A peer-reviewed version of this preprint was published in PeerJ on 24 October 2017.

View the peer-reviewed version (peerj.com/articles/3864), which is the preferred citable publication unless you specifically need to cite this preprint.

Tuller J, Oliveira KN, Silva JO, de Faria ML, do Espírito-Santo MM, Serrão JE, Zanuncio JC. 2017. *Glycaspis brimblecombei* (Hemiptera: Psyllidae) attack patterns on different *Eucalyptus* genotypes. PeerJ 5:e3864
https://doi.org/10.7717/peerj.3864
**Glycaspis brimblecombei** (Hemiptera: Psyllidae) attack patterns on different *Eucalyptus* genotypes

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**Background.** The red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), an eucalypt insect pest from Australia, was reported in Brazil in 2003. This study evaluated damage patterns of this pest and the parasitism rates of *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) on *E. camaldulensis* and its hybrids *E. urophylla* X *E. camaldulensis* and *E. urophylla* X *E. grandis*. **Methods.** Plantation plots of three eucalypt genotypes were evaluated over one year. The eucalypt leaves were collected and examined. **Results.** Damage by *G. brimblecombei* was lower on the hybrid genotypes and on the adaxial surface of the eucalypt leaves. *Glycaspis brimblecombei* egg and nymph density correlated negatively with monthly rainfall. Nymph parasitism by *P. bliteus* was low (2.9%) independent of genotype and did not vary throughout the year. **Discussion.** The use of less susceptible eucalypt genotypes (e.g., hybrids) seems to be an alternative to *G. brimblecombei* management because mortality rates for this pest resulting from *P. bliteus* parasitism were low.
Running-title: Attack patterns of a psyllid herbivore on different eucalypt genotypes

*Glycaspis brimblecombei* (Hemiptera: Psyllidae) attack patterns on different *Eucalyptus* genotypes

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ABSTRACT

Background. The red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), an eucalypt insect pest from Australia, was reported in Brazil in 2003. This study evaluated damage patterns of this pest and the parasitism rates of *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) on *E. camaldulensis* and its hybrids *E. urophylla* × *E. camaldulensis* and *E. urophylla* × *E. grandis*.

Methods. Plantation plots of three eucalypt genotypes were evaluated over one year. The eucalypt leaves were collected and examined.

Results. Damage by *G. brimblecombei* was lower on the hybrid genotypes and on the adaxial surface of the eucalypt leaves. *Glycaspis brimblecombei* egg and nymph density correlated negatively with monthly rainfall. Nymph parasitism by *P. bliteus* was low (2.9%) independent of genotype and did not vary throughout the year.

Discussion. The use of less susceptible eucalypt genotypes (e.g., hybrids) seems to be an alternative to *G. brimblecombei* management because mortality rates for this pest resulting from *P. bliteus* parasitism were low.

Keywords Hybrid eucalypt, Biological control, *Psyllaephagus bliteus*, Red gum lerp psyllid, Water stress

INTRODUCTION

Introduced insect species are the major cause of native biodiversity loss (Gaertner et al., 2009; Hejda, Pysek & Jarosík, 2009). These organisms compete for nutrients, water, and light, change soil nutrient cycling, affect community structure (Levine et al., 2003), and reduce pollinator numbers (Totland et al., 2006). In addition, introduced phytophagous insects may
become pests (Queiroz-Santana & Burckhardt, 2007).

The Australian genus *Eucalyptus* is one of the most cultivated around the world. In Brazil, this genus was introduced in the second half of the 19th century (Couto & Betters, 1995), and its plantation covers more than 4.8 million ha (ABRAF, 2013). This genus comprises more than 600 species, besides hybrids, with more than 30 cultivated in Brazil (Queiroz, Burckhardt & Majer, 2012) for cellulose, charcoal, essential oils, furniture, planks, and paper production. *Eucalyptus* has a remarkable native phytophagous fauna in Brazil (Guedes et al., 2000), which can be explained by its phylogenetic proximity to the Brazilian flora, with high Myrtaceae diversity (Zanuncio et al., 2001).

