

Feeding preference of *Thaumastocoris peregrinus* on several *Eucalyptus* species and the relationship with the profile of terpenes in their essential oils

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Abstract The feeding preference by *Thaumastocoris peregrinus*, Carpintero and Dellapé (Heteroptera: Thaumastocoridae) on different *Eucalyptus* species was evaluated through choice tests in a paired comparisons design. Twenty individuals per combination were tested over a 48 h period, and each one was offered leaves of two species at a time, in all possible combinations. Preference was determined by counting the number of insect excrements on each plant as a proxy for effective feeding, and then ranked through a paired comparison matrix. A linearity index was calculated to test for hierarchy preferences and, after that, a cardinal index (David's score) was calculated to assign a preference value for each species. The essential oils were extracted from leaves of each *Eucalyptus* species, and their chemical composition determined through GC-MS. The results show a significant and strictly linear

feeding preference ranking between the species tested. The order of preference between species was *E.viminalis*>*E.grandis*>*E.tereticornis*>*E.dunnii*>*E.maidenii* > *E.globulus*. The qualitative and quantitative chemical compositions of the essential oils of these *Eucalyptus* species differed. A significant correlation was found between the feeding preferences and the concentrations of 1,8-cineole as a repellent and *p*-cymene as a feeding stimulant. These results underline the importance of selecting the right *Eucalyptus* species in areas infested by *T. peregrinus*.

Keywords Antixenosis · Bronze bug · *p*-cymene · 1,8-cineole

Introduction

Thaumastocoris peregrinus Carpintero and Dellapé, 2006 (Heteroptera: Thaumastocoridae) is an invasive species native of Australia that has spread to South Africa (Jacobs and Nesser, 2005), Argentina (Noack and Coviella 2006), Brazil (Wilcken et al. 2010), Uruguay (Martínez and Bianchi 2010), Chile (Ide et al. 2011), New Zealand (Sopow et al. 2012), Italy (Laudonia and Sasso 2012), Portugal (Garcia et al. 2013), Israel (Novoselsky and Freidberg 2016), Mexico (Jiménez-Quiroz et al. 2016) and California (Arakelian 2016). Known as the Bronze bug because of the damage and the threatening economic loss it produces, this species feeds exclusively on *Eucalyptus* trees (Souza et al. 2012; de Souza et al. 2016). *T. peregrinus* can reach

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very high population densities, thus becoming a threat to *Eucalyptus* plantations worldwide, feeding on more than 40 species and hybrids of this plant (Chiaradia and Bearzi 2010; Nadel et al. 2010; Lima et al. 2016), even facing native natural enemies (Dias et al. 2014). The genus *Eucalyptus* is native to Australia with over 500 species (Coppen 1995). In northeastern Argentina, *Eucalyptus* plantations occupy more than 130,000 ha with *E. grandis* (Hill) ex Maiden as the most economically important species for Argentina and Uruguay (Carpinetti et al. 1995). In Argentina, *Eucalyptus* is used for reforestation and as a wood resource since the 1950s (FAO 1995; Marcó and Harrand 1999).

Plant selection as food resource by herbivorous insects is complex, involving plant characteristics and insect behavior (Bernays and Chapman 1994; Schoonhoven et al. 2005), besides feeding capacity (Souza et al. 2014). Different behavioral patterns can be recognized in a predictable sequence before actual feeding behavior can start: the search for the host plant habitat, host plant location at the distance, recognition of the suitable host, probing, and acceptance (Dethier 1982; Metcalf and Luckmann 1994). The entire process of host-plant selection and specificity depends on the insects' behavioral responses to plant defenses and, therefore, behavioral differences are likely a central factor in the evolution of host-plant specificity (Bernays and Chapman 1994). Moreover, the complex defense system protecting the plants against insect pests can be divided in physical or chemical barriers with toxic, repellent, antifeedants or antinutritive properties (Mithöfer and Boland 2012). Painter (1951) categorized the mechanisms of tolerance, antibiosis, and non preference (now antixenosis, Kogan and Ortman 1978). Since then, the categories of resistance for plants against herbivorous insects are being characterized as antibiosis, antixenosis, and tolerance (Smith 1989). Essential oils from *Eucalyptus* leaves are defined as steam-volatile compounds with a molecular weight lower than 500 Da as a mixture of hydrocarbons, terpenes and sesquiterpenes, and oxygenated compounds such as alcohols, esters, ethers, aldehydes, ketones, lactones and phenols (Brophy and Southwell 2002; Coppen 1995). The oil glands are located deeply within the *Eucalyptus* leaves, well below the epidermal cuticle and other cells forming the foliage surface layers of the leaf (Denny 2002).

