20), *E. urophylla* × *E. grandis* (H-13 and 1404), *E. grandis* × *E. camaldulensis* (1277) and *E. urophylla* × *E. camaldulensis* (VM-1)). Each treatment was applied to thirty caterpillars. Each experimental unit consisted of a single caterpillar in a plastic container. Adult leaves were washed under running water, dried and then offered to the insects. The leaves were replaced with fresh leaves every two days. The plastic containers containing the leaves and caterpillars were stored in acclimatized rooms (12:12 L:D, 26±2 °C; 60±10% RH). A completely randomized design was used and each caterpillar considered one repetition (thirty repetitions per treatment, totalling 270 caterpillars).

Insect development was monitored daily. In the larval stage, larval survivorship (ratio of live/total individuals per treatment), instar duration (number of days the caterpillars remained in each instar), and number of instars (presence of exuviae in the containers) were assessed. At the end of this phase, the pupae were weighed (24 h after pupation), sexed and individually placed in plastic containers with the bottom lined with filter paper to maintain moisture.

Finally, recently emerged adults from each treatment were split in pairs (1 male and 1 female), to obtain eggs. Each pair was placed in one carton cage (10 cm in diameter and 20 cm high) sealed at the top and bottom with Petri dishes and lined with silk paper. A container with cotton soaked in a 10% aqueous honey solution was placed inside cages as food supply and replenished daily to prevent contamination or fermentation by microorganisms. Variables assessed in the adult stage were longevity, percentage of individuals with deformities (live individuals with wing curling or wings that did not fully expand), number of eggs per female and their eclosion rate.

The independent variables were tested for residual normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests, respectively. Data on pupal weight, adult longevity and fecundity, and eclosion rate met these assumptions. Next, one-way ANOVA was carried out, followed by Tukey's test for comparison of the means. Data that did not meet the assumptions of residual normality and homogeneity of variance, such as larval survivorship, instar and larval phase duration, and adult deformity, were compared using the Generalized Linear Model (GLM) with binomial distribution (proportion) or Poisson distribution (count). GLM tests were followed by pairwise comparisons. All statistical analyses were performed using R software (R Development Core Team, 2019).

Results

Feeding on different *Eucalyptus* treatments affected *S. violacens* development. In all treatments, larval mortality remained under 25%, irrespectively of the eucalyptus species, or of the hybrids used, except for *E. globulus*, which caused 100% mortality of first instar *S. violascens* caterpillars ($\chi2 = 132$; $df = 8$, $P < 0.01$) (Fig. 1). The duration of the first to third instar was shorter on *E. urophylla* and generally longest on *E. grandis* (except for the third instar, where the duration was slightly longer in two of the other treatments). These differences were significant when compared to some, but not all, treatments (First instar: $\chi2 = 44.79$; $df = 7$; $P < 0.01$, Second instar: $\chi2 = 19.42$; $df = 7$; $P < 0.01$, Third instar: $\chi2 = 18.00$; $df = 7$; $P < 0.01$) (Table 1). The fourth, fifth and sixth instars did not differ between the *Eucalyptus* material (fourth: $\chi2 = 8.06$; $df = 7$; $P = 0.33$, fifth: $\chi2 = 5.01$; $df = 7$; $P = 0.66$, six: $\chi2 = 12.30$; $df = 7$; $P = 0.09$) (Table 1). Only those reared on *E. grandis*, *E. camaldulensis,* and VM-1 leaves reached the seventh instar, with no significant difference in its duration between treatments ($\chi2 = 2.60$; $df = 2$; $P = 0.27$). The number of instars varied between five and eight, with only one caterpillar fed on *E. grandis* leaves reaching this instar. Insects that consumed *E. urophylla* leaves had the shortest mean larval period duration, followed by 1404, H-13, 117–20, and 1277. *Eucalyptus grandis*, *E. camaldulensis,* and hybrid VM-1 prolonged the larval phase of *S. violascens* ($\chi2 = 169.23$; $df = 7$; $P < 0.01$) (Table 1).

Regarding weight, male pupae weighed less when fed on VM-1, *E. camaldulensis,* and *E. grandis* and more when fed on hybrids 1404, 117–20, and *E. urophylla* leaves ($F = 20.51$; $df = 7$, 101; $P < 0.01$). A similar trend was observed for female pupae, which were lighter in the VM-1, *E. camaldulensis,* and *E. grandis* treatments and heavier for hybrid 1404 ($F = 30.64$; $df = 7$, 96; $P < 0.01$) (Table 2).