Exotic phytophagous insects causing economic losses in Brazilian eucalypt plantations (Pereira et al., 2001) include the red gum lerp psyllid *C. eucalypti* (Hemiptera: Psyllidae), *Goniapterus gibberus, G. scutellatus* and *Phoracantha semipunctata* (Coleoptera: Cerambycidae) (Ribeiro et al., 2001, Queiroz-Santana DL, Burckhardt D. 2007), *Leptocybe invasa* (Hymenoptera: Eulophidae) (Fernandes et al., 2014), and the Australian red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) (Ferreira Filho et al., 2014). *Glycaspis brimblecombei*, the major problem in eucalyptus plantations in Brazil, first recorded in São Paulo State in 2003 (Wilcken et al., 2003) and has spread throughout the country (Pereira et al., 2013; Queiroz et al., 2013). As *G. brimblecombei* can cause 20% to 30% defoliation, crown thinning, and tree mortality (Wilcken et al., 2003, Queiroz et al., 2013), there is an urgent need to develop strategies aiming to manage this pest in Brazil.

*Glycaspis brimblecombei* shows sexual reproduction depositing 6-45 eggs per eucalyptus leaf, preferentially on young leaves (Firmino-Winckler et al., 2009). Psyllid nymphs eliminate honeydew after initial feeding and use it with a wax secretion to build tapered protective white
shelters (lerp) on the leaf surface (Sharma et al., 2013). *Glycaspis brimblecombei* has nymph instars, with a complete lifecycle from 15 to 34 days and several generations per year (Firmino-Winckler et al., 2009). In its original habitat in Australia, the red gum lerp psyllid prefers feeding on *Eucalyptus camaldulensis* (Moore, 1975). In Australia, many psyllid species co-occur with natural enemies controlling most of them in native eucalypt areas (Collett, 2001).

*Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) parasitizes *G. brimblecombei* in Australia. This koinobiont parasitoid prefers to oviposit on the third-instar *G. brimblecombei* nymphs, but its offspring development is delayed until the psyllid reaches the fifth instar (Daane et al., 2005). The exoskeleton of the dead parasitized hosts forms an easily recognized mummy, by which time, the psyllid nymph is completely consumed and the parasitoid has nearly finished its development. The white *P. bliteus* larva can be seen throughout the mummified exoskeleton of *G. brimblecombei*, which becomes transparent (Sullivan et al., 2006). *Psyllaephagus bliteus* was accidentally introduced into Brazil and first reported in 2003 (Berti-Filho et al., 2003). This parasitoid is specific to *G. brimblecombei*, but it has a low parasitism rate (Ferreira-Filho et al., 2015). However, in 2003, it was the first time a mass *P. bliteus* rearing program was used for biological control to help manage this psyllid in California, United States (Dahlsten et al., 2005). In Brazil, *P. bliteus* parasitism may reach up to 80% with mass releasing programs (Ferreira-Filho et al., 2015).

Integrated pest management (IPM) combines different control methods, such as physical, chemical (Zanetti et al., 2003), cultural, and biological methods (Grosman et al., 2005), to reduce pest damage (Kogan, 1998). However, IPM efficiency depends on understanding the pest lifecycle, susceptibility to environmental conditions, female preference and offspring performance on host plants and habitats, population dynamics, and mortality from natural
enemies (VanLenteren et al., 2003; Lockwood et al., 2004; Pereira et al., 2013).

Chemical control has low efficiency against *G. brimblecombei* (Queiroz-Santana & Burckardt, 2007), but entomopathogenic fungi (DalPogetto et al., 2011), predators (Dias et al., 2012, 2014), and its main natural enemy from Australia, the parasitoid *P. bliteus* (Daane et al., 2005; Daane, Sime & Paine, 2012), have been studied to manage this insect. However, the efficiency of *P. bliteus* to control *G. brimblecombei* in eucalypt plantations in different Brazilian regions and climates needs to be further studied.

Resistant genotypes can be an alternative to pesticides for insect management. Hybrid eucalypt plants were developed for greater commercial value (such as wood density for furniture and construction and lignin content for charcoal) or increasing resistance to stress by water and pests (Gonçalves et al., 2013). The susceptibility of *Eucalyptus* genotypes to insect species varies (Firmino-Winckler et al., 2009; Queiroz, Burckhardt & Majer, 2012) and plant mechanisms responsible for reducing damages by these organisms must be further investigated. *Glycaspis brimblecombei* populations are larger during the dry months, but diminish during the rainy season (Oliveira et al., 2012).

