



Development of *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae) on *Eucalyptus camaldulensis* Dehnh. and *Eucalyptus dunnii* Maiden

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- Abstract**
- 1 The red gum lerp psyllid *Glycaspis brimblecombei* is an invasive insect species, native from Australia, that specifically feeds on *Eucalyptus* trees. It has invaded several countries throughout the world. In Argentina, it was first recorded in 2005, although little is known about its ecology in the region.
 - 2 We assessed *G. brimblecombei* population development on *Eucalyptus camaldulensis* and *Eucalyptus dunnii* using samples of branches for the immature stages and yellow sticky traps for the adults. We also identified the meteorological variables associated with changes in the red gum lerp psyllid abundance.
 - 3 The abundance of eggs, nymphs and adults stages of *G. brimblecombei* was significantly greater on *E. camaldulensis* than on *E. dunnii* in the 2 years of the survey.
 - 4 *Glycaspis brimblecombei* development was complete on *E. camaldulensis* where all instars were present, even in the unfavourable seasons. The full development of the psyllid population was not observed in *E. dunnii* where a high mortality of the first and second nymphal instars was detected.
 - 5 Temperature and relative humidity were the variables that mostly affected red gum lerp psyllid abundance, whereas no effect of rainfall was detected.

Keywords Eucalypt, forest pest, invasive species, population abundance, red gum lerp psyllid.

Introduction

The red gum lerp psyllid *Glycaspis brimblecombei* Moore is a small sap-sucking insect that specifically feeds on *Eucalyptus* leaves. Native from Australia, this invasive species has spread to several countries and become a major *Eucalyptus* pest worldwide. In America, it was first recorded in the U.S.A. in 1998 (Brennan *et al.*, 1999); thereafter, it was detected in Mexico (Cibrián, 2002), Chile (Sandoval & Rothmann, 2002), Brazil (Wilcken *et al.*, 2003), Argentina (Bouvet *et al.*, 2005), Ecuador (Onore & Gara, 2007), Venezuela (Rosales *et al.*, 2008) and Peru (Burckhardt *et al.*, 2008). In its native range, *G. brimblecombei* occurs on eight *Eucalyptus* species in the sections Exsertaria and Maidenaria of the subgenus Symphyomyrtus (*Eucalyptus blake-lyi* Maiden, *Eucalyptus brassina* Blake, *Eucalyptus camaldulensis* Dehnh., *Eucalyptus camphora* Baker, *Eucalyptus dealbata* Cunn. ex Schauer, *Eucalyptus mannifera* ssp *maculosa* Baker, *Eucalyptus nitens* Deane & Maiden and *Eucalyptus tereticornis*

Smith) but, outside its home range, it is found feeding on other species of *Eucalyptus* and their hybrids (Brennan *et al.*, 2001). The susceptibility of these host plants to the red gum lerp psyllid varies from highly susceptible to near resistant (Hodkinson, 2009).

Glycaspis brimblecombei infestations are easily recognizable by the presence of conical white shields known as lerps, each inhabited by a single nymph and attached to the foliage covering both surfaces of the leaves. These lerps are built from faecal excretions of the nymphs that harden upon exposure to the air (White, 1972), providing protection against natural enemies (predators and parasitoids) and avoiding desiccation during development (Sullivan *et al.*, 2006; Sharma *et al.*, 2013). The damage caused by this psyllid is mainly a result of nymphs and adults feeding by sucking phloem from the leaves. In the case of light infestations, there is some discoloration and wilting of the foliage, whereas heavy infestations cause extensive discoloration leading to leaf necrosis and defoliation, which reduces growth and tree vigour (Collett, 2000). Sooty mold thriving from psyllid honeydew may also contribute to this defoliation by

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reducing the leaf surface available for photosynthesis (Brennan *et al.*, 2001). When psyllid attack is severe and repeated, tree susceptibility to other insects and diseases may increase, eventually leading to the death of the weakened plants (Garrison, 1998; Collett, 2000; Paine, 2006).