Adult deformity was significantly higher for insects fed on *E. grandis*, differing from all treatments, except to H-13; and followed by 1404, 117–20, and 1277 ($\chi2 = 35.25$; $df = 7$; $P < 0.01$) (Fig. 2). Deformed adults were not observed for *E. urophylla*, *E. camaldulensis,* and VM-1 treatments. The highest longevity was observed for *E. urophylla*, differing significantly from *E. camaldulensis*, *E. grandis,* 117–20, H-13 and VM-1 ($F = 3.59$; $df = 7$; $P < 0.01$) (Table 2).

Oviposition of *S. violascens* was significantly higher in treatments 1404, *E. urophylla,* and 1277 ($F = 2.35$; $df = 7$, 77; $P < 0.05$) than on treatments *E. grandis and* H-13 (Table 3). Successful egg eclosion

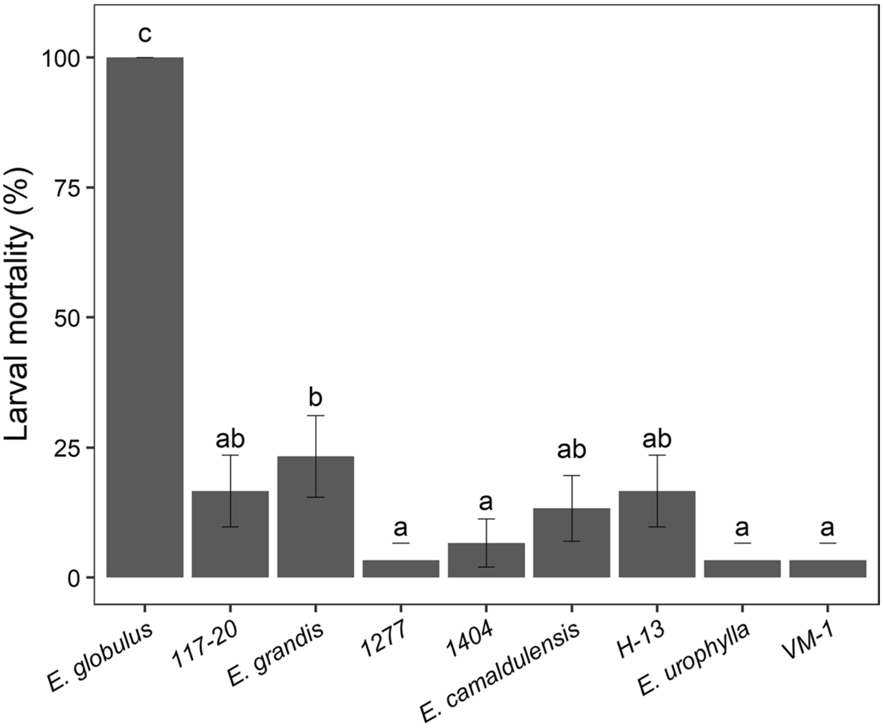

Fig. 1 Larval mortality of *Sarsina violascens* when reared on *Eucalyptus* species and hybrids (12:12 L:D; 26 ± 2 °C; 60 ± 10% RH). Parental hybrids: 117–20 (*E. urophylla*), 1277 (*E. grandis* × *E. camaldulensis*), VM-1 (*E. urophylla* × *E. camaldulensis*), H-13, and 1404 (*E. urophylla* × *E. grandis*). Bars followed by the same lowercase letter do not differ significantly (GLM with Binomial distribution ($P \geq 0.05$), followed by pairwise comparisons)

Table 1 Average instar duration (± SE) of *Sarsina violascens* fed on the leaves of *Eucalyptus* species and hybrids (12:12 L:D; 26 ± 2 °C; 60 ± 10% RH)