Chemical defenses with biosynthesis, accumulation and terpenoid profiles play an important role in chemical

ecology as repellents or deterrents (Barrios-San Martín et al. 2014; Edwards et al. 1993) and as feeding stimulants (Delrio et al. 1983). The role that *Eucalyptus* spp. essential oils play on herbivorous insects has been long recognized (Morrow and Fox 1980). VOCs released by *Eucalyptus benthamii* plants affected *T. peregrinus* herbivory, and the preference of its virgin and mated females to these compounds have been studied (Martins and Zarbin 2013). Different terpene profiles released VOCs from damaged plants or plants with mechanical damage were compared. Mated *T. peregrinus* females preferred undamaged plants over herbivore-damaged plants, while virgin females did not exhibit any preference between conspecific-damaged or undamaged plants.

This study examines differences in essential oil profiles between six *Eucalyptus* species and the relationship between the feeding preference of *T. peregrinus* and individual essential oil constituents found on the *Eucalyptus* species tested.

The aims of the present work were: 1) to identify *T. peregrinus* relative preferences on six *Eucalyptus* species, and 2) to show the relationship between these preferences and the chemical composition of the essential oils.

Materials and methods

Feeding preference

Feeding preference by *T. peregrinus* over a range of different *Eucalyptus* species was evaluated in the laboratory. Choice experiments were performed with paired comparisons (David 1988; de Vries 1998). In each assay, a pair of leaves of the *Eucalyptus* species tested (six) paired in all possible combinations (fifteen) were offered to adult insects. Twenty replications were performed for each combination. This design was preferred over a cafeteria type design (Miller and Clifton 1964; Gross et al. 1991), allowing to offer more than two items because it is not possible to ensure a complete evaluation of the whole set by the insect before a choice is made (Pirk and de Casenave 2011). The *Eucalyptus* species evaluated were: *E. viminalis* (Labill), *E. dunnii* (Maiden), *E. tereticornis* (Smith), *E. maidenii* (F. v. Muell), *E. globulus* (Labill), and *E. grandis* (Hill) ex Maiden. These species cover the highest land surface in Argentina (Villegas and Rivera 2002).

Assays were performed in the Agro Zoology laboratory, Universidad Nacional de Luján, (UNLu), Argentina. A JEIO Tech GC-300/1000 growth chamber was used under 24 ± 2 °C temperature, $60 \pm 2\%$ RH, and 12 L:12D photoperiod.

The insects were collected on *E. camaldulensis* Dehnh at UNLu experimental fields, and reared in the laboratory on the same species until adult emergence. The experiments were performed with recently emerged adults of the same cohort. Plant material, used for the bioassays, was collected on the same day, and placed in 5 cm³ containers with water. The arenas consisted in a 1 L jar with two of these containers, each one of them with plant material of a single *Eucalyptus* species.

For each assay, the items were placed at the same distance between each other, and randomizing the position, left or right, from the point the adult insect was placed as seen from the light source.

One adult was placed per jar between the two small containers after a 2 h period without feeding. After 48 h, insect selection was determined by counting the presence and number of insect excrements on the leaves of each species tested as proxy for effective feeding.

Plant material and extraction of essential oils:

Fresh leaves from the six *Eucalyptus* species were collected during August 2014, and used for the bioassays. The leaves were harvested from an experimental plot formed by *Eucalyptus* species planted in different years; – 2002: *E. dunnii* and *E. grandis* - 1996: *E. maidenii* - 1998: *E. globulus* - 1985: *E. viminalis* and *E. tereticornis* in the Universidad Nacional de Luján, Argentina (34° 35' 41" S, 59° 04' 30" W, 37 m above sea level). The essential oils were obtained by hydrodistillation using a modified Clevenger-type device. The extraction procedure was performed for 70 min, time at which the yield remained constant (Lucia et al. 2008). After extraction, the essential oils were separated from water, dried over anhydrous sodium sulfate and maintained at –4 °C until use. Also, the essential oils were extracted in two independent distillations for each sampling procedure.

Chemical standards and GC-MS essential oil analyses:

All chemical standards were purchased from Sigma-Aldrich® Argentina: α -pinene (98%), (*R*)-(+)-camphene (80%), (*S*)-(-)- β -pinene (98%), α -terpinene

(85%), *p*-cymene (99%), (*S*)-(-)-limonene (96%), 1,8-cineole (99%), γ -terpinene (97%), linalool (97%), (*S*)-(-)-borneol (90%), (*S*)-(+)-4-terpineol (96%), (\pm)- α -terpineol (99%), caryophyllene oxide (99%).

The essential oils were analyzed in a Shimadzu GC-17A gas chromatograph interfaced to a Shimadzu quadrupole mass spectrometer (GCMS-QP5050A), and equipped with two equal DB-WAX columns (J&W Scientific, Agilent Technologies, 30 m \times 0.32 mm i.d. \times 0.25 μ m film thickness) serially connected (Connector HP 5041–2174). The essential oil samples were diluted in hexane (0.5 mg/ml) and 0.5 μ l were injected using a split ratio of 1:35. The temperature ramp started at 50 °C (hold time: 10 min), then increased to 68 °C (1 °C/min), and finally up to 230 °C (3 °C/min) with a final hold time of 3 min. Column inlet pressure was set up at 61.6 kPa (linear velocity: 31.43 cm/s), flow column was 1.5 ml/min. Injector and detector were set at 240 °C and 245 °C, respectively.