Glycaspis brimblecombei has quickly spread throughout Argentina from 2005 onwards, with a current distribution that covers from the Northern provinces to North Patagonia (Bouvet *et al.*, 2005; Holgado *et al.*, 2005; Dapoto *et al.*, 2007; Diodato & Venturini, 2007; Quintana de Quinteros *et al.*, 2008; Ganci & Lanatti, 2011). Although the presence of *G. brimblecombei* has been widely recorded, little is known about its biology and ecology under local environmental conditions. The area of *Eucalyptus* plantations in Argentina has grown in recent years and now covers more than 250 000 ha (Ministerio de Agricultura, Ganadería y Pesca, 2015). The most widely planted species in this region include *Eucalyptus camaldulensis* Dehnh. and *Eucalyptus dunnii* Maiden (Ministerio de Asuntos Agrarios, 2010). *Eucalyptus camaldulensis* has been identified in several studies as one of the most susceptible species to the red gum lerp psyllid attack because it allows full development from egg to adult stages and exhibits heavy defoliation (Collett, 2000; Brennan *et al.*, 2001; Pereira *et al.*, 2012). In Australia, *E. dunnii* is considered susceptible to a wide range of insect pests of which psyllids are a major threat. Whyte *et al.* (2011) identified three psyllids genera damaging *E. dunnii* foliage and the genus *Glycaspis* was noted as one of the taxa responsible for foliar necrosis in this *Eucalyptus* species. The value of both *Eucalyptus* species is significant with respect to providing raw material for manufacturing different products, as well as having desirable features, such as resistance to frost in *E. dunnii* (McMahon *et al.*, 2010a) or a wide adaptability to a range of climatic conditions in *E. camaldulensis* (McMahon *et al.*, 2010b). These characteristics make these species very valuable in programmes of genetic improvement and the development of hybrids (Harrand, 2005).

The present study aimed to compare the development of *G. brimblecombei* on *E. camaldulensis* and *E. dunnii* under the local conditions in Argentina, as well as identify the main abiotic factors that affect their population biology.

Materials and methods

The present study was carried out at the experimental field of the 'Instituto Nacional de Tecnología Agropecuaria' (34°36'21"S 58°40'14"O) Castelar, Buenos Aires province, Argentina. The climate in this region, according to the Koppen classification, is Cfa Subtropical without a dry season and with a warm summer. The mean monthly temperature is 16.9 °C, the mean annual rainfall is 1024.3 mm and the mean relative humidity (RH) is 69.1%.

The population development of *G. brimblecombei* was assessed on 10 *E. camaldulensis* and 10 *E. dunnii* trees by recording the number of eggs, nymphs and adults every 15 days, from 26 December 2012 until 22 December 2014. The *Eucalyptus* plantations used for the surveys were spaced 300 m from each other and were subject to minimum pruning and weeding and managed without chemical control.

The immature stages (eggs and nymphs) of the psyllid were counted in a randomly selected branch/tree of approximately

40 cm long, bearing 30–50 leaves (Ozanne, 2005). Each branch was cut off from a height of 4 m, isolated into a plastic bag and analyzed in the laboratory. The adults were monitored using yellow sticky traps (6 × 7 cm) placed on a branch at a height of 1.8 m. The variations on the age structure of *G. brimblecombei* population were analyzed every 15 days, from 7 October 2013 until 30 September 2014, by recording the number of nymphs from each developmental stage (instar). Instars were identified from the number of antennal segments (Sánchez *et al.*, 2003) after the lerp was carefully removed using an entomological needle. The first and second instars were combined because there are no differences in the number of antennal segments. All observations were carried out in the laboratory under a stereoscopic microscope.

Daily data of minimum and maximum temperature, RH and rainfall were provided by the 'Instituto de Clima y Agua' – INTA, Castelar.

Statistical analysis

For each abundance period, the number of eggs, nymphs and adults was compared between the two species of *Eucalyptus* by a generalized linear model using R, version 2.15.3 (<http://www.r-project.org>). An abundance period was defined by the dates in which the number of individuals of *G. brimblecombei* in *E. dunnii* was greater than zero. We used the function generalized least squares of the linear and nonlinear mixed effects models (Gaussian family and identity link function). The data were normalized using the Box-Cox transformation. An error structure was chosen where a correlation between the dates was assumed with the shape of a compound symmetric matrix. The structure of the variances-covariances matrix was evaluated with Akaike's information criterion and likelihood ratio tests were built to assess the factor effects. In the cases in which interaction between factors was significant, means were separated using Tukey's multiple comparison test (*lsmeans* package in R).