The present study aimed to evaluate the damage patterns of *G. brimblecombei* and the parasitism by the wasp *P. bliteus* on *Eucalyptus camaldulensis* and its hybrids *E. urophylla* X *E. camaldulensis* (urocam) and *E. urophylla* X *E. grandis* (urograndis). The questions addressed were: i) Does the *G. brimblecombei* egg and nymph density vary according to the host plant, leaf surface, and month? ii) Does the *P. bliteus* parasitism rate on *G. brimblecombei* nymphs vary according to these factors? iii) Is the temporal variation of *G. brimblecombei* related to the rainfall cycle?
MATERIALS AND METHODS

Study area

The study was conducted at Extrema farm (17º15' S  43º39' W) of Vallourec & Mannesman Florestal S.A. in Olhos D'Água, Minas Gerais State, Brazil. This farm is located at an altitude of 800 m and has an area of 9,655.61 ha, 6,597.72 ha (68%) with an eucalypt plantation, and 1884.22 ha (32%) of native vegetation remnants (e.g., Cerrado fragments). This region has a tropical climate, with a dry winter (Aw in Köppen's classification) from June to September, and a rainy season from November to March. The mean annual temperature is 24ºC and the total annual rainfall is 1,246 mm, according to data collected at a weather station at the Extrema farm. The selected plots had 6 year old trees of the river red gum *Eucalyptus camaldulensis* Dehn, and two hybrids: *Eucalyptus urophylla* x *Eucalyptus camaldulensis* (urocam) and *Eucalyptus urophylla* x *Eucalyptus grandis* (urograndis).

Sampling

Sampling was conducted in two plantations per eucalypt plant genotype (*E. camaldulensis*, urocam and urograndis). Twenty eucalyptus trees were randomly selected each month, from December 2006 to November 2007, and 10 leaves collected every month from each tree selected were individually caged and taken to the laboratory. The number of *G. brimblecombei* eggs and nymphs, and nymph development were recorded for both the upper and lower leaf surfaces and the percentage of *P. bliteus* parasitism determined based on the ratio between parasitized and total nymphs (3-5th instar). The viability of *G. brimblecombei* eggs was determined using the percentage of nymph hatched. Each leaf was scanned with the Image J software (Rasband, 2006) to determine its area. *Glycaspis brimblecombei* eggs and nymph density per cm² of the leaf surface and per tree were calculated.
Statistical Analyses

A linear mixed effect models (LME) was constructed with the influence of host plant (Eucalyptus genotypes and host leaf surfaces), and time (month) for G. brimblecombei egg and nymph density and percentages and the first ones parasitized by P. bliteus (response variables) during one year. The models were tested against null models and followed by residual inspection of the error distribution (McCullagh & Nelder, 1989). The LME models (one per response variable) were used owing to the random effects (Crawley, 2007), such as nested structure into different host plants/leaf surface. Time was only included as an explanatory variable in the models for E. camaldulensis owing to the low G. brimblecombei density on the two hybrid genotypes. Generalized linear models (GLM; one per response variable) were employed to test the influence of average monthly rainfall on G. brimblecombei egg and nymph density and the percentage of nymphs parasitized by P. bliteus.

The non-significant variables were progressively (one-by-one) removed with the backward method, starting from the complete models containing all the explanatory ones and their interactions, until the minimal adequate models were obtained (Crawley, 2007). After this procedure, the differences between the levels of all categorical variables were tested with contrast analyses. All the models were also tested for adequacy through residual analysis. The analyses were conducted with the software R version 2.14 (R Development Core Team, 2011).

RESULTS

A total of 481,212 eggs and 42,785 nymphs of G. brimblecombei was found on 14,388 eucalypt leaves. Eucalyptus camaldulensis had the higher G. brimblecombei egg and nymph densities, followed by Eucalyptus “urograndis” and “urocam” (Table 1; Figure 1).
Glycaspis brimblecombei egg viability was 4%, 2% and 1% on E. camaldulensis, urocam and urograndis, respectively. The G. brimblecombei egg density was higher on the abaxial surface of E. camaldulensis and urograndis and no differences were found for urocam leaves (Table 1; Figure 1).

Nymphs of G. brimblecombei parasitized by P. bliteus were 976, two and one on E. camaldulensis, urocam and urograndis, respectively. The availability of 3-5th instar nymphs was low and parasitism reached 2.28%, 4.44% and 2.08% on E. camaldulensis, urocam and urograndis, respectively. The percentage of parasitized eggs was similar between the Eucalyptus genotypes (Table 1).

