ORIGINAL ARTICLE

Development of the red gum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) in *Eucalyptus* spp.

Desenvolvimento do psilídeo-de-concha Glycaspis brimblecombei (Hemiptera: Aphalaridae) em Eucalyptus spp.

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Abstract

The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), is considered one of the most important pests of *Eucalyptus* worldwide. Since its detection in Brazil in 2003, the insect has caused defoliation in forests, twig dieback and sooty mold. Several types of management were evaluated, but few studies focused on the search for resistant *Eucalyptus* species and hybrid clones. The present study aimed to assess the biological performance of *G. brimblecombei* on *E. camaldulensis*, *E. urophylla*, and *E. grandis* and on the hybrids *E. grandis* x *E. camaldulensis* (1277 and 3025), *E. urophylla* x *E. camaldulensis* (VM-01), and *E. urophylla* x *E. grandis* (C-219, H-13, GG-100 and I-144), in order to determine resistance mechanisms. Under laboratory conditions (T= 26 ± 2°C; RH= 60 ± 10%; photoperiod= 12 h), psyllids on each of the ten *Eucalyptus* genotypes were evaluated daily until adult emergence. Genotypes C-219 and H-13 (*E. urophylla* x *E. grandis*) exhibited high levels of antibiosis and/or antixenosis (non-preference) resistance to *G. brimblecombei*, drastically reducing nymph viability (mortality > 80%). In turn, genotypes 3025 (*E. grandis* x *E. camaldulensis*) and *E. camaldulensis* were highly susceptible to the biological development of red gum lerp psyllids. Our results may contribute to genetic improvement programs aimed at obtain *G. brimblecombei* resistant *Eucalyptus* genotypes.

Keywords: Antibiosis; Antixenosis; Forest pest; Host plant resistance.

Resumo

O psilídeo-de-conha *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), é considerado uma das mais importantes pragas de eucalipto em todo o mundo. Desde a sua detecção no Brasil, em 2003, o inseto causa desfolha em florestas, secamento de ponteiros e possibilita o desenvolvimento de fumagina. Várias estratégias de controle foram avaliadas, mas poucos estudos têm-se centrado na busca de clones de espécies e híbridos de eucalipto resistentes. O presente estudo teve como objetivo avaliar o desempenho biológico do *G. brimblecombei* nas espécies *E. camaldulensis*, *E. urophylla*, *E. grandis* e híbridos *E. grandis* x *E. urophylla* (C-219, H-13, GG-100 e I-144), *E. uropylla* x *E. camaldulensis* (VM-01) e *E. grandis* x *E. camaldulensis* (1277 e 3025), a fim de determinar mecanismos de resistência. Sob condições de laboratório (T = 26 ± 2°C; UR = 60 ± 10%; fotoperíodo = 12 h), a biologia do psilídeos foi avaliada nos dez genótipos de eucalipto, diariamente até a emergência de adultos. Os genótipos C-219 e H-13 (*E. urophylla* x *E. grandis*) expressaram altos níveis de antibiose e/ou antixenose (não preferência) resistência a *G. brimblecombei*, reduzindo drasticamente a viabilidade ninfal (mortalidade > 80%). Os genótipos 3025 (*E. grandis* x *E. camaldulensis*) e *E. camaldulensis* foram considerados altamente suscetíveis, pois foram

favoráveis ao desenvolvimento biológico do psilideo-de-conha. Nossos resultados podem contribuir para programas de melhoramento genético visando obter genótipos de eucalipto resistentes a *G. brimblecombei*.

Palavras-chave: Antibiose; Antixenose; Praga florestal; Resistência de plantas.

INTRODUCTION

The red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), native to Australia, has spread rapidly to other regions. *G. brimblecombei* was first reported in Brazil in June 2003, in the state of São Paulo, and quickly spread to other states (Santana & Burckhardt, 2007). The insect has several generations a year, with populations dropping under unfavorable conditions (Laudonia et al., 2014). The infestation is easily confirmed by the presence of conical coverings (lerps) secreted by the nymphs (Cuello et al., 2018).