The relationship between *G. brimblecombei* abundance and meteorological variables was assessed by a Spearman correlation test using INFOTAT (Di Rienzo *et al.*, 2015). Precipitation effects were analyzed using the rainfall of the month prior to the psyllid number assessment to enable consideration of a possible time delay on insect population development.

Results

A cyclic pattern was observed with two periods of distinct abundance in each year. The abundance of *G. brimblecombei* increased in early spring (September), reaching its maximum abundance between the end of this season (November) and the beginning of the summer (December). Subsequently, it decreased in early Autumn (March) and remained low during winter (June to August) (Figs 1–3).

Temperature and RH were the variables that mostly influenced psyllid population (Fig. 3 and Table 1). The abundance of *G. brimblecombei* showed a significant positive correlation with maximum temperature (MxT) for all psyllid instars in both species of *Eucalyptus*. The same correlation was detected with minimum temperature (MnT), except for the nymphs on

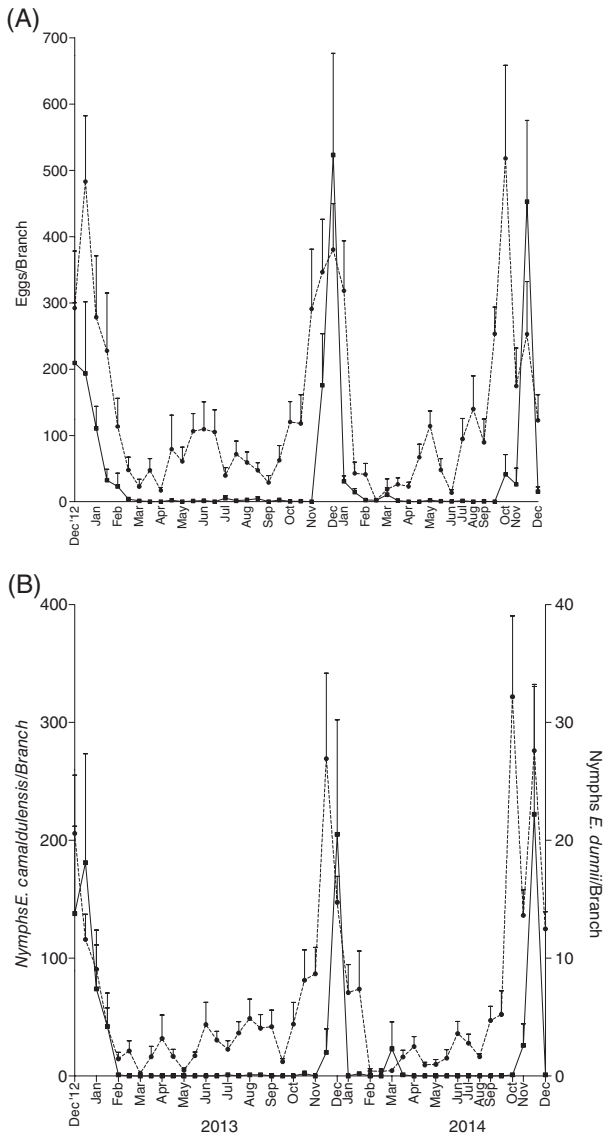


Figure 1 Number of *Glycaspis brimblecombei* eggs (A) and nymphs (B) on *Eucalyptus camaldulensis* (dotted lines) and *Eucalyptus dunnii* (full line) branches (mean \pm standard error). In (B), the left axis represents *E. camaldulensis* nymphs and the right axis represents *E. dunnii* nymphs.

E. camaldulensis and the adults on *E. dunnii* where the correlation was not significant. By contrast, RH was negatively correlated with the three developmental stages in both host plants. No significant correlation was observed between the rainfall of the previous month and *G. brimblecombei* abundance (Table 1), nor was there any correlation with the rainfall occurring 2 months before psyllid population assessment (data not shown).

