

Volatile Organic Compounds of Conspecific-Damaged *Eucalyptus benthamii* Influence Responses of Mated Females of *Thaumastocoris peregrinus*

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Abstract Plants respond to herbivory by synthesizing and releasing novel blends of volatile organic compounds (VOCs). Natural enemies are attracted to these VOCs, but little is known about the effects of these chemicals on the herbivores themselves. We studied the effect of *Thaumastocoris peregrinus* herbivory on VOCs released by *Eucalyptus benthamii* plants and the responses of this herbivore to the VOCs. In total, 12 compounds released by *E. benthamii* were identified. Five compounds (β -pinene, linalool, 9-epi-(*E*)-caryophyllene, viridiflorol, and one unidentified compound) emitted after herbivore and mechanical damage were not detected in collections from undamaged plants. The three most abundant VOCs, α -pinene, aromadendrene, and globulol, were released in greater quantities from herbivore-damaged plants compared to plants with mechanical damage, which, in turn, released greater amounts than undamaged (control) plants. The VOCs emitted after herbivore damage did not differ during the photophase and scotophase in either quantity or quality. In an olfactometer, mated female *T. peregrinus* showed a preference for undamaged plants over herbivore-damaged plants, and also for hexane over α -pinene at an amount equivalent to that released by a herbivore-damaged plant. In the olfactometer, virgin females did not exhibit any preference between conspecific-damaged or undamaged plants.

Keywords Bronze bug · Forestry pest · Herbivore-induced plant volatiles (HIPVs) · terpenes · VOCs · Heteroptera · Thaumastocoridae

Introduction

Plants respond to herbivory by synthesizing and releasing novel blends of volatile organic compounds (VOCs) (Turlings et al., 1990), which may be specific to the herbivore (Turlings and Wäckers, 2004). These specific VOC blends may also attract specific natural enemies (Turlings and Wäckers, 2004). However, to date only a few studies have explored the response of herbivores themselves to the induced plant volatiles. For example, female moths of *Heliothis virescens* (Lepidoptera, Noctuidae) are repelled by volatiles emitted during the night by tobacco plants, *Nicotiana tabacum* (Solanaceae), infested with conspecific larvae, suggesting that females avoid ovipositing on damaged plants (De Moraes et al., 2001). Likewise, four-arm olfactometer assays revealed that alate *Aphis gossypii* Glover, 1877 (Hemiptera, Aphididae) spent more time in the presence of odor from uninfested cotton seedlings compared to clean air, but less time in the presence of odor from *A. gossypii*-infested plants (Hegde et al., 2011). Herein, we studied a new system of the herbivore *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) and its host plant *Eucalyptus benthamii* (Myrtaceae).

Known as the bronze bug, *T. peregrinus* is a sap-feeding pest of *Eucalyptus* plantations in several countries (e.g., South Africa, Argentina, Uruguay, and Brazil). It was probably introduced accidentally to South America from Australia (Nadel and Slippers, 2009; Wilcken et al., 2010). In Brazil, there is no effective method for controlling this pest (Wilcken et al., 2010); attempts at introducing and establishing the egg parasitoid *Cleruchoides noackae* (Hymenoptera, Mymaridae) are underway.

So far, the interactions of *T. peregrinus* and its host plant have not been investigated. Thus, we aimed to identify and compare the VOCs released by undamaged, herbivore-

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damaged and mechanically damaged *E. benthamii* plants. The quantitative and qualitative differences among the VOCs released during the photophase and scotophase, following herbivory, were also compared. Finally, we tested the responses of mated and virgin females to these VOCs. The ultimate aim of this work is to develop new control measures for this pest in *Eucalyptus* spp. plantations.

Methods and Materials

Rearing of *Thaumastocoris peregrinus* Insects were obtained from a colony kept at EMBRAPA Forestry, Colombo, Paraná, and maintained at 25 ± 2 °C and a 12 L: 12 D photoperiod in the Laboratório de Semioquímicos, Departamento de Química of the Universidade Federal do Paraná (UFPR). Adults and nymphs were reared on branches of *E. benthamii* inside acrylic boxes (30×30×30 cm) until use. This plant is suitable for the development of *T. peregrinus*. To obtain adults for experimentation, fifth instars were placed individually on a leaf disc of *E. benthamii* in a small, round plastic container (2.5 cm diam.), containing a gel (Hydroplan–EB/HyC, produced by SNF S.A. Floger) to maintain humidity and to discourage insects from leaving the leaf perimeter. Leaf discs were changed every other day. To obtain mated females, couples were allowed to form within 2 d of emergence. The laying of eggs confirmed the mated status of females used in the bioassays. Using the same conditions as for nymphs, virgin and mated females were grouped separately in Petri dishes (5 cm diam.) until experiments were conducted.