The mass spectra were recorded between 40 and 350 amu and ionization energy of 70 eV. Quantification was performed using normalized relative peak areas obtained from the GC chromatograms. Individual components were identified by different methods: (a) Mass spectrums obtained from each compound were compared with database information (Wiley mass spectral database ver.7, NIST database); (b) Comparing retention times of each compound with those of chemical standards, when available; and (c) Linear retention indexes, obtained by co-injection with a homologous series of *n*-alkanes (C9–C17), and comparison with bibliographic data.

Statistical analyses

Differences in feeding preference among the *Eucalyptus* species by *T. peregrinus* were tested using a Chi-sq. test (χ^2 , $p < 0.05$) (Zar 1999).

The *Eucalyptus* species were ranked according to *T. peregrinus* preferences with the data analyzed with paired comparison matrices to study dominance relationships (Appleby 1983; Boyd and Silk 1983; de Vries 1995, 1998; de Vries et al. 2006). A linearity index (Landau index) was determined and statistical significance was calculated to test for a linear hierarchy. The linearity in the preferences and statistical significance was also tested (de Vries 1995; Landau 1951). The Landau linearity index “h” was then calculated along with its associated value “d”, which is the average number of loops among all comparisons (Landau 1951). This linearity index “h” ranges from

0 to 1, where 1 implies a completely linear preference hierarchy among all the species tested and 0 indicates that all the species are equally preferred. The significance p value, associated to the linearity index, was then calculated (Appleby 1983).

Linearity calculations were performed with the R program (R Development Core Team 2006). The David's score (1988) was used to calculate an additional preference ranking, using the statistical package 'steepness' (Leiva and de Vries 2011).

The preferences detected were then tested against the chemical composition of the essential oil with regression analyses and GLM. The regression analyses were performed using Statistical Graphics SGWIN® software (Statgraphics Plus 4.0; Statistical Graphics Corporation, 1994–1999, Herndon, VA, USA). The Generalized Linear Model (GLM), using the 'Binomial' distribution to model the response variable (Feeding preference (%)) and the 'logit' link function, was used to analyze differences between the species with either the higher or the lower 1,8-cineole content (Pinheiro and Bates 2006). The threshold for significance was at $P < 0.05$. Statistical analyses were performed using Infostat (<http://infostat.com.ar>, di Rienzo et al. 2014). The two-way choice tests used the results from each paired comparison expressed as percentage of insects that preferred one species over another. The value of feeding preference (%) of one species or another was selected with the pair tested. Each paired *Eucalyptus* species was also identified as the species with the higher or the lower percentage of 1,8-cineole of the tested pair. The tested pairs, *E. maidenni* vs *E. globulus* and *E. grandis* vs *E. tereticornis*, were eliminated because their contents of 1,8-cineole were similar. The values of relative concentration of 1,8-cineole (%) in the essential oils were transformed and considered to be significantly different, if the 95% confidence limits did not overlap (GraphPad InStat® Vers. 3.01, Copyright 1992–1998, San Diego, California, USA).

Results

Insect preference The Chi-square analysis showed differences in the feeding preferences of *T. peregrinus* over the range of species tested. The two-way choice tests for the feeding preferences are shown in Fig. 1. These results of *Eucalyptus* feeding preferences were used to calculate the additional ranking of preferences

expressed and ordered by the David's score values (DS). The order of preferred *Eucalyptus* species was *E. viminalis* > *E. grandis* > *E. tereticornis* ≈ *E. dunnii* > *E. maidenni* > *E. globulus* (Fig. 2). The results of the statistical analysis show a Landau linearity index $h = 1$, and a complete absence of loops ($d = 0$). These results show that there is a significant ($P = 0.022$) and strict feeding preference hierarchy of *T. peregrinus* on the six *Eucalyptus* species tested.