Both adults and nymphs cause damage by sucking the sap from the phloem of plants. Their attack can cause leaf deformation and small leaves as well as twig dieback, with severe infestations leading to plant death. Psyllids also produce large amounts of honeydew, favoring the occurrence of sooty mold and increasing the *Eucalyptus* susceptibility to other insects (Paine et al., 2006; Peris-Felipo et al., 2011). The productivity of eucalyptus plantations declined considerably after the establishment of this insect in Brazil (Wilcken et al., 2015). Defoliation of 20 to 30% was observed in the *E. grandis* x E. *urophylla* clone (Wilcken et al., 2003). For *E. camaldulensis*, tree mortality ranges from 20 to 95% (Wilcken et al., 2015).

Psyllids feed on a wide variety of *Eucalyptus* species, but some *G. brimblecombei* feed exclusively on *Eucalyptus,* section Exsertaria (Collett, 2000), especially *E. camaldulensis*, although this psyllid has been recorded in over twenty *Eucalyptus* species (Brennan et al., 2001).

Studies have been performed worldwide with a view to developing chemical, cultural and biological management strategies to control red gum lerp psyllid populations, but few assess the resistance of eucalyptus species to this insect (Brennan et al., 2001; Petro et al., 2017).

Host plant resistance is an important tool in integrated pest management (IPM) systems and reduces insect pest populations to below the economic injury level without causing an environmental imbalance, in addition to being compatible with other control methods (Henery, 2011; Smith & Clement, 2012). Resistant plants can negatively affect insect biology and behavior, altering plants' ability to withstand large pest populations without significant yield losses (Smith & Clement, 2012).

Brennan et al. (2001) studied the resistance of 21 *Eucalyptus* species to *G. brimblecombei* based on the presence of eggs, nymphs and defoliation. The species *E. cinerea*, *E. cladocalyx*, *C. ficifolia*, *E. globolus* (juvenile leaves), *E. paniculata*, *E. polyanthemos*, *E. pulverulenta*, *E. robusta*, *E. saligna* and *E. spathulata* were deemed resistant, but *E. camaldulensis* and *E. tereticornis* were rated highly susceptible. Leaf epicuticular wax appears to be involved in resistance, since the resistant species have glaucous leaves.

Firmino-Winckler et al. (2009) evaluated the biological development of red gum lerp psyllids on eucalyptus species (*E. camaldulensis*, *E. grandis*, *E. urophylla* and *C. citriodora*) and one *E. urophylla* x E. *grandis* hybrid. The low attractiveness and oviposition observed in *E. grandis* and *E. urophylla* suggests non-preference resistance (Pereira et al., 2013).

Considering the expansion of *Eucalyptus* cultivation in Brazil (Indústria Brasileira de Árvores, 2018) and the growing importance of *G. brimblecombei* in forests (Wilcken et al., 2015), it is important to investigate the resistance of *Eucalyptus* hybrids to the red gum lerp psyllid. This study assessed the development of *G. brimblecombei* on ten *Eucalyptus* genotypes to identify possible resistance mechanisms.

MATERIAL AND METHODS

Obtaining *Eucalyptus* spp. genotypes

The biological performance of *G. brimblecombei* on approximately 100-day-old seedlings of several *Eucalyptus* genotypes was monitored. This assay used the species and hybrids described in Table 1. The *Eucalyptus* genotypes were chosen based on information regarding their resistance (*E. grandis*) or susceptibility (*E. camaldulensis*), and the importance of the commercially planted area. Commercial hybrid clones and their associated species were also used.

Table 1. *Eucalyptus* genotypes used in tests for resistance to *Glycaspis brimblecombei* and their genealogies.