Volatile Collection Headspace volatile collections were performed using glass chambers (11.5×35 cm), inside a controlled-temperature room (24 ± 2 °C, 12 L: 12 D). Samples were collected using a humidified and charcoal-filtered airflow at $1 \text{ l} \cdot \text{min}^{-1}$. VOCs were captured on glass columns containing 20 mg of HayeSep Q 80–100 mesh (Altech, Lokeren, Belgium), and eluted with 180 μl of double-distilled HPLC-grade hexane (Zarbin et al., 1999). An internal standard (IS) of tridecane (C13; 20 μl of a solution of 100 ppm) was added to the final extract. The final IS concentration was calculated for each extraction, and the extract was quantified based on IS peak area. Plants used in experiments were approximately 120 d old (28–35 cm, with 10–12 leaves). They were kept inside the glass chambers for 2 d to acclimate before beginning the collection of VOCs. To reduce the capture of volatiles from soil, plant roots were covered with aluminium foil.

We tested the responses of plants to herbivory and mechanical damage. For both treatments, three replicates were performed, with an undamaged plant as a control. For the herbivory treatment, 10 male *T. peregrinus* were placed

inside each glass chamber. After 24 h of feeding, males were removed. The collection of VOCs began after the removal of males. Extracts were produced every 24 h for 5 d. For the mechanical-damage treatment, a leaf of *E. benthamii* was cut in half, and the VOCs were collected beginning 24 h later. Collections were produced every 24 h over 4 d. Collections were performed at the same time each day until the end of the experiment.

We also analyzed the emission of VOCs from plants after herbivory, during the photophase and scotophase, using the same methodology described, except that collectionn were made every 12 h, according to the photoperiod, over 5 d. All collections were initially analyzed by gas chromatography (GC) and stored at -20 °C until further analysis/testing.

Analytical Procedures Collections were analyzed by gas chromatography/mass spectrometry (GC/MS) (on a Shimadzu QP 2010 Plus) and gas chromatography-infrared spectroscopy (GC/FTIR). The GC and GC/MS were equipped with a RTX-5 column (30 m×0.25 mm i.d., 0.25 mm film thickness; Restek, Bellefonte, PA, USA). Injections of 1 μl were performed in the splitless mode, with an injector temperature of 250 °C. The column oven temperature was held at 40 °C for 1 min, increased to 180 °C at 5 °C·min⁻¹, then increased to 250 °C at 10 °C·min⁻¹, and held for 2 min. Helium was the carrier gas at a column head pressure of 170 kPa. The same parameters were used for all analyses. The GC/FTIR spectra were recorded on a DiscovIR Spectra Analysis spectrophotometer coupled to a Shimadzu GC (model 2010). The GC used helium as carrier gas, was operated in splitless injection mode, and was equipped with a DB-5 (0.25 μm , 0.25 m×30 m; J&W Scientific, Folsom, CA, USA) capillary column. The column oven temperature program was the same as that used for GC/MS analysis. A liquid-nitrogen-cooled photoconductive mercury-cadmium-telluride (MCT) detector was used with a FT/IR resolution of 8 cm^{-1} . The mean quantity of VOCs released was calculated in ng, based on the peak areas of the GC chromatograms of the three replicates of treatments.

Identification of Compounds VOCs were identified by mass and infrared spectra, Kovat's indices (KI), and co-injections with commercially available standards or essential oil extracts. α -Pinene, β -pinene, limonene, *p*-cymene, and linalool were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Aromadendrene, globulol, epiglobulol, viridiflorol, and 9-epi-(*E*)-caryophyllene were identified by mass spectral analyses and KIs. Aromadendrene, globulol, and epiglobulol are constituents of the essential oil of *E. benthamii* (Döll-Boscardin et al., 2010), and were further identified by co-injection of this oil. The essential oil used for the co-injections was the same produced and evaluated by Döll-Boscardin et al. (2010).

Bioassays These were conducted in a 17-cm-long Y-tube olfactometer with two 13-cm arms. An odor source was placed, inside a glass tube (1 cm diam., 4 cm long), at the end of each arm. For each replicate, a new filter paper (1 cm²), with treatment (1 μ l of treatment+9 μ l of hexane) was used as an odor source for mated and virgin females (3–20 d old). α -Pinene solutions (Aldrich Chemical Company) were prepared so that their concentrations were equivalent to that in extracts from plants that had herbivore damage (180 ng) or no damage (15 ng). We tested the attraction of mated or virgin females to: A) collection of control plant vs. collection of plant with herbivore damage, B) plant with herbivore damage vs. control plant, and C) α -pinene (180 ng) vs. hexane. For the mated females, a further trial was run: D) α -pinene (15 ng) vs. hexane. The response of an insect was considered positive if a female chose one of the arms of the olfactometer (3 cm limit). The time limit for an insect response was 10 min. For each female type (mated or virgin), 20–35 replicates were performed. α -Pinene solution was chosen in this experiment because it is the major compound released by herbivore-induced plants, and has been described as an insect repellent (Batish et al., 2008; Liu et al., 2011).