Chemical composition of essential oils The qualitative and quantitative chemical composition of the essential oils of these *Eucalyptus* species is shown in Table 1. The main component of both *E. maidenni* and *E. globulus* essential oils was the monoterpene 1,8-cineole, with 88.9% and 89.9%, respectively. Moreover, the following major monoterpenes for these essential oils were α -pinene (3.2% and 4.4%), limonene (2.5% and 1.6%), and other minor constituents, respectively. The *E. dunnii* essential oil, in addition to 1,8-cineole as the main component (60.1%), had a complex composition, characterized by α -pinene (6.5%), globulol (5.2%), *trans*-pinocarveol (5.1%), α -terpinyl acetate (5.7%), and other minor compounds. In contrast, the essential oil extracted from *E. grandis* was dominated by α -pinene (48.8%) and 1,8-cineole (18.1%), followed by limonene (2.2%), *p*-cymene (7.9%), γ -terpinene (13.4%), α -terpineol (13.4%), and isobutyl isobutyrate (1.3%). The essential oil of *E. viminalis* presented three main compounds; 1,8-cineole (34.3%), globulol (25.1%) and α -pinene (17.3%), followed by limonene (2.2%), *p*-cymene (2.1%), aromadendrene (2.78%), *trans*-pinocarveol (0.9%), α -terpineol (1.8%), epiglobulol (3.9%), and viridiflorol (3.9%). The *E. tereticornis* essential oil showed *p*-cymene (30.1%), α -terpinyl acetate (24.5%), and 1,8-cineole (18.4%) as the main constituents. The compound 1,8-cineole was a common component in all samples, with different proportions according to the species. The percentage of 1,8-cineole of *E. maidenni* (88.9%) and *E. globulus* (89.9%) was similar and they differed from the other species tested. No differences were found between *E. grandis* (18.1%) and *E. tereticornis* (18.4%), but they differed from the other species tested. Moreover, the content of 1,8-cineole in the *E. viminalis* essential oil (34.3%) and *E. dunnii* (60.1%) were different from each other.

Relationship between feeding preferences and essential oils A negative correlation between David's score

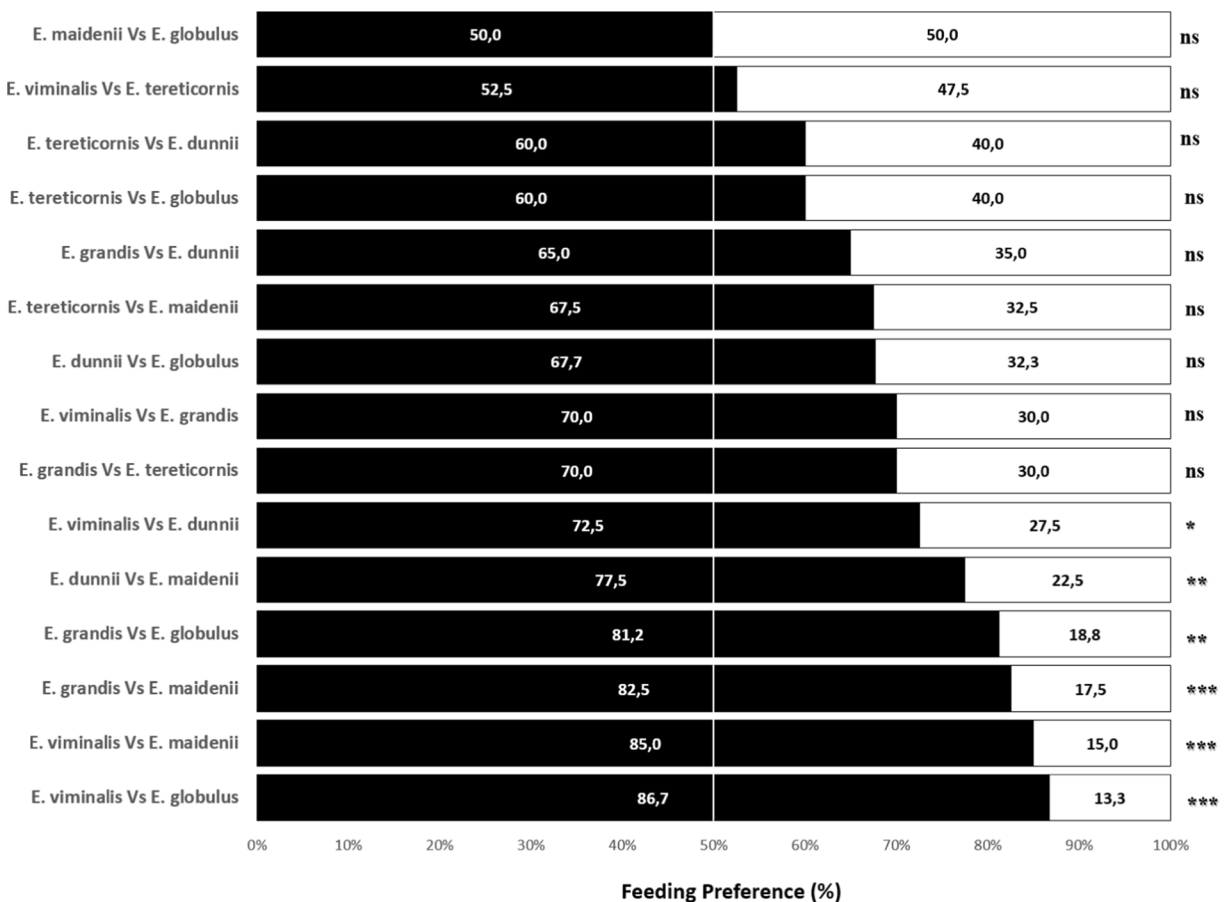


Fig. 1 The bars show the insects preference percentage of one *Eucalyptus* species over another in the pairwise comparison tests ($N = 20$). The black bar shows the first name and the white bar the