Treatment	Duration (days)*							Larval phase*
	1° (n)	2° (n)	3° (n)	4° (n)	5° (n)	6° (n)	7° (n)	
E. camaldulensis	8.2 ± 1.1 abcd (29)	6.5 ± 1.1 ab (28)	6.9 ± 1.4 a (27)	6.7 ± 1.1 (27)	7.4 ± 1.1 (27)	8.7 ± 1.1 (27)	11.6 ± 1.0 (14)	50.8 ± 5.3 a (26)
E. grandis	10.3 ± 1.1 a (29)	7.6 ± 1.1 a (25)	6.8 ± 1.1 ab (24)	7.3 ± 1.1 (24)	7.5 ± 1.1 (23)	9.7 ± 1.1 (21)	10.6 ± 1.4 (8)	53.0 ± 5.9 a (23)
E. urophylla	6.1 ± 1.1 d (30)	5.0 ± 1.1 b (29)	5.1 ± 0.7 b (29)	6.1 ± 1.1 (29)	7.8 ± 1.1 (29)	9.6 ± 1.1 (13)	-	34.5 ± 8.4 d (29)
H-13	7.9 ± 1.1 bcd (30)	6.2 ± 1.1 ab (26)	6.7 ± 1.3 ab (25)	6.1 ± 1.1 (25)	8.8 ± 1.1 (25)	9.6 ± 1.1 (10)	-	39.7 ± 3.6 bc (25)
VM-1	8.2 ± 1.1 abc (30)	6.2 ± 1.1 ab (30)	6.9 ± 1.5 a (30)	6.8 ± 1.1 (29)	8.3 ± 1.1 (29)	11.4 ± 1.1 (15)	11.2 ± 1.7 (4)	45.3 ± 8.4 ab (29)
117–20	9.4 ± 1.1 ab (30)	5.9 ± 1.1 ab (27)	5.6 ± 0.8 ab (26)	6.2 ± 1.1 (26)	8.4 ± 1.1 (25)	9.1 ± 1.1 (15)	-	40.7 ± 3.2 bc (25)
1277	7.9 ± 1.1 cd (30)	5.5 ± 1.1 b (30)	5.6 ± 0.9 ab (30)	5.6 ± 1.1 (30)	7.9 ± 1.1 (30)	10.9 ± 1.1 (20)	-	40.2 ± 4.7 c (29)
1404	6.8 ± 1.1 cd (30)	5.3 ± 1.1 b (28)	5.4 ± 0.9 ab (28)	6.4 ± 1.1 (28)	8.3 ± 1.1 (28)	10.8 ± 1.1 (13)	-	37.4 ± 5.8 cd (28)
CV (%)	20.3	26.6	18.3	17.6	18.5	19.2	11.1	13.1

Parental hybrids: 117–20 (*E. urophylla*), 1277 (*E. grandis* × *E. camaldulensis*), VM-1 (*E. urophylla* × *E. camaldulensis*), H-13, and 1404 (*E. urophylla* × *E. grandis*)

*Means followed by the same lowercase letter in the column do not differ significantly (GLM with Poisson distribution ($P \geq 0.05$), followed by pairwise comparisons)

Table 2 Mean pupal weight (±SE) and longevity of *Sarsina violascens* adults fed on the leaves of *Eucalyptus* species and hybrids (12:12 L:D; 26 ± 2 °C; 60 ± 10% RH)

Treatment	Pupal weight (g)*		Longevity (n)*
	Male (n)	Female (n)	
E. camaldulensis	0.267 ± 0.005 ab (12)	0.582 ± 0.019 a (14)	10.2 ± 0.6 a (26)
E. grandis	0.289 ± 0.006 abc (8)	0.590 ± 0.023 a (15)	10.6 ± 0.8 a (23)
E. urophylla	0.331 ± 0.007 de (17)	0.726 ± 0.023 bc (12)	13.2 ± 0.6 b (29)
H-13	0.305 ± 0.014 cd (11)	0.766 ± 0.016 c (14)	10.5 ± 0.8 a (25)
VM-1	0.263 ± 0.007 a (17)	0.652 ± 0.014 ab (12)	10.6 ± 0.5 a (27)
117–20	0.334 ± 0.008 de (15)	0.789 ± 0.033 c (10)	10.1 ± 0.6 a (25)
1277	0.305 ± 0.010 bcd (12)	0.702 ± 0.019 bc (17)	12.1 ± 0.5 ab (28)
1404	0.369 ± 0.008 e (17)	1.010 ± 0.042 d (11)	12.3 ± 0.6 ab (27)
CV (%)	10.00	11.93	28.8

Parental hybrids: 117–20 (*E. urophylla*), 1277 (*E. grandis* × *E. camaldulensis*), VM-1 (*E. urophylla* × *E. camaldulensis*), H-13, and 1404 (*E. urophylla* × *E. grandis*)