Glycaspis brimblecombei attacks on E. camaldulensis varied throughout the year, which egg and nymph densities of this insect higher during the dry season (May to October) and lower in the rainy season (December to March) (Figure 2). Glycaspis brimblecombei egg and nymph density were negatively correlated to monthly rainfall (Table 1; Figure 3).

**DISCUSSION**

Eucalyptus genotypes affected G. brimblecombei egg (female preference) and nymph densities, particularly, compared to E. camaldulensis and hybrids. On a small scale (i.e., leaf surface), there were spatial differences in the oviposition preference between E. camaldulensis and urograndis. The marked influence of climatic factors on G. brimblecombei density and the low nymph mortality resulting from P. bliteus parasitism have potential consequences on the IPM of this psyllid. Higher E. camaldulensis susceptibility to the red gum lerp psyllid corroborates the field and laboratory studies for this pest in Brazil (Wilcken et al., 2003; Pereira et al., 2013) and other parts of the world (Wilcken et al., 2003; Valente & Hodkinson, 2009). The
oviposition preference of *G. brimblecombei* females for *E. camaldulensis* confirms its higher egg viability and nymph survival on this eucalypt species (Firmino-Winckler et al., 2009). Nevertheless, the mechanisms responsible for such differential preference and performance are still poorly studied for *E. camaldulensis* and hybrid lineages. It may be due to the coexistence of this host and *G. brimblecombei* in Australia (Phillips, 1992) favoring the successful attack on the natural genotype, showing that this insect probably evolved the capacity to deal with chemical and physical characteristics of *E. camaldulensis* leaves. This also indicates the necessity of studying leaf morphology and physiology to identify the resistance mechanism of hybrid *Eucalyptus* genotypes for *G. brimblecombei*. Interspecific and phenotypic differences related to leaf traits (e.g. texture, roughness, and trichome density) occur between *Eucalyptus* genotypes (Reifenrath, Riederer & Müller, 2005) and may play a role in psyllid preference and performance, especially affecting the first instar nymph adhesion to the leaf surface. Higher lerp numbers and lower *G. brimblecombei* nymph and adult mortality on *Eucalyptus globulus* leaves were found when epicuticular wax was removed (Brennan & Weinbaum, 2001a). The level of epicuticular wax varies among *Eucalyptus* genotypes and is important for the adhesion of psyllid nymphs (Brennan & Weinbaum, 2001a) and stylet probing (Brennan & Weinbaum, 2001b) on waxy resistant eucalypt leaves.

A similar *G. brimblecombei* nymph density and parasitism rate between the leaf surfaces of all *Eucalyptus* genotypes indicates that some nymphs may migrate to the adaxial surface to reduce intraspecific competition, contributing to their homogenization on the leaf surfaces. Alternatively, nymph mortality is higher on the leaf abaxial surface; however, this was not owing to parasitism by *P. bliteus*. Moreover, other causes of psyllid mortality, such as lerp removal by rain and wind and nymph predation, require further study. Physical defense may also explain the
higher red gum lerp psyllid oviposition preference on the abaxial leaf surface, as observed on two of the *Eucalyptus* genotypes. The preference of this species to oviposit near leaf mid-ribs or lateral veins on the abaxial surface had been explained by the higher nutrient flow for nymph development and reduced desiccation, especially during insect molt (Phillips, 1992; Firmino-Winkler et al., 2009; Oliveira et al., 2012).

The reduced parasitism rate by *P. bliteus* showed the low efficacy of this natural enemy to control *G. brimblecombei*. This parasitoid has been successfully reared in the laboratory and released in eucalyptus plantations in Brazil for *G. brimblecombei* biological control; however, its parasitism under field conditions is low (Daane et al., 2005). This suggests that *P. bliteus* might not be adapted to the environmental conditions of Brazilian eucalypt plantations, although it reduced more than 40% of the red gum lerp abundance in North America (Dahlsten et al., 2005). Thus, the warmer temperatures of tropical regions may have hampered *P. bliteus* performance and efficacy in *G. brimblecombei* classical biological control (Daane, Sime & Paine, 2012).