(feeding preference) and the 1,8-cineole concentration was observed ($P < 0.01$), but no correlation was found for the other components. Regression analysis showed a statistically significant relationship between feeding preference and the 1,8-cineole concentration (David's score = $7.44 - 0.14 \times 1,8\text{-cineole } (\%)$; $p < 0.05$; $R^2: 75.65$; $F: 12.43$; Correlation Coefficient: -0.86). No significant relationship between concentration and feeding preference was observed for *p*-cymene alone ($p > 0.1$). However, the *p*-cymene showed a statistically significant relationship ($p < 0.05$) when included in the previous linear model. Then, in the new multiple regression model, the two independent variables showed a significant relationship with *T. peregrinus*' feeding preference (1,8-cineole, $p < 0.01$; *p*-cymene, $p < 0.05$). Therefore, *p*-cymene was included in the model, resulting in the equation: David's score = $12.76 - 0.21 \times 1,8\text{-cineole } (\%) - 0.27 \times 291 \text{ } p\text{-cymene } (\%)$; ($p < 0.01$; $F: 31.14$; $R^2: 95.40$).

second one. Asterisks indicate significance levels in Chi-square tests (ns; non-significant., * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$)

Discussion

The feeding preference results presented here are consistent with field observations of insect density and damage, where *E. viminalis*, *E. grandis*, and *E. tereticornis* are the preferred species (Cuello et al. 2014; Jacobs and Nesser 2005), while the less preferred species have lower insect densities and damage (Ide et al. 2011). These results agree with *T. peregrinus*' performance on the same *Eucalyptus* species, with higher survival from first instar nymph to adult *T. peregrinus* on the most preferred species *E. viminalis*, and lowest in the two least preferred species, *E. maidenii* and *E. globulus* (Santadino unpublished).

The chemical composition of *Eucalyptus* essential oils was also similar to those of other studies. *Eucalyptus maidenii* has results similar to those obtained by Lucia et al. (2008), which contained 1,8-cineole (77.91%), α -pinene (5.84%), α -terpineol (6.04%), γ -