Both *Eucalyptus* species showed differences regarding their suitability as hosts for *G. brimblecombei*. On *E. camaldulensis*, all developmental stages were present the entire year, even during the unfavourable winter season, although with a lesser abundance compared with the spring–summer period (Figs 1 and 2). In one sampling date (19 May 2014), no adults were captured in

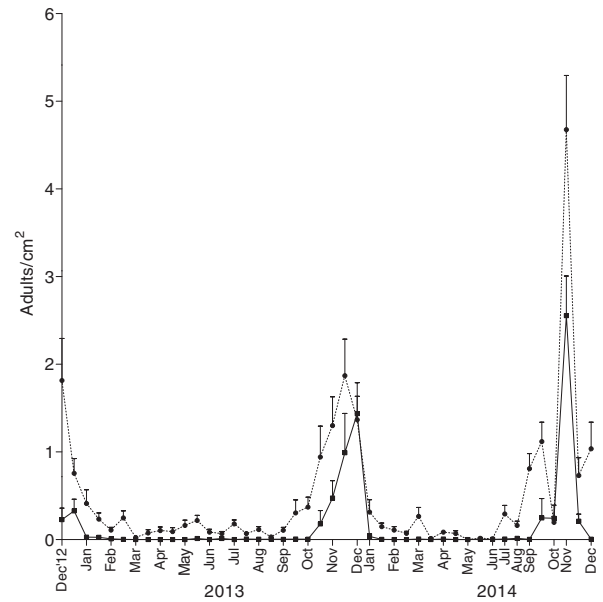


Figure 2 The number of adults of *Glycaspis brimblecombei* on yellow sticky traps on *Eucalyptus camaldulensis* (dotted line) and *Eucalyptus dunnii* (full line) (mean \pm SE).

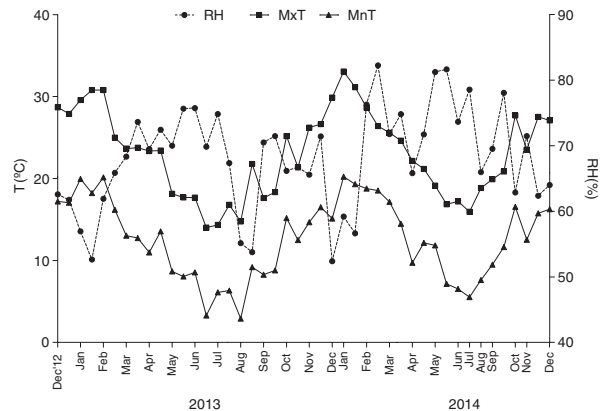


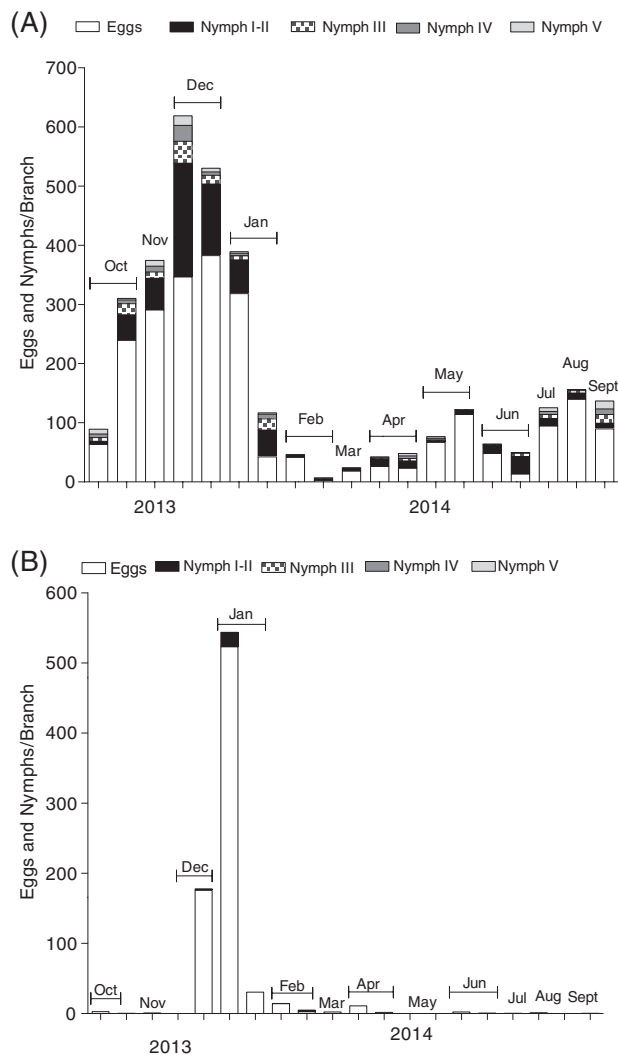
Figure 3 Maximum temperature (MxT), minimum temperature (MnT) and relative humidity (RH) in Castelar, Buenos Aires province, Argentina.