The temporal changes in the psyllid population density with monthly rainfall and the negative correlation of *G. brimblecombei* egg and nymph densities on *E. camaldulensis* with rainfall corroborate the population peaks for this pest during the dry season (Wilcken et al., 2003, Ferreira-Filho et al., 2015). Drought stress causes physiological changes in the host plants by increasing the nitrogen concentration (White, 1984; Wilcken et al., 2003; Huberty & Denno, 2004) and reducing chemical defenses (Gutbrodt, Dorn & Mody, 2012). This increases the herbivorous insect performance on stressed plants (White, 1969, 1984). Furthermore, the mechanical removal of the psyllid lerps by water droplets and/or lerp solubilization by leaf moisture may decrease its population (Oliveira et al., 2012). In addition, the higher humidity during the rainy season increases entomopathogenic fungi occurrence, which may kill psyllid
nymphs (Ramirez, Mancera & Guerra-Santos, 2002). Thus, plantation timing may be an important management tool to, at least, reduce infestation of highly vulnerable eucalypt seedlings. Although irrigation can physically remove lerps from juvenile and adult trees during the dry season, its use is less feasible owing to high costs and environmental impacts.

This is the first field study in Brazil investigating the ecological aspects of *G. brimblecombei* and eucalypt genotypes interactions. Management recommendations for this insect pest include: (i) planting less susceptible *Eucalyptus* spp. or hybrids to reduce the red gum lerp psyllid populations with the aid of production and releasing its parasitoid; (ii) abiotic factors such as rainfall decrease psyllid abundance; and (iii) synchronization of planting with the rainy season helps during the most susceptible stage of *Eucalyptus* spp. to this pest.

**ACKNOWLEDGEMENTS**

We thank Vallourec & Mannesman S. A. for logistical support, in special to Josefredo D. Moreira, Vilmar de A. Izidoro and Bianca V. Fernandes. We are grateful to Fabiene M. de Jesus, Ronald R. M. Santos and Sarah F. M. Silva for help during field and lab work. We also thank Thais G. Pellegrini for helpful suggestions in the first drafts of this manuscript. This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG - Process EDT-318/05). We gratefully acknowledge the scholarships to Karla N. Oliveira (Probic-FAPEMIG), Jhonathan de O. Silva (Bic-UNIMONTES) and Mário Marcos do Espírito-Santo (BIPDT-FAPEMIG). This paper was partially produced during the course PEC 527 - Publicação Científica of the Graduate Program in Applied Ecology of the Universidade Federal de Lavras. To “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)”. Dr. Phillip Villani
revised and corrected the English language used in this manuscript.

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Table 1. Results of data analyses performed to evaluate *Glycaspis brimblecombei* attack patterns on different *Eucalyptus* genotypes

| Response variable                              | Explanatory variable     | n   | F       | p    |
|-----------------------------------------------|--------------------------|-----|---------|------|
| *G. brimblecombei* egg abundance              | *Eucalyptus* genotype    | 1140| 537.86  | <0.001 |
| *G. brimblecombei* nymph abundance            | *Eucalyptus* genotype    | 1140| 250.00  | <0.001 |
| Percentage of parasitized nymphs              | *Eucalyptus* genotype    | 1140| 0.24    | 0.665 |
| *G. brimblecombei* egg abundance on *E. camaldulensis* | Leaf surface     | 480 | 21.77   | <0.001 |
| *G. brimblecombei* egg abundance on *urograndis* | Leaf surface     | 480 | 0.83    | 0.363 |
| *G. brimblecombei* egg abundance on *urocam*  | Leaf surface            | 480 | 0.02    | 0.878 |
| *G. brimblecombei* egg density                | Monthly rainfall         | 12  | 67.43   | <0.001 |
| *G. brimblecombei* nymph density              | Monthly rainfall         | 12  | 83.48   | <0.001 |
Figure 1 Number of *Glycaspis brimblecombei* (Hemiptera: Psyllidae) eggs (a) and nymphs (b) on leaf surfaces of different eucalypt genotypes. Bars indicate standard error. Means followed by the same letter above the bars are similar ($\alpha = 0.05$).
Figure 2 Monthly total rainfall and temporal variation of *Glycaspis brimblecombei* (Hemiptera: Psyllidae) on *Eucalyptus camaldulensis* from December 2006 to November 2007. Mean density of this psyllid (a) eggs and (b) nymphs on *E. camaldulensis*. Bars indicate standard error. The shading indicates the dry season.
Figure 3. Number of *Glycaspis brimblecombei* (Hemiptera: Psyllidae) eggs (a) and nymphs (b) on *Eucalyptus camaldulensis* related to monthly total rainfall (n= 12). The parameter estimates is used to fit the curve obtained with the minimal adequate GLM model and adjusted for linear and exponential function, respectively (α= 0.05).