Genotypes	Provenance or Genealogy
E. camaldulensis IPEF (seeds)	Itatinga, SP, Brazil
E. grandis (seeds)	Lençóis Paulista, SP, Brazil
E. urophylla (seeds)	Flores, Indonesia
3025	*E. grandis* x *E. camaldulensis*
1277	*E. grandis* x *E. camaldulensis*
VM-1	*E. urophylla* x *E. camaldulensis*
H-13	*E. urophylla* x *E. grandis*
I-144	*E. urophylla* x *E. grandis*
C-219	*E. urophylla* x *E. grandis*
GG-100	*E. urophylla* x *E. grandis*

Biology assessment

The present study was performed under controlled conditions (T= 26 ± 2°C, RH= 60 ± 10% and photo-phase= 12 h). The seedlings were grown in 200 mL plastic pots containing sand. Eggs (leaf disk) collected in the field were placed in Petri dishes (100 × 20 mm) containing moistened filter paper and monitored daily until hatching. First instar nymphs were then transferred with a brush to the adaxial side of leaves on the middle third of seedlings of each *Eucalyptus* genotype tested.

Four seedlings were used per genotype, each containing on average of 70 nymphs. A completely randomized experimental design was used, with each seedling corresponding to a replicate. Assessments were carried out daily, always at the same time, using a stereomicroscope (40x magnification). Lerp size was measured at each instar, on the second day after nymph stage change of stage.

The nymphs of *G. brimblecombei* secrete a conical covering (lerp) composed of solidified honeydew (Sharma et al., 2013), which protects them until they reach adulthood (Sullivan et al., 2006). The lerp expands as nymphs grow and lerp size can therefore be easily used to characterize changes between instars (Stivanelli et al., 2009). The number of antennal segments has previously been used to determine differences between instars of *G. brimblecombei* (Firmino-Winckler et al., 2009). However, lerp size can be easily observed and allows the nymph development stage to be established without removing the lerp.

In the juvenile phase, the duration of stages, total duration of the juvenile phase, and total mortality were measured. Instar changes were determined based on differences in lerp size (Stivanelli et al., 2009), the presence of exuviae, and nymph morphological characteristics. To prevent emergent adults on different *Eucalyptus* genotypes from interacting, the infested leaves were covered with individual voile cages following the appearance of fifth instar nymphs.

After adult emergence, individuals were sexed by observing the terminal part of the abdomen and forceps, which are only present in males. Recently emerged red gum lerp psyllid

pairs from different *Eucalyptus* genotypes were then placed separately in cages, consisting of plastic Petri dishes (60 mm × 15 mm) attached to the leaves of the respective genotype seedlings, for egg laying and to measure longevity with food (seedlings). The number of eggs laid and their viability were determined. Other recently emerged adults were placed in test tubes (one individual per tube) containing cotton moistened with distilled water and sealed with parafilm evaluate longevity without food.

Statistical analysis

The data obtained were submitted to analysis of variance (ANOVA) using the F test treatment means compared using Tukey's test (P≤0.05). Nymph mortality and adult longevity data were transformed with arcsin $(x/100)^{1/2}$ and $(x+1)^{1/2}$, respectively, to meet ANOVA assumptions, but the original values are presented in Figures 1 and 2.

RESULTS AND DISCUSSION

Following nymph infestation of the adaxial side of *E. camaldulensis* leaves and hybrids of this species (3025, 1277 and VM-1), some individuals moved to the abaxial side, while others remained on the upper surface. However, in *E. grandis*, *E. urophylla* and *E. urophylla* x *E. grandis* (C-219, H-13, I-144 and GG-100), most individuals migrated to the abaxial side.

Nymph migration to the abaxial surface can be explained by their oviposition behavior. *G. brimblecombei* females prefer to lay their eggs on the abaxial surface of eucalyptus leaves (Silva et al., 2010, Pereira et al., 2013; Tuller et al., 2017).

Five *G. brimblecombei* nymph instars were observed in all the genotypes tested. The mean lerp diameter was 0.95 ± 0.01 mm for first instar nymphs, and 1.11 ± 0.02, 1.57 ± 0.04, 2.30 ± 0.03, and 2.69 ± 0.05 mm for second, third, fourth and fifth instars, respectively.