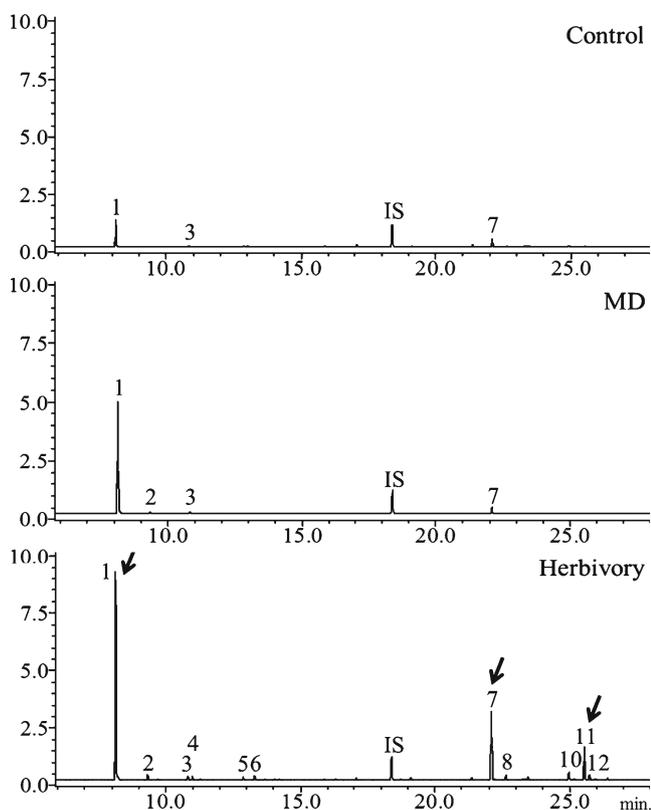


Fig. 1 Representative chromatograms of volatiles (1–12) released by *Eucalyptus benthamii* plants 24 h following different treatments: undamaged (control), herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae), and mechanical damage (MD). The arrows highlight the increases in the three major compounds produced after herbivory (compounds 1, 7, and 11)

Statistical Analyses A multivariate analysis of variance (*MANOVA*) was performed on all compounds, comparing treatments (herbivory, mechanical damage, and control), and also comparing the herbivore-induced VOCs emitted during the photophase and scotophase. Individual *ANOVAs*, followed by the *post-hoc Tukey test*, were performed for the main compounds (α -pinene, aromadendrene, and globulol) to compare effects of treatment and time. *ANOVAs* and *MANOVAs* were performed using R version 2.13 (R Development Core Team, 2011). Statistical analyses for the bioassays were performed using the *Binomial Test* in the statistical program BioEstat 3.0 (Ayres et al., 2003).

Results

VOCs of *E. benthamii*: Identification, Analysis and Comparison of Treatments GC profiles of the collections revealed major differences between the control, herbivory, and mechanical damage treatments. In particular, three compounds (1, 7, and 11) were present in much larger amounts in the herbivore-damaged plant collections compared to those of the control and mechanically damaged plants (Fig. 1). *MANOVA* revealed that the mean amount of VOCs emitted after herbivore damage was different (Wilks' $\lambda=0.063$; $F=5.74$; $P<0.001$) from that from control and mechanically damaged plants (Fig. 2).

The same approach was employed in the identification of all the compounds. However, identification of compounds 1, 7, and 11 only is described. Retention times (Rts) and Kovats Indices (KIs) of the three compounds on the RTX-5 column were: 1: Rt=8.13 min, KI=928; 7: Rt=22.09 min, KI=1448; and 11: Rt=25.5 min, KI=1593. The mass spectrum of compound 1 showed a base peak at m/z 93, fragments at m/z 41,

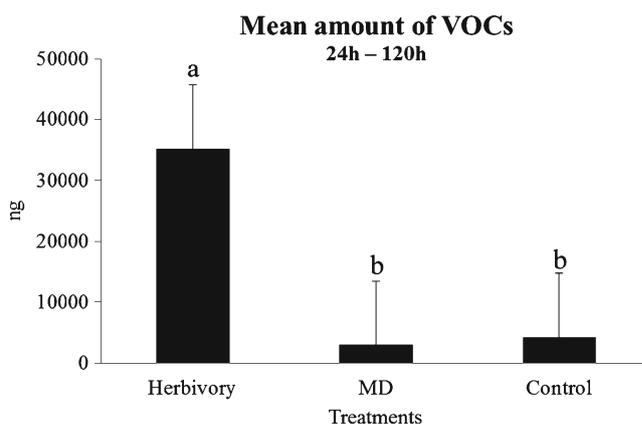


Fig. 2 Mean amount of volatile organic compounds (VOCs) released by *Eucalyptus benthamii* plants (24–120 h following treatment) induced by herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae), compared with plants with no (control) or mechanical damage. Different letters indicate differences ($P<0.05$) among treatments

m/z 77, m/z 91, m/z 105, m/z 121, and a molecular ion of 136 Da (Fig. 3, 1A). Comparison of this mass spectrum with the NIST library revealed that compound **1** might be the monoterpene α -pinene. The most important signal in the GC/FTIR spectrum of **1** (Fig. 3, 1B) was a *cis* C-H stretch asymmetric vibration band at 3028 cm^{-1} (Smith, 1999), supporting the identity of the compound as α -pinene. The chemical structure was confirmed after co-injection with authentic α -pinene.