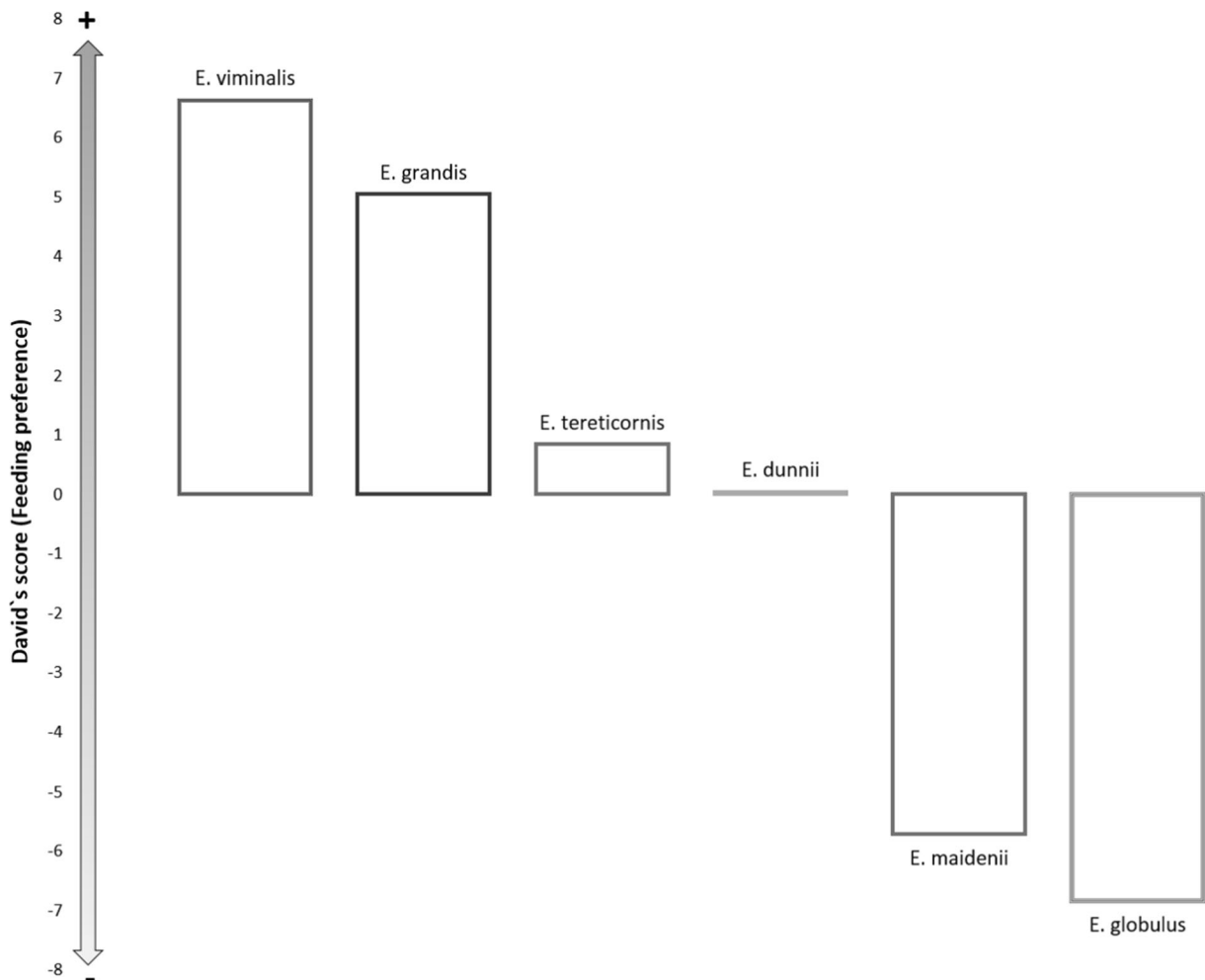


Fig. 2 Significant *T. peregrinus* preferences between the tested *Eucalyptus* species. Linearity value (h): 1 indicates a strict linear hierarchy in the preferences tested. d value: 0 indicates no loops in the linear hierarchy of preferences). $P = 0.022$

terpinene (4.82%), and *p*-cymene (2.31%). In the same work, they determined that the main compounds of *E. globulus* essential oil were 1,8-cineole (76.66%), α -pinene (11.09%) and α -terpineol (1.45%). Results from other published work show the chemical composition of *E. dunnii* essential oil similar to the one presented here (Lucia et al. 2008; Mizrahi et al. 1997; Toloza et al. 2010). *Eucalyptus grandis* had α - and β -pinene as the major components (Dagne et al. 2000), although α -pinene and 1,8-cineole (Lucia et al. 2007 and Mora Martinez et al. 2002), and γ -terpinene, ocimene, and β -pinene (Estanislau et al. 2001) were determined to be the main compounds in the essential oil of this plant. *Eucalyptus viminalis* showed a high concentration of 1,8-cineole (83.8–85.6%) (Müller da Silva et al. 2006), similar to those published by Lucia et al. (2008). Finally,

p-cymene was reported as the main oil component in *E. tereticornis* (Alitonou et al. 2004; Bossou et al. 2013).

The feeding preference of *T. peregrinus* was lower on *Eucalyptus* with high content of 1,8-cineole in their essential oil (*E. dunnii*, *E. maidenii*, and *E. globulus*).

In 13 of the 15 choice experiments with paired comparisons the insects preferred those with lower 1,8-cineole content. Therefore, the feeding preference was analyzed considering each *Eucalyptus* species tested as that species with the higher or lower percentage of 1,8-cineole of each tested pair. *Thaumastocoris peregrinus* individuals strongly preferred *Eucalyptus* species with lower concentrations of 1,8-cineole ($64.53 \pm 0.02\%$) in their essential oil ($28.10 \pm 0.02\%$) ($P < 0.0001$; Deviance residual: 135.3070; df:11) (Fig. 3).

Table 1 Chemical composition (%) of *Eucalyptus* essential oils analyzed by gaschromatography