In general, the first and fifth instars lasted longer than the remaining instars (Table 2). The first instar was significantly shorter for genotypes 3025 (*E. grandis* x *E. camaldulensis*) and *E. camaldulensis* and longer for C-219, while the second instar was longer for C-219, I-144, H-13 (*E. urophylla* x *E. grandis*) and *E. urophylla*. The second instar was shorter for *E. camaldulensis*, 1277 and 3025 (*E. grandis* x *E. camaldulensis*). The third instar was longer for *E. urophylla* and shorter for 3025 (*E. grandis* x *E. camaldulensis*). No significant differences were observed between fourth and fifth instar duration.

Table 2. Duration in days (mean ± SE) of the instars and nymph periods of *Glycaspis brimblecombei* in *Eucalyptus* genotypes (12:12 L:D, 26 ± 2 °C, 60 ± 10% RH).

Genotype[1]	1st instar[2]	2nd instar[2]	3rd instar[2]	4th instar[2]	5th instar[2]	Nymphal period[2]
C-219	4.19 ± 0.32 a	2.72 ± 0.20 a	2.74 ± 0.10 ab	2.83 ± 0.09 a	3.96 ± 0.29 a	15.20 ± 0.65 a
E. grandis	3.99 ± 0.31 ab	2.36 ± 0.07 ab	2.55 ± 0.17 ab	2.46 ± 0.17 a	3.24 ± 0.23 a	13.89 ± 0.16 ab
I-144	3.90 ± 0.30 ab	2.76 ± 0.06 a	2.54 ± 0.08 ab	2.43 ± 0.15 a	3.61 ± 0.15 a	14.34 ± 0.17 ab
E. urophylla	3.55 ± 0.15 ab	2.71 ± 0.19 a	2.78 ± 0.19 a	2.70 ± 0.11 a	3.12 ± 0.11 a	14.22 ± 0.54 ab
H-13	3.67 ± 0.29 ab	2.73 ± 0.14 a	2.72 ± 0.31 ab	2.73 ± 0.30 a	3.75 ± 0.43 a	14.06 ± 0.46 ab
VM-1	3.41 ± 0.15 ab	2.32 ± 0.06 ab	2.36 ± 0.11 ab	2.71 ± 0.21 a	3.46 ± 0.71 a	13.86 ± 0.26 ab
GG-100	3.34 ± 0.13 ab	2.39 ± 0.07 ab	2.74 ± 0.09 ab	2.73 ± 0.09 a	3.57 ± 0.09 a	14.37 ± 0.26 ab
1277	3.30 ± 0.07 ab	2.18 ± 0.02 b	2.49 ± 0.08 ab	2.71 ± 0.09 a	3.71 ± 0.19 a	13.90 ± 0.26 ab
E. camaldulensis	3.06 ± 0.03 b	2.06 ± 0.02 b	2.17 ± 0.02 ab	2.37 ± 0.03 a	3.89 ± 0.11 a	13.41 ± 0.11 b
3025	3.05 ± 0.03 b	2.08 ± 0.02 b	2.06 ± 0.02 b	2.51 ± 0.07 a	3.54 ± 0.08 a	13.16 ± 0.14 b
$F_{9, 30}$	3.37**	6.76**	3.06*	1.07ns	1.61ns	2.59*

[1]C-219, I-144, H-13 and GG-100 (*E. urophylla* x *E. grandis*); 1277 and 3025 (*E. grandis* x *E. camaldulensis*); VM-1 (*E. urophylla* x *E. camaldulensis*). [2]Original data. Means followed by the same letter in the column do not differ significantly according to Tukey's test ($P \geq 0.05$). nsNon-significant.