The mass spectrum of compound **7** revealed a base peak at m/z 91, fragments at m/z 41, m/z 105, m/z 119, m/z 133,

m/z 161, and a molecular ion of 204 Da (Fig. 3, 7A), which strongly suggested a sesquiterpene with formula $\text{C}_{15}\text{H}_{24}$. The GC/FTIR spectrum (Fig. 3, 7B) showed the existence of a vinyl CH_2 asymmetric stretch band at 3083 cm^{-1} and a $\text{C}=\text{C}$ stretch band at 1638 cm^{-1} (Smith, 1999). A comparison of the mass spectrum and KI of the natural product with literature values (Adams, 2007) suggested that the compound might be aromadendrene. Co-injection with an essential oil extract of *E. benthamii* containing aromadendrene (Döll-Boscardin et al., 2010) confirmed its identity.

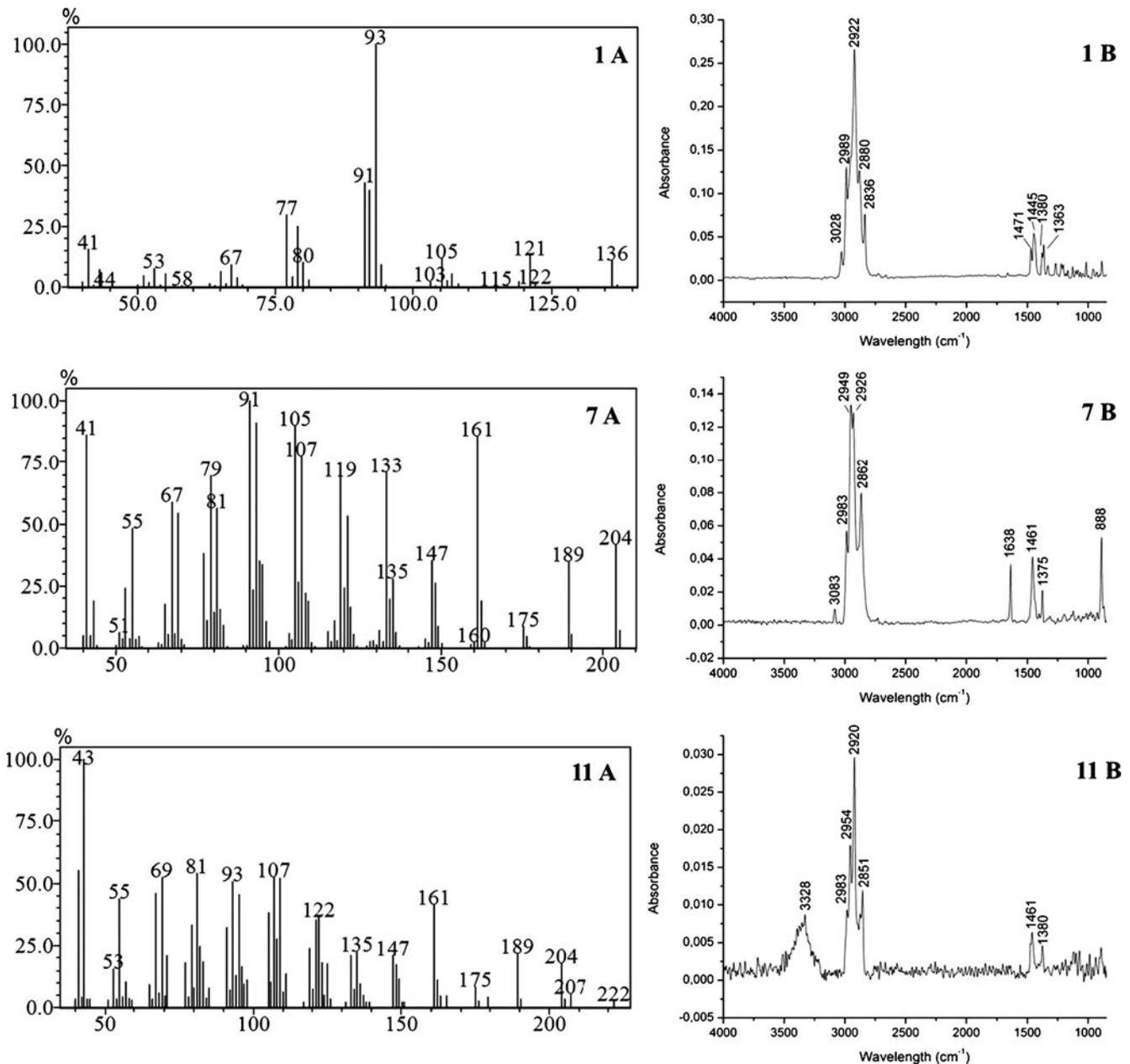


Fig. 3 Mass (left, 1A, 7A, and 11A) and infrared (right, 1B, 7B, and 11B) spectra of the three major compounds (**1**= α -pinene, **7**=aromadendrene, and **11**=globulol) produced by *Eucalyptus benthamii* after herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae)