Compounds	LRI ^a	Identification							
			Methods ^b	E.v.	E.gr.	E.d.	E.t.	E.m.	E.gl.
α -pinene	1017	LRI, MS, RC		17.27	48.82	6.49	2.37	3.21	4.38
α -thujene	1019	LRI, MS		-	-	-	0.66	-	-
camphene	1054	LRI, MS, RC		-	0.16	-	-	-	-
isobutylpropanoate	1075	LRI, MS		-	0.26	-	-	-	-
isobutylisobutyrate	1089	LRI, MS		-	1.34	-	-	-	-
β -pinene	1102	LRI, MS, RC		-	-	0.11	0.09	0.08	0.17
sabinene	1113	LRI, MS		-	-	-	0.33	-	-
α -felandrene	1154	LRI, MS		-	-	-	0.12	-	-
β -myrcene	1157	LRI, MS		-	-	-	0.20	0.16	-
α -terpinene	1167	LRI, MS, RC		-	-	-	0.11	-	-
limonene	1190	LRI, MS, RC		2.18	2.19	1.71	3.18	2.48	1.58
isobutylisopentanoicacidester	1194	LRI, MS		-	0.31	-	-	-	-
β -phellandrene	1198	LRI, MS		-	-	-	0.52	-	-
1,8-cineole	1205	LRI, MS, RC		34.32	18.06	60.13	18.41	88.98	89.96
trans-beta-ocimene	1229	LRI, MS		-	0.20	-	0.70	-	0.16
γ -terpinene	1235	LRI, MS, RC		-	13.42	0.28	1.72	0.07	-
<i>p</i> -cymene	1259	LRI, MS, RC		2.10	7.96	1.56	30.16	0.07	-
α -terpinolene	1272	LRI, MS		-	0.24	-	-	-	-
isoamylisovalerate	1294	LRI, MS		-	0.20	0.10	-	-	-
α -thujone	1432	LRI, MS		-	-	-	0.19	-	-
linalool	1553	LRI, MS, RC		-	0.37	-	0.44	0.03	-
pinocarvone	1567	LRI, MS		-	-	2.15	-	-	0.56
fenchol	1588	LRI, MS		-	0.25	0.20	-	-	-
<i>trans</i> -caryophyllene	1593	LRI, MS		-	-	0.12	-	0.08	-
aromadendrene	1605	LRI, MS		2.78	-	-	-	-	-
4-Terpineol	1605	LRI, MS, RC		-	0.96	0.52	2.02	0.56	0.26
<i>allo</i> -aromadendrene	1647	LRI, MS		-	-	-	-	0.03	-
<i>trans</i> -pinocarveol	1663	LRI, MS		0.90	-	5.13	-	0.08	0.55
cryptone	1675	LRI, MS		-	-	-	1.72	-	-
α -terpinylacetate	1701	LRI, MS		-	-	5.71	24.46	-	-
α -terpineol	1705	LRI, MS, RC		1.82	2.64	1.54	-	2.70	0.78
borneol	1711	LRI, MS, RC		-	0.66	0.24	-	-	-
phellandranal	1726	LRI, MS		-	-	-	0.30	-	-
carvone	1742	LRI, MS		-	-	-	0.11	-	-
cuminaldehyde	1789	LRI, MS		-	-	-	2.37	-	-
cis-p-Mentha-1(7),8-dien-2-ol	1807	LRI, MS		-	-	0.89	-	-	0.13
trans-Carveol	1844	LRI, MS		-	-	0.25	-	-	0.06
cis-Carveol	1876	LRI, MS		-	-	0.09	-	-	-
trans-p-Mentha-1(7),8-dien-2-ol	1899	LRI, MS		-	-	0.64	-	-	0.10
epiglobulol	2030	LRI, MS		3.93	-	0.76	-	0.08	0.09
globulol	2098	LRI, MS		25.10	-	5.21	0.50	0.53	0.55
viridiflorol	2105	LRI, MS		3.98	-	0.62	-	0.11	0.08
spathulenol	2140	LRI, MS		-	-	-	5.06	-	-
				94.38	97.99	94.38	95.69	99.22	99.37

^a LRI: Linear retention index of each component in the essential oils (DB-WAX column), ^b MS: Mass spectrum comparison with bibliography, CS: Comparison of retention time with a chemical standard, LRI: comparison with those of bibliography. ^c Essential oils: E.v.: *E. viminalis*; E.gr.: *E. grandis*; E.d.: *E. dunnii*; E.t.: *E. tereticornis*; E.m.: *E. maidenii*; E.gl.: *E. globulus*

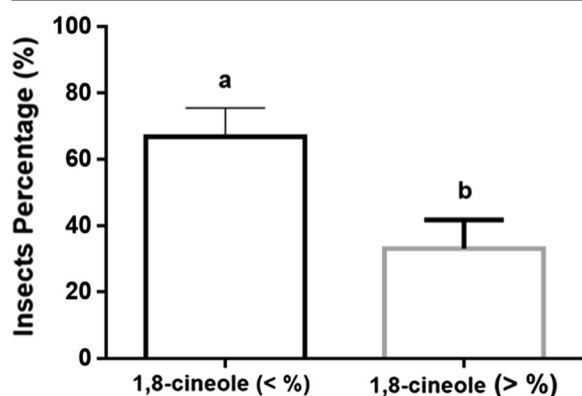


Fig. 3 Percentage of insects preferring the species with the higher 1,8-cineole over the one with the lower content for all two-way choice tests. N (total): 280 insects. $P < 0.0001$; Deviance residual: 135.3070; df:11. 1,8-cineole (>%) represents the species with the higher content of 1,8-cineole and 1,8-cineole (<%) represents the species with lower content of 1,8-cineole in the essential oils for all the two-way choice tests. The bars marked with different letters are significantly different

The 1,8-cineole is a natural bioactive repellent. The no-choice, choice/no-choice, and manipulative experiments with leaf consumption by Christmas beetles (*Anoplognathus pallidicollis* Blanchard) was negatively correlated with the concentrations of 1,8-cineole, and artificial increases in the concentration of this compound reduced leaf consumption (Matsuki et al. 2011). This effect was also observed for *Eucalyptus* trees with a high 1,8-cineole content showing lower susceptibility to herbivory by Christmas beetles (Edwards et al. 1993). In addition, the bioactivity of 1,8-cineole against a wide spectra of insect pests has been reported. The 1,8-cineole, the major monoterpenoid present in the volatile oil of *Hemizonia fitchii* A. Gray (Asteraceae), was moderately effective as a feeding repellent and highly effective as an ovipositional repellent against *Aedes aegypti* L. adults, the dengue and yellow fever mosquito (Klocke et al. 1987).