The nymph stage was shorter for nymphs grown on *Eucalyptus* genotype 3025 (*E. grandis* x *E. camaldulensis*) *and E. camaldulensis*, and longer for C-219 (*E. urophylla* x *E. grandis*). Considering the entire nymph stage (Figure 1), genotypes *E. camaldulensis* and 3025 (*E. grandis* x *E. camaldulensis*) exhibited significantly lower mortality and H-13 (*E. urophylla* x *E. grandis*) provided greater nymph mortality (96.31%).
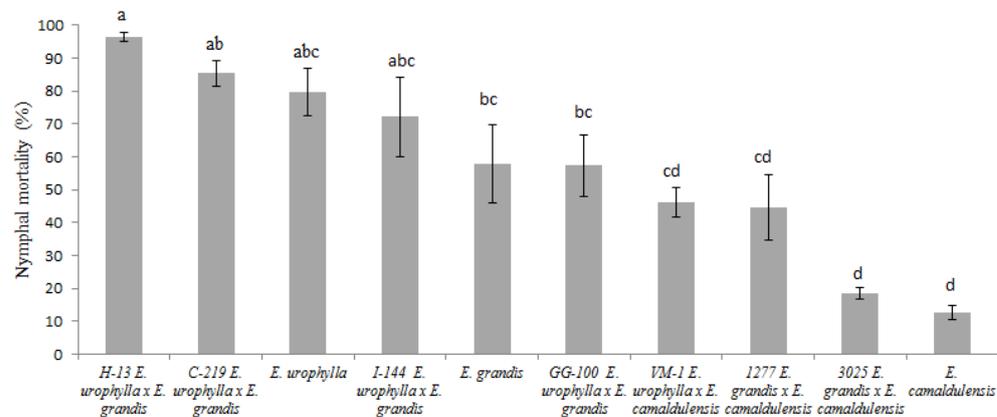
Figure 1. Nymph mortality (%) of *Glycaspis brimblecombei* in *Eucalyptus* genotypes (12:12 L:D, 26 ± 2 °C, 60 ± 10% RH). Means followed by the same letter in the column do not differ significantly according to Tukey's test ($P \geq 0.05$). Original data. Data were transformed to arc sin (x/100)^{½} for analysis. ($F_{9, 30}$ = 15.10, $P < 0.01$). C-219, I-144, H-13 and GG-100 (*E. urophylla* x *E. grandis*); 1277 and 3025 (*E. grandis* x *E. camaldulensis*); VM-1 (*E. urophylla* x *E. camaldulensis*).

Nymphs fed with *E. camaldulensis* and 3025 (*E. grandis* x *E. camaldulensis*) exhibited shorter nymphal stages, indicating that these genotypes were more suitable for their development, corroborating the findings of Firmino-Winckler et al. (2009), Ribeiro et al. (2015), and Cuello et al. (2018). The longer nymph stage observed for C-219 (*E. urophylla* x *E. grandis*) indicates the occurrence of antibiosis and/or antixenosis.

In addition, the high mortality rates (Figure 1) in the nymph stage of all the C-219, H-13, I-144 (*E. urophylla* x *E. grandis*) and *E. urophylla* individuals demonstrate a high level of antibiosis and/or antixenosis for these genotypes. *E. grandis* and *E. urophylla* display non-preference as a resistance mechanism to *G. brimblecombei*, due to low attractiveness and oviposition (Pereira et al., 2013).

Longer insect development stages or life cycles indicate that the plant has a negative effect on insect biology, characteristic of antibiosis resistance. However, extended developmental stages may also be related to antixenosis, due to lower food intake by insects (Smith & Clement, 2012). These resistance mechanisms (antibiosis or antixenosis) could not be differentiated because the volume of insect droppings was not quantified.

E. urophylla and *E. grandis* and some of their hybrids showed high nymphal mortality, which could explain why Petro et al. (2017) found no *G. brimblecombei* on *E. grandis*. In *E. dunni*, Cuello et al. (2018) observed high *G. brimblecombei* mortality in the first and second nymph instars. Insect mortality may increase with poor diet; as such, low concentrations of certain nutrients may be plant defense strategies to which insects respond by ingesting more food over longer time periods (Haukioja, 1990).