The mass spectrum of compound **11** had a base peak at m/z 43, fragments at m/z 69, m/z 81, m/z 109, m/z 122, m/z 161, m/z 189, m/z 204, and a molecular ion of 222 Da (Fig. 3, 11A), suggesting a sesquiterpenoid with formula $C_{15}H_{26}O$. GC/FTIR analysis revealed a signal at 3328 cm^{-1} , indicative of an O–H stretch of an alcohol (Smith, 1999) (Fig. 3, 11B). Additionally, we observed no band indicative of a terminal double bond in the IR spectra, as apparent in the aromadendrene IR data. The higher retention time of **11**, together with KI and mass spectral data from the literature (Adams, 2007), suggested that this compound was a sesquiterpenoid closely related to aromadendrene **7**, namely globulol. Co-injection with *E. benthamii* essential oil extract, which contains globulol (Döll-Boscardin et al., 2010), confirmed the identification.

A total of twelve compounds, mainly monoterpenes and sesquiterpenes, were detected in the headspace extracts of *E. benthamii* plants (Fig. 4). The amounts of the major compounds **1**, **7**, and **11** emitted by plants 24 h after herbivory increased approximately 11-fold, 69-fold, and 59-fold, respectively, over that from control plants. After 120 h, the increases, relative to the control, were only 3-fold, 10-fold, and 11-fold, respectively (Table 1). Five other compounds, not detected in control plants, were released by plants after

herbivory: β -pinene **2**, linalool **5**, 9-epi-(*E*)-caryophyllene **8**, viridiflorol **12**, and the minor compound **9**. These compounds also were produced in lower quantities following mechanical damage, than in plants with 24 h of herbivory. For example, β -pinene was emitted 150-fold more after herbivory than by mechanical damage (Table 1). For most of the compounds, the amounts emitted increased after 24 h of herbivory, and then decreased slowly in subsequent days.

For α -pinene, the main compound released by *E. benthamii*, the amounts ranged from $2.9\pm 1.1\text{ }\mu\text{g}$ in collections of control plants to $36.1\pm 0.9\text{ }\mu\text{g}$ in collections of plants after 24 h of herbivory. Control and mechanically damaged plants did not emit any additional compounds from those emitted by herbivore-damaged plants. Although the amount of α -pinene emitted increased after mechanical damage, it was still lower than after herbivory; this effect was similar for the other compounds. Herbivore-damaged plants differed from control and mechanically damaged plants for amounts of all three major compounds (α -pinene, aromadendrene, and globulol; $P<0.001$). For α -pinene ($P=0.011$) and globulol ($P<0.001$), collections after the first 24 h, for each treatment, did not differ from those at 48 h, but differed from those from 72 to 120 h. The amounts of