*Means followed by the same lowercase letter in the columns do not differ significantly (Tukey's test, $P \geq 0.05$)

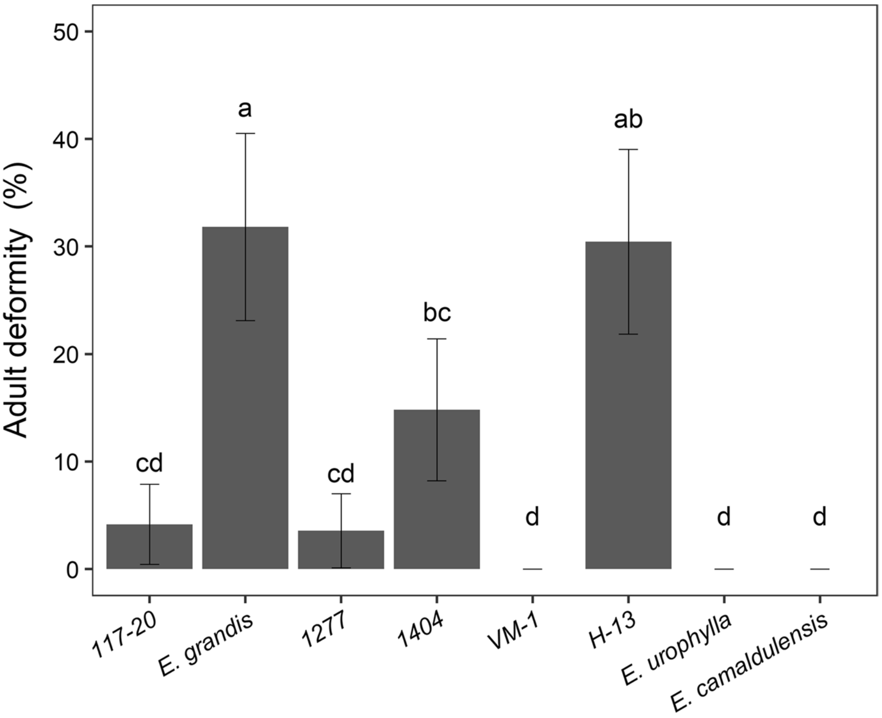

Fig. 2 Adult deformity of *Sarsina violascens* fed on *Eucalyptus* species and hybrids (12:12 L:D; 26 ± 2 °C; 60 ± 10% RH). Parental hybrids: 117–20 (*E. urophylla*), 1277 (*E. grandis* × *E. camaldulensis*), VM-1 (*E. urophylla* × *E. camaldulensis*), H-13, and 1404 (*E. urophylla* × *E. grandis*). Bars followed by the same lowercase letter do not differ significantly (GLM with Binomial distribution ($P \geq 0.05$), followed by pairwise comparisons)

was higher among females reared on VM-1 significantly different only from those fed on *E. grandis* leaves ($F = 2.25$; $df = 7, 77$; $P < 0.05$) (Table 3).

Discussion

Although *S. violascens* is commonly associated with *Eucalyptus* (Bernardi et al., 2011; Kowalczuck et al., 2012; Ribeiro et al., 2016), few studies have

Table 3 Mean number of eggs/female and eclosion rate (±SE) of *Sarsina violascens* fed on the leaves of *Eucalyptus* species and hybrids (12:12 L:D; 26±2 °C; 60±10% RH)

Treatment	Eggs/female (n)*	Eclosion rate (%)*
E. camaldulensis	136.0±16.9 ab (13)	44.6±8.7 ab
E. grandis	114.1±18.4 a (9)	17.0±10.7 a
E. urophylla	208.1±24.4 b (12)	55.5±6.9 ab
H-13	105.9±27.1 a (10)	27.9±10.2 ab
VM-1	173.1±32.9 ab (11)	61.4±9.8 b
117–20	149.8±29.2 ab (6)	36.6±16.3 ab
1277	195.2±27.6 b (13)	36.7±6.1 ab
1404	211.9±35.5 b (11)	37.6±10.1 ab
CV (%)	53.81	62.39

Parental hybrids: 117–20 (*E. urophylla*), 1277 (*E. grandis*×*E. camaldulensis*), VM-1 (*E. urophylla*×*E. camaldulensis*), H-13, and 1404 (*E. urophylla*×*E. grandis*)

*Means followed by the same lowercase letter in the columns do not differ significantly (Tukey's test, $P \leq 0.05$)

investigated how different species and hybrids can affect its development and survival. *Eucalyptus grandis* prolonged the larval phase, reduced the weight of female pupae, and produced more deformed adults and lower eclosion rate. *Eucalyptus camaldulensis* and VM-1 prolonged the larval cycle and reduced the pupal weight of males and females. These treatments were unfavorable to the development of *S. violascens*. By contrast, *E. urophylla* favor its development based on a shorter larval period, lower number of larval instars, high adult longevity and fecundity.