Some host plant characteristics may be associated with differences in nymph development and oviposition. In addition, *Eucalyptus* leaves also display high levels of secondary compounds such as tannins, phenols and essential oils, which can be considered defenses against phytophagous insects (Ohmart & Edwards, 1991). However, *G. brimblecombei* adults avoid the oil glands, directing their stylets around them (Brennan & Weinbaum, 2001a). According to Ribeiro et al. (2015), the high mortality of *G. brimblecombei* nymphs in eucalyptus genotypes may be related to greater leaf lignin concentrations (*C. citriodora*), high total phenol

content and the presence of epicuticular wax. In this context, Lucia et al. (2016) recorded elevated 1,8-cineole levels in eucalyptus that exhibited a low *G. brimblecombei* abundance, whereas terpenes α-and ß- phellandrene were the main components in eucalyptus with a high abundance of psillyds.
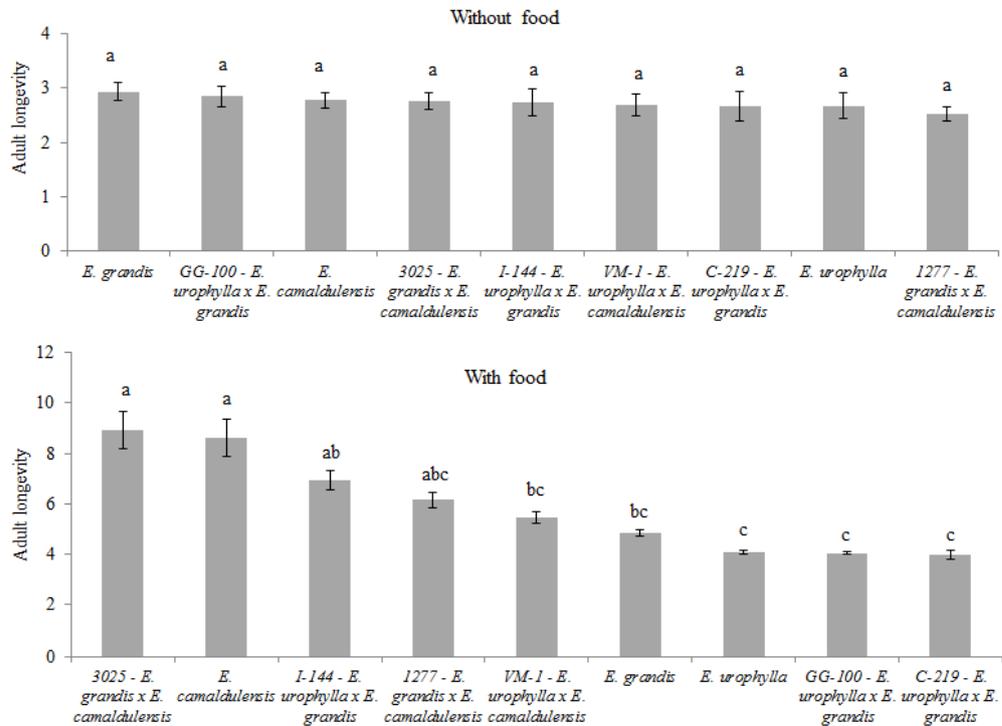
Figure 2. Longevity in days (mean ± SE) of adult *Glycaspis brimblecombei* fed or not with *Eucalyptus* genotypes (12:12 L:D, 26 ± 2 °C, 60 ± 10% RH). Means followed by the same letter in the column do not differ significantly according to Tukey's test ($P \geq 0.05$). *Original data. Data were transformed $(x + 1)^{1/2}$ for analysis. Without food ($F_{9, 28} = 0.62$, $P = 0.77$) and with food ($F_{8, 25} = 8.20$, $P < 0.01$). C-219, I-144 and GG-100 (*E. urophylla* x *E. grandis*); 1277 and 3025 (*E. grandis* x *E. camaldulensis*); VM-1 (*E. urophylla* x *E. camaldulensis*). Genotype H-13 (*E. urophylla* x *E. grandis*) was excluded from this assessment due to its high nymphal mortality.