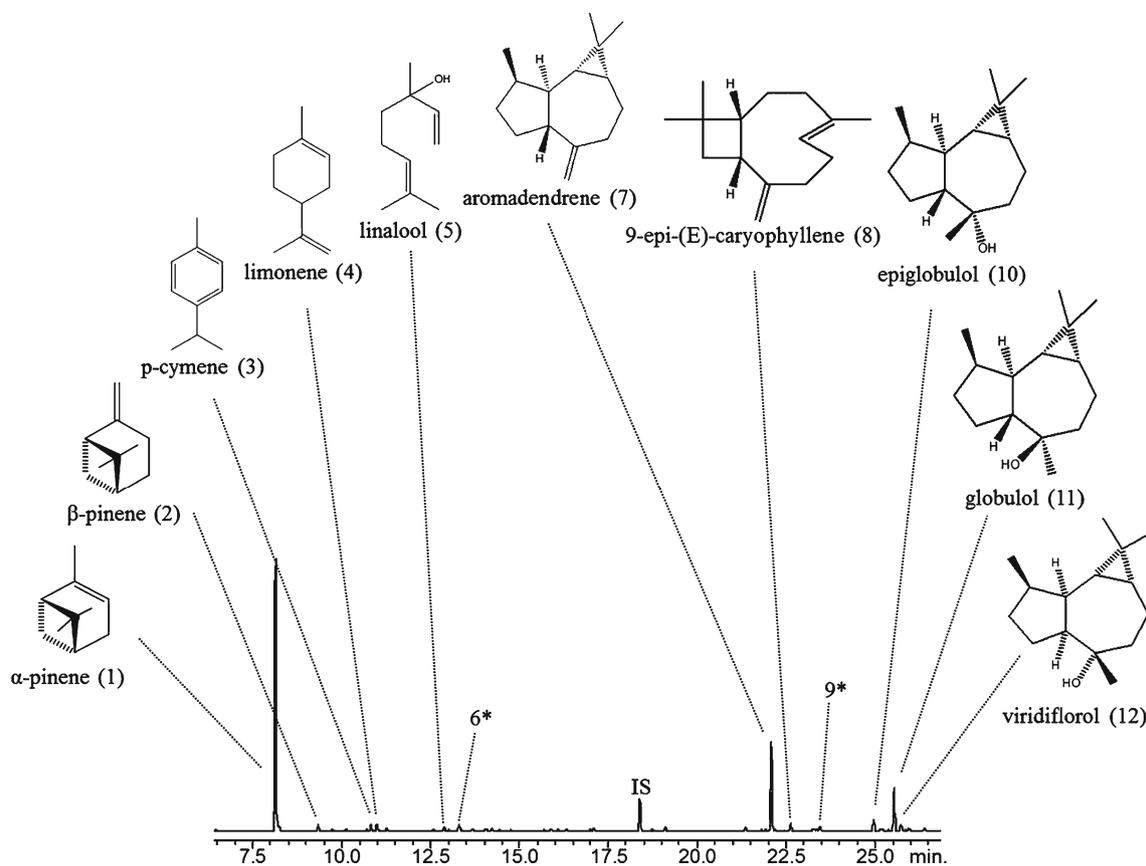


Fig. 4 Volatiles released by *Eucalyptus benthamii* plants 24 h after herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae). *Unidentified compounds. IS=internal standard

Table 1 Identification and quantification (mean±SE) (ng) of compounds released by *Eucalyptus benthamii* plants from 24 to 120 h following treatment [control (untreated), herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae), mechanical damage]. N=peak number (see Fig. 4), RT=retention time, nd=non-detectable, X= unidentified compound, *=compounds tentatively identified based on mass spectra and Kovats Indices

N	RT	KI ^a	KI - Lit. ^b	Compounds	Control		MD		Herbivory			
					Mean SE	Mean SE	Mean SE	Mean SE	24 h	48 h	72 h	96 h
1	8.134	928	939	α-pinene	2900.01±1159.79	2141.15±656.70	36116.41±921.17	20051.63±2471.11	24009.17±4171.83	23833.09±5297.19	11181.55±1699.73	
2	9.331	977	979	β-pinene	nd	4.53±2.78	766.47±16.62	332.44±50.45	351.66±34.79	315.24±56.71	152.31±36.80	
3	10.603	1025	1024	para-cymene	682.23±287.62	344.46±101.98	161.51±49.55	124.65±36.90	1405±580.89	93.29±25.58	25.56±8.58	
4	10.822	1030	1029	limonene	280.41±265.93	46.79±14.14	760.47±61.72	316.61±61.01	406.74±82.54	488.78±121.65	209.72±43.98	
5	12.873	1102	1096	linalool	nd	15.58±8.59	532.28±144.42	197.04±56.38	204.74±82.54	165.29±50.68	105.56±36.76	
6	13.315	1116	x	x	16.51±5.85	129.87±42.67	74.83±16.92	69.75±19.03	83.77±25.38	174.31±63.65	201.62±78.46	
7	22.093	1448	1441	aromadendrene	240.15±98.49	326.80±110.09	16502.26±1315.42	6272.95±1084.73	4964.08±1103.31	3310.29±874.76	2360.70±659.76	
8	22.631	1470	1466	epi-(E)-cartholene*	nd	2.11±2.11	1492.65±156.77	429.73±74.04	317.41±77.55	179.99±57.24	117.26±40.34	
9	23.447	1505	x	x	nd	6.20±6.07	732.82±221.84	211.00±49.37	160.65±38.40	75.89±22.87	42.69±17.26	
10	24.937	1569	1564	epiglobulol	23.29±18.03	0.27±0.27	2440.78±302.74	777.04±58.15	242.84±80.55	472.32±87.24	223.23±43.70	
11	25.532	1593	1590	globulol	69.12±31.23	16.87±13.04	3845.56±492.83	815.42±240.52	487.32±182.08	1442.62±432.06	786.18±253.86	
12	25.719	1602	1592	viridiflorol*	nd	6.42±3.85	502.36±135.31	11.49±5.14	0.94±0.42	150.59±67.35	81.17±36.30	
Total (ng) - Control					4211.72±1423.54							
Total (ng) - Mechanical damage					3041.05±821.15							
Total (ng) - Herbivory							63928.40±4341.64		29609.75±8456.30		32634.31±12396.38	
							30701.69±13070.93		30701.69±13070.93		15487.54±3584.20	