the yellow sticky traps, although some adult individuals were observed in the trees. The lack of trap captures might have been a result of not only the low abundance of psyllids in the field, but also the reduced activity of adults during winter. Instead, on *E. dunnii*, the psyllid was absent for most of the year (Figs 1 and 2) and was unable to complete its development. In this regard, the presence of psyllids on *E. dunnii* was restricted to adults, eggs and the first and second nymphal instars, whereas, on *E. camaldulensis*, all nymphal instars were present (Nymphs I–V) (Fig. 4). Moreover, a great difference was observed in the number of alive nymphs (I–II) with respect to the number of eggs laid in the two *Eucalyptus* species (e.g. 380.00 ± 69.58 eggs and 120.80 ± 19.12 nymphs for *E. camaldulensis* and 523.00 ± 153.32 eggs and 20.50 ± 9.72 nymphs for *E. dunnii* in December 2013) (Fig. 4).

Table 1 Spearman correlation test between *Glycaspis brimblecombei* abundance and weather variables in *Eucalyptus camaldulensis* and *Eucalyptus dunzii*

Instar	Host	MxT (°C)		MnT (°C)		RH (%)		Rainfall (mm)	
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Eggs	<i>Eucalyptus cam</i>	0.39	0.01	0.31	0.04	-0.41	0.01	-0.23	0.14
	<i>Eucalyptus dun</i>	0.62	< 0.001	0.58	< 0.001	-0.47	0.001	0.12	0.43
Nymphs	<i>Eucalyptus cam</i>	0.33	0.03	0.21	0.17	-0.56	< 0.001	0.04	0.79
	<i>Eucalyptus dun</i>	0.53	< 0.001	0.46	0.002	-0.54	< 0.001	0.18	0.25
Adults	<i>Eucalyptus cam</i>	0.4	0.01	0.35	0.02	-0.36	0.02	0.08	0.59
	<i>Eucalyptus dun</i>	0.32	0.03	0.22	0.15	-0.46	0.002	-0.04	0.81

r, Spearman correlation coefficient, *P* < 0.05. MxT, maximum temperature; MnT, minimum temperature; RH, relative humidity.

**Figure 4** Mean number of *Glycaspis brimblecombei* eggs and nymphs on *Eucalyptus camaldulensis* (A) and *Eucalyptus dunzii* (B) branches.

There were significant differences in the abundance of eggs, nymphs and adults of the red gum lerp psyllid between *Eucalyptus* species in the three periods analyzed (Table 2). In the cases in which the interaction between factors (*Eucalyptus* species and date) was not significant, the presence of *G. brimblecombei* was

greater on *E. camaldulensis* than on *E. dunzii*. For periods in which the interaction between factors was significant, the multiple comparison test detected more psyllids on *E. camaldulensis* compared with *E. dunzii*, except on three dates (eggs: 16 December 2013 and 9 December 2014; adults: 16 December 2013); however, these differences were not significant.

Discussion

In Argentina, *Glycaspis brimblecombei* is a multivoltine species with continuous and overlapping generations. The number of generations that a psyllid species can produce per year depends on its specific biology, as well as the environmental conditions and the availability of suitable host plants (Collett, 2000). In its home range, *G. brimblecombei* shows two to four generations per year (Morgan, 1984), whereas, for the same region, other species of lerp forming psyllids can have four generations (*Glycaspis* sp. on *E. sideroxylon*) or six to seven generations per year (*G. baileyi* on *E. saligna*) (Moore, 1961; Sharma *et al.*, 2013). If we take into account the number of degree-days (DD) estimated by Nogueira *et al.* (2009) for *G. brimblecombei* (377.49 ± 16.09 DD) and the temperature values measured in this region, it is possible to estimate that there should be six generations per year under local environmental conditions. This value is within the range cited in previous studies and consistent with the findings of Firmino-Winckler *et al.* (2009) who reported *E. camaldulensis* comprising the most suitable host on which *G. brimblecombei* exhibited multiple generations.