In general, the choice and establishment of phytophagous insects in eucalyptus is related to the presence of secondary compounds (Branco et al., 2019). Possibly such substances may be related to the unfavorable development of *S. violacens* in the species *E. grandis* and *E. camaldulensis*. Similarly, *E. camaldulensis* showed resistance to defoliation by coleopteran *Anoplognathus* spp. (Leach) (Edwards et al., 1993), but *E. grandis* was classified as highly susceptible to *T. arnobia*, another important *Eucalyptus* defoliator caterpillar (Jesus et al., 2015). *Eucalyptus urophylla* was best suited to *S. violascens* development, as observed for *Hylesia paulex*, a lepidopteran from the family Saturniidae (Pereira et al., 2009).

Our findings show that *E. globulus* is an unsuitable host for *S. violascens* larvae, while species such as *E. urophylla* favor its development mostly due to a shorter larval period and higher adult longevity. We observed 100% of *S. violascens* mortality for larvae fed on *E. globulus* leaves. For autumn gum moth larvae *Mnesampela privata* (Guenée, 1857), the high tannin levels of *E. globulus* leaves have been associated with reduced survival (Rapley et al., 2008). These authors suggested that tannins act as toxins and/or antifeedants for the larval phase of *M. privata*. Additionally, wax compounds are indicated as a possible source of *E. globulus* resistance to *M. privata* (Jones et al., 2002).

Caterpillars reared on *E. grandis*, *E. camaldulensis,* and VM-1 had more instars than immature individuals fed on other materials. The variation in the number of *S. violascens* instars demonstrates that some *Eucalyptus* species and hybrids do not favor the insect's development. Moreover, lengthening of the developmental phases of the insect or its cycle may indicate that the plant has an unfavorable effect on its biology, characterizing antibiosis-type resistance. However, this may also be related to the non-feeding preference, due to low levels of food intake by the insect (Baldin et al., 2019).

Caterpillars fed with *E. grandis* and *E. camaldulensis* leaves produced the lightest female pupae, and females reared on *E. grandis* and H-13 produced fewer eggs. In general, the weight and/or size of females is positively correlated with egg production (Haukioja & Neuvonen, 1985; Marshall, 1990; Tammaru, 1998). However, only for 1404 we observed higher weight of female pupae and higher fertility.

Eucalyptus urophylla and hybrids 1277 and 1404 produced females with the highest fecundity. These values are higher than that reported previously for *S. violascens,* namely approximately 150 eggs per female (Nascimento et al., 2000; Zanuncio et al., 1992b). Unexpectedly, eggs on *E. urophylla* exhibited a lower eclosion rate than that observed by Zanuncio et al. (1992b) (72.45%) on the same species. A lower eclosion rate was recorded for eggs laid by females reared on *E. grandis* leaves, less than half the 49.5% reported by Nascimento et al. (2000) on the same species.

Interestingly, hybrid 1277 (between *E. grandis* and *E. camaldulensis*) favored larval development, while its parental species had a negative effect on the larval stage of *S. violascens*. Hybrids seem to be more susceptible to pests than pure species. Therefore, intense selection to obtain elite hybrids is important (Potts & Dungey, 2004). Species *E. grandis* and *E. urophylla* and their hybrids (*E. grandis* x *E. urophylla*) comprise most *Eucalyptus* forest crops in Brazil (Assis et al., 2015). *Eucalyptus* hybrids are important in forest crops, particularly in subtropical and tropical regions, since clonal propagation is frequently used.

Plant resistance to insects is an alternative strategy to decrease pest populations and can be adopted in conjunction with other control techniques, contributing to reducing insect infestation in the field. The current trend is sustainable pest management, seeking to limit the use of chemical insecticides in forestry. The results obtained here may be useful in breeding programs focused on developing *Eucalyptus* resistant to *S. violascens*.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare that they have no conflicts or competing interest.

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