^a Kovats indices on a DB-5 column; ^b Kovats indices on a DB-5 column for compounds 1–9, and 11–12 (from Adams, 2007), and compound 10 (from Su et al., 2006)

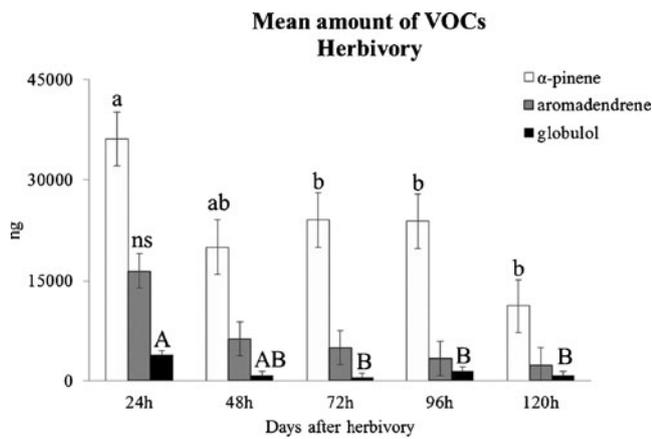


Fig. 5 Comparison of mean amounts (\pm SEM) of the main volatile organic compounds (VOCs; α -pinene, aromadendrene, and globulol) released by *Eucalyptus benthamii* plants from 24 to 120 h after herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) with those released by control plants. Different letters indicate differences ($P < 0.05$) in amount of each compound among treatments

these compounds in the 48-h collection did not differ from the amounts in extracts from the following days (72–120 h). For aromadendrene, there were no differences ($P = 0.778$) in amount in collections from 24 to 120 h (Fig. 5).

Effects of Photophase Period on Emission of Herbivore-induced VOCs In herbivore-damaged plants, all compounds were released during both the photophase and scotophase (Table 1). *MANOVA* indicated that collections from the photophase and scotophase were not different at any time (treatments: Wilks' $\lambda = 0.24$; $F_{12,2} = 0.41$; $P = 0.87$; time:

Wilks' $\lambda = 0.006$; $F_{45,6.7} = 0.65$; $P = 0.81$; time and treatment: Wilks' $\lambda = 0.009$; $F_{45,6.7} = 0.55$; $P < 0.88$) (Fig. 6).

Bioassays The attraction of mated and virgin females to different extracts and plants was tested. Bioassays revealed that mated females preferred the control plant collection ($P = 0.019$; Fig. 7a) and the control plant odor ($P = 0.03$; Fig. 7b) over those from the herbivore-damaged plant extract and herbivore-damaged plant, respectively. Virgin females did not exhibit a preference between control and herbivore-damaged plant collections ($P = 0.62$; Fig. 8a), and between herbivore-damaged and control plant odors ($P = 0.85$) (Fig. 8b). Mated females were attracted to hexane over α -pinene (180 ng) ($P = 0.004$; Fig. 7c); however, they did not show any preference to hexane over α -pinene (15 ng) ($P = 0.5$; Fig. 7d). Virgin females did not show a preference for hexane over α -pinene (180 ng) ($P = 0.5$; Fig. 8c). Non-responsive females were less than 30 % of the total females tested in each bioassay.

Discussion

Isoprene, and monoterpenes in particular, are the predominant compounds in biogenic emissions in nature (Kesselmeier and Staudt, 1999). Among *Eucalyptus* species, α -pinene is the main monoterpene emitted, followed by 1,8-cineole and β -pinene (He et al., 2000). In this study, α -pinene was the most abundant compound, followed by aromadendrene (a sesquiterpene) and globulol (a sesquiterpenoid), released by *E. benthamii* in all treatments. These three compounds were emitted in larger amounts after herbivory by *T.*