Temperature and RH were the climatic variables that mostly affected the abundance of *G. brimblecombei*. Low temperatures and high RH were correlated with low psyllid abundance, whereas high temperatures and low RH showed the opposite effect. A similar relationship among these two meteorological variables and the red gum lerp psyllid abundance was reported by Lima da Silva *et al.* (2013) in the state of Mato Grozo, Brazil. In the present study, *G. brimblecombei* abundance (all developmental stages) dropped drastically between January and February. This fall in the psyllid abundance was more notorious in *E. dunzii*, where eggs, nymphs and adults reached a value close to zero and did not recover until the next spring. The decline of psyllid abundance in *E. camaldulensis* during this period was less marked because the number of all instars continued fluctuating. In laboratory studies, Firmino (2004) showed that temperatures above 30 °C can limit the development and reproduction of

Table 2 Generalized linear model results for differences in the three periods of maximum abundance of eggs, nymphs and adults between *Eucalyptus camaldulensis* and *Eucalyptus dunnii*

Population abundance	Period	Instar	Species		Date		Interaction	
			F	P	F	P	F	P
First peak	26 December 2012 to 18 February 2013	Egg	10.74	0.001	2.96	0.024	0.53	0.712
	26 December 2012 to 5 February 2013	Nymph	49.58	< 0.001	3.81	0.014	0.78	0.507
	26 December 2012 to 7 January 2013	Adult	40.4	< 0.001	0.54	0.468	7.14	0.011
Second peak	2 December 2013 to 20 January 2014	Egg	17.79	< 0.001	18.5	< 0.001	3.83	0.013
	2 December to 16 December 2013	Nymph	113.44	< 0.001	9.67	0.004	19.39	< 0.001
	4 November to 16 December 2013	Adult	19.09	< 0.001	11.75	< 0.001	4.33	0.007
Third peak	28 October to 22 December 2014	Egg	25.66	< 0.001	3.89	0.012	5.49	0.002
	17 November to 9 December 2014	Nymph	91.92	< 0.001	9.99	0.003	1.93	0.173
	14 October to 9 December 2014	Adult	30.74	< 0.001	39.4	< 0.001	3.62	0.017

G. brimblecombei. During our observations, the months of December, January and February included several days with temperatures above 30 °C and this probably contributed to the fall in the abundance of the psyllid on both species of *Eucalyptus*. On the other hand, it is unlikely that food availability was responsible for this decline because the trees remained vigorous and healthy during these warm periods. A reduction of psyllid numbers as a result of high temperatures had also been recorded for other psyllids, such as *Cardiaspina* species (Hall *et al.*, 2015) and *Heteropsylla cubana* (Geiger & Gutierrez, 2000).

Rainfall did not impact the abundance of *G. brimblecombei* at the study site. Several studies have shown that precipitation influence can be seen with time delay on psyllid population development on *Eucalyptus*. Gherlenda *et al.* (2016) demonstrated that rainfall stimulates *Eucalyptus* leaf production, which in turn promotes population growth of a *Glycaspis* species. However, the increase on psyllid numbers occurs after a dry period during the previous year. These findings are explained by the plant stress hypothesis (White, 1969), which suggests that herbivorous insects exhibit enhanced performance on water stressed host as a result of an increase in available plant nitrogen. However, phloem feeders require positive turgor pressure to benefit from these stress-induced increases in plant nitrogen (Huberty & Denno, 2004). Similarly, Laudonia *et al.* (2013), when studying seasonal occurrence of *G. brimblecombei* in Italy, showed that rain during summer and after a dry period was favourable for psyllid population increase. The results of the present study do not support, nor do they reject the influence of rainfall in psyllid abundance, presumably because there are no distinct periods of rainfall and drought in the study area. A similar situation was observed in other regions where the red gum lerp psyllid has spread (Ferreira Filho *et al.*, 2008).