The resistance of *Eucalyptus* genotypes may be linked to the presence of leaf wax, which can improve or prevent insect oviposition, movement and feeding in addition to affecting locomotion and fixation to the surface (Eigenbrode & Espelie, 1995). Epicuticular wax has been found to negatively affect the survival and feeding behavior of *G. brimblecombei* on the young leaves of *E. globulus* (Brennan & Weinbaum, 2001b). The larger amounts of epicuticular wax on the leaves of *E. globulus* resulted in lower adhesion of *G. brimblecombei* to the leaf surface (Brennan & Weinbaum, 2001c). This wax may be a possible cause of the resistance observed in this study, since its quantity and structure varies among different *Eucalyptus* species (Wirthensohn & Sedgley, 1996), which may also be related to the migration of nymphs observed in *E. grandis* and *E. urophylla* and their hybrids.

The *Eucalyptus* genotype on which the psyllids developed did not influence adult longevity in the treatment without food and survived for approximately 3 days (Figure 2). However, adult longevity with food differed among *Eucalyptus* genotypes. Suitable genotypes for insects (*E. camaldulensis* and 3025) enable greater longevity. The genotypes GG-100, C-219 (*E. urophylla* x *E. grandis*) and *E. urophylla* stood out in terms of reducing the longevity of adult psyllids.

Only a few 3025, 1277 (E. *grandis* x E. *camaldulensis*), *E. camaldulensis* and I-144 (*E. urophylla* x *E. grandis*) females oviposited (Table 3). The number of eggs was higher for clone

3025 and *E. camaldulensis*. This result was expected because these genotypes show a high oviposition preference (Pereira et al., 2013).

Table 3. Number of eggs per female (means ± SE) and egg viability (means ± SE) of *G. brimblecombei* on *Eucalyptus* genotypes (12:12 L:D, 26 ± 2 °C, 60 ± 10% RH).

Genotypes[1]	Eggs/female[2]	Viability (%)[2]	Number of females
E. camaldulensis	36.83 ± 22.77	85.60 ± 5.72	6
3025	31.27 ± 6.05	81.24 ± 7.82	11
I-144	15.17 ± 4.77	59.72 ± 13.84	6
1277	15.00 ± 2.57	81.25 ± 9.65	8
$F_{3, 27}$	1.15[ns]	1.23[ns]	-

[1]I-144 (*E. urophylla* x *E. grandis*); 1277 and 3025 (*E. grandis* x *E. camaldulensis*). [2]Original data. Means followed by the same letter in the column are do not differ significantly according to Tukey's test ($P \geq 0.05$). [ns]Non-significant

In addition, *E. camaldulensis* Section Exsertaria species promotes the biological development of *G. brimblecombei* nymphs and adults, whereas *E. urophylla* and *E. grandis* did not favor insect development and belong to Section Transversaria. This result was observed in the *E. urophylla* and *E. grandis* hybrids, evident in the lower viability of eggs on I-144 (*E. urophylla* x *E. grandis*) and the absence of oviposition in C-219 and GG-100 (*E. urophylla* x *E. grandis*). The genotypes *E. urophylla* x *E. grandis* (C-219 and H-13) showed greater resistance to *G. brimblecombei*, indicating the occurrence of antibiosis and/or antixenosis.

CONCLUSION

The species *E. urophylla* and *E. grandis* and their hybrids (C-219, I-144, GG-100, and H-13) are unfavorable to the biological development of *Glycaspis brimblecombei,* indicating the occurrence of antibiosis and/or antixenosis resistance. *E. camaldulensis* and 3025 (*E. grandis* x *E. camaldulensis*) are susceptible, favoring the development and reproduction of *G. brimblecombei*; demonstrating the need for frequent monitoring in the field.

ACKNOWLEDGMENTS

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