The 1,8-cineole evoked strong repellent action against *Sitophilus granarius* L. and *S. zeamais* Motsch, but was moderately repellent to *Tribolium castaneum* (Herbst) and *Prostephanus truncatus* Horn., (Obeng-Ofori et al. 1997). As a main compound of *Cyperus rotundus* L. and *Eucalyptus robusta* labill essential oils, the 1,8-cineole repelled the German cockroach *Blattella germanica* L. (Liu et al. 2011). The essential oil of *Tanacetum vulgare* L. had a strong repellency effect on *Ixodes ricinus* L., the castor bean tick (Pålsson et al. 2008).

The fumigant toxicity of *Eucalyptus* essential oils against different insects was also attributed to the 1,8-

cineole for *Musca domestica* L. (Tarelli et al. 2009), *Rhodnius prolixus* Stahl (Sfara et al. 2009), *Pediculus humanus capitis* De Geer (Tolozza et al. 2008), *Haematobia irritans* L. (Juan et al. 2011), *A. aegypti* (Lucia et al. 2008, 2009, 2012), and *B. germanica* (Alzogaray et al. 2011).

Unlike the 1,8-cineole, the *p*-cymene concentration was higher in the most preferred *Eucalyptus* species (*E. viminalis*, *E. grandis*, and *E. tereticornis*). Some essential oil constituents exhibit insect attractant properties, as *p*-cymene with attractant activity in the laboratory on olive fly females *Bactrocera oleae* (Gmelin) (Delrio et al. 1983) and the blueberry maggot fruit fly, *Rhagoletis mendax* Curran (Lugemwa et al. 1989). In addition, the asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama was attracted to the young flush of citrus plants rich in *p*-cymene, where it feeds on the sap and uses it as a site for mating, oviposition, and development of the nymphs (Coutinho-Abreu et al. 2014). The attractiveness of airborne terpenes from mango fruit (*Mangifera indica* L.) to *Ceratitis capitata* Wiedemann was evaluated in laboratory cages (Hernández-Sánchez et al. 2001). This monoterpene was also described as a phagostimulant compound of the horse-chestnut leafminer *Cameraria ohridella* Deshka & Dimic (Kordan et al. 2013). Plant breeding may change the characteristics of the plant reducing insect pest levels but it has received low attention in most breeding programs, with priority given to yielding capacity and product quality.

The interspecific hybrids of *Eucalyptus* have been used in forestry for decades (Griffin et al. 1988), mainly focusing in propagation, coppicing, frost, drought and salt resistance, wood density, resistance to pests and pulp yield (Potts and Dungey 2004). The main *Eucalyptus* hybrid plantations are in Brazil and other South American countries, besides Congo, China, Indonesia, South Africa, and Asia (Dungey and Nikles 2000).

Eucalyptus hybridization can improve the quality and quantity of essential oils (Farah et al. 2002), because differences in the terpene profiles from parental species (*E. grandis*, *E. tereticornis* and *E. camaldulensis*) and their hybrids (*E. grandis* x *E. tereticornis* and *E. grandis* x *E. camaldulensis*; respectively) were reported. The essential oils of interspecific hybrid progeny had intermediate compositions between their parents (Tolozza et al. 2010). This was also reported for the interspecific hybrid of *E. grandis* x *E. camaldulensis* (Doran et al. 2002) and in the hybrids *E. ovata* x *E. crenulata* and *E. alpina* x *E. baxteri* (Farah et al. 2002).

Moderate to strong genetic control of oil concentration (yield) and composition has been reported (Külheim et al. 2015; Naidoo et al. 2013; Oates et al. 2015; Webb et al. 2013). Six of the uninfested species by *Glycaspis brimblecombei* Moore, exhibited very high contents of the terpene 1,8-cineole, ranging from 49 to 91% of the total oils (Lucia et al. 2016). Moreover, the infestation of *E. tereticornis* (446 lerps/individual) and *E. camaldulensis* (356 lerps/individual) was higher than the hybrids *E. grandis* x *E. tereticornis* and *E. grandis* x *E. camaldulensis*, with 25 and 21.4 lerps/individual respectively. Their results show that the abundance of this insect was lower in those *Eucalyptus* species with very high content of 1,8-cineole content.

Using interspecific hybrids with high 1,8-cineole concentrations in their essential oils could reduce the need of controlling *T. peregrinus*. However, this should be considered with caution, and the relationship between feeding preference and the essential oil chemical composition should be further studied to test feeding preference using pure 1,8-cineole and *p*-cymene.

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