One peak of maximum abundance of *G. brimblecombei* was identified per year of surveys. The peak was simultaneous in both *Eucalyptus* species but, in *E. camaldulensis*, the population density was significantly higher than in *E. dunnii*. However, the infestation levels of *E. dunnii* appeared to rely on changes in the abundance of the psyllids in the *E. camaldulensis* plantation. Even though *E. dunnii* did not allow the full development of *G. brimblecombei*, the psyllid population recovered each year in this tree species. Psyllid in *Eucalyptus* plantations are likely to disperse when the population increases or when host plant

food quality becomes less suitable (Hodkinson, 2009); therefore, when the *G. brimblecombei* population on *E. camaldulensis* increased, the adult stage dispersed to the *E. dunnii* trees, given the proximity of both plantations (300 m). By contrast, when the psyllid population on *E. camaldulensis* declined, the dispersion also diminished and the psyllid abundance on *E. dunnii* reached values close to zero. A similar phenomenon was observed by García *et al.* (2014) when studying the presence of two *Eucalyptus* psyllid species (*Ctenarytaina spatulata* and *Ctenarytaina eucalypti*) in apple orchards with an overlapping distribution with *Eucalyptus* trees, which are the actual host plant of these psyllids.

Several studies have singled out *E. camaldulensis* as one of the most susceptible host of *G. brimblecombei* (Brennan *et al.*, 2001; Hidalgo Reyes, 2005; Huerta *et al.*, 2010; Pereira *et al.*, 2012; Camargo *et al.*, 2014; Ribeiro *et al.*, 2014). This susceptibility lies on a high preference of *G. brimblecombei* females to oviposit on this species (Pereira *et al.*, 2012) and on the suitability for psyllid development (Brennan *et al.*, 2001). These two characteristics are supported by the results of the present study. By contrast, the psyllid population on *E. dunnii* barely survived beyond the first and second instars. In this respect, in the lapse of 1 year (December 2013 to December 2014), 76% of 2004 nymphs recorded on this species were found dead, without lerp formation and with a desiccated appearance (data not shown). These observations suggest that there is some kind of resistance mechanism in *E. dunnii* that negatively affects the nymphal survival and development of *G. brimblecombei*, although it does not appear to affect female oviposition given the abundance of eggs and adults recorded. This resistance involves distinctive anatomical, chemical or physiological characteristics among species that are not closely related, such as *E. camaldulensis* and *E. dunnii*, which are phylogenetically separated into two different sections in the subgenus *Symphomyrtus* (Steane *et al.*, 2011). Resistance mechanisms of different *Eucalyptus* species to *Glycaspis* have been investigated in several studies (Brennan & Weinbaum, 2001a, 2001b; Ribeiro *et al.*, 2014; Lucia *et al.*, 2016). Lucia *et al.* (2016) found that *Eucalyptus* species with high levels of 1,8 cineole, which is considered to be responsible for the fumigant toxicity of *Eucalyptus* essential oils against different insects (Batish *et al.*, 2008; Alzogaray *et al.*, 2011; Juan *et al.*, 2011), had a significantly lower abundance of psyllids (e.g. *E. dunnii*) than

species with terpenes α - and β -phellandrene as major components (e.g. *E. camaldulensis*). On the other hand, when Brennan and Weinbaum (2001a) studied stylet probing behaviour on psyllid species, they found that *G. brimblecombei* adults avoid oil glands of the leaves by directing their stylet around them. However, it is not clear whether the nymphs are capable of repeating this behaviour. Leaf-settling may also be related to the survival of psyllids by affecting its adhesion to the leaf surface (Brennan & Weinbaum, 2001a, 2001b). Further studies will be necessary to identify the mechanisms (structural or physiological) underlying the psyllid resistance of *E. dunnii* observed in the present study.

The present study comprises the first exploration of the population biology of *G. brimblecombei* in Argentina. Understanding the development of the red gum lerp psyllid population under local environmental conditions would be useful for the design of control strategies with respect to this pest.

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