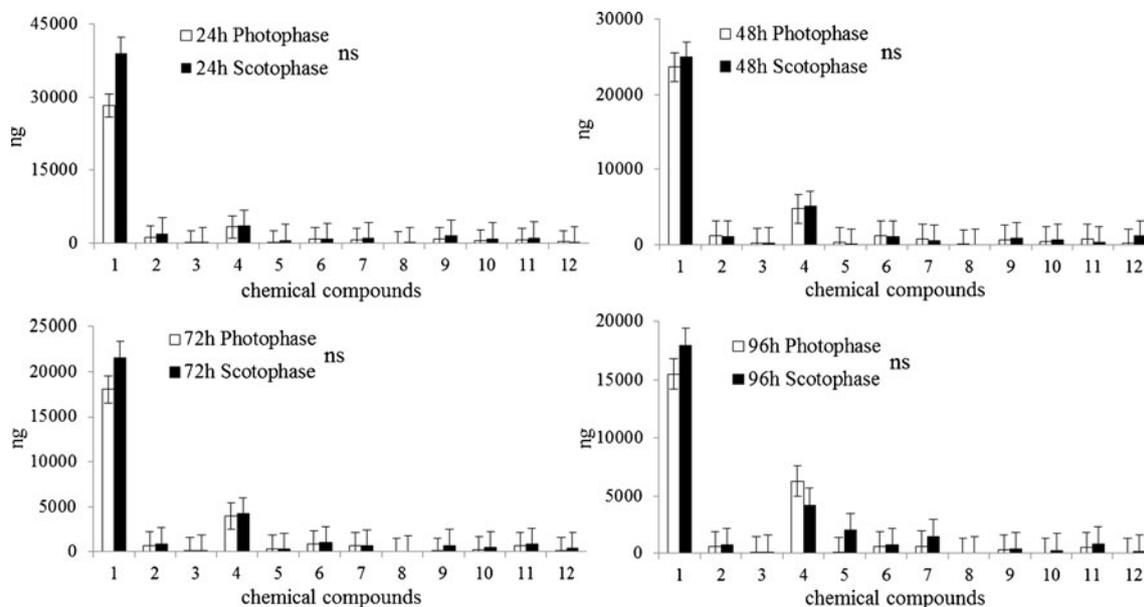
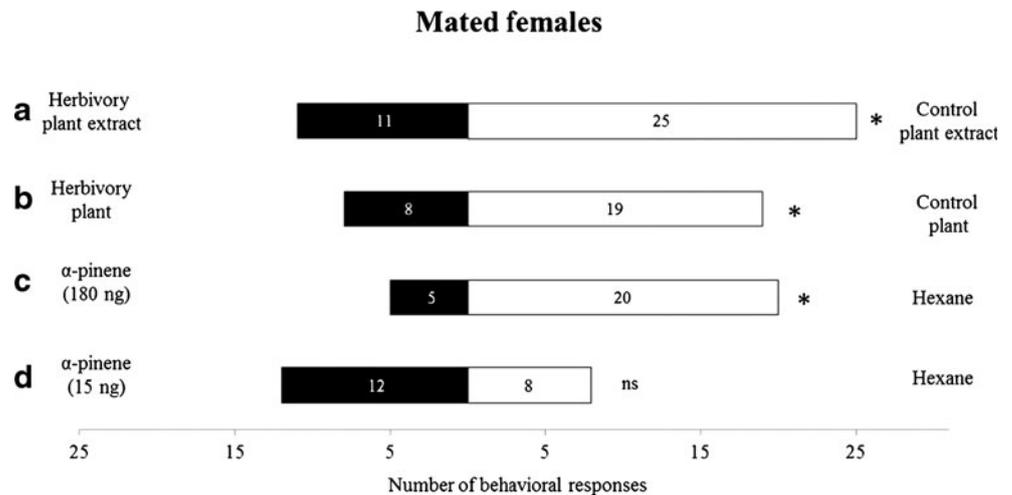


Fig. 6 Comparison of mean (\pm SEM) amounts of volatiles (1–12; see Fig. 4 for identity) released by *Eucalyptus benthamii* plants during the photophase and scotophase at different times following herbivory by *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae)

Fig. 7 Number of responses of individual mated females of *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) to (a) volatile collections of plants, and (b) plants, of *Eucalyptus benthamii* with herbivore damage or no damage (control), and to (c, d) α -pinene at two different amounts (180 and 15 ng) vs. a hexane control, in a Y-tube olfactometer. * = Statistically different, ns = not different at $P \leq 0.05$



peregrinus. A solid-phase microextraction study (Zini et al., 2002) on VOCs emitted by *Eucalyptus citriodora*, *E. dunni*, and *E. saligna*, identified some of the same compounds we identified from *E. benthamii*: α -pinene, β -pinene, *p*-cymene, limonene, linalool, aromadendrene, and globulol.

Cotton plants also emit α -pinene and caryophyllene after 24 h of herbivory by *Spodoptera exigua* (Lepidoptera, Noctuidae). However, after 48 h, different terpenes are emitted, including (*E*)-1-ocimene, linalool, (*E*)- β -farnesene, and (*E,E*)- α -farnesene, and there is a diurnal periodicity in their emission (Loughrin et al., 1994). In another study, on *N. tabacum* infested with *H. virescens* (De Moraes et al., 2001), terpenes such as linalool, (*E*)- β -cymene, β -caryophyllene, α -humulene, and (*E,E*)- α -farnesene, were emitted only during the scotophase. Monoterpene synthesis is influenced by light and temperature (Staudt et al., 1997). In our study, there were no differences between VOCs emitted during the photophase and scotophase following herbivory. Several *Eucalyptus* species, such as *E. grandis*, *E. globulus*, *E. camaldulensis*, and *E. viminalis*, also have shown no differences in monoterpene emission during the photophase and scotophase, suggesting that monoterpene emission for this genus is not light dependent, and that it might be regulated predominantly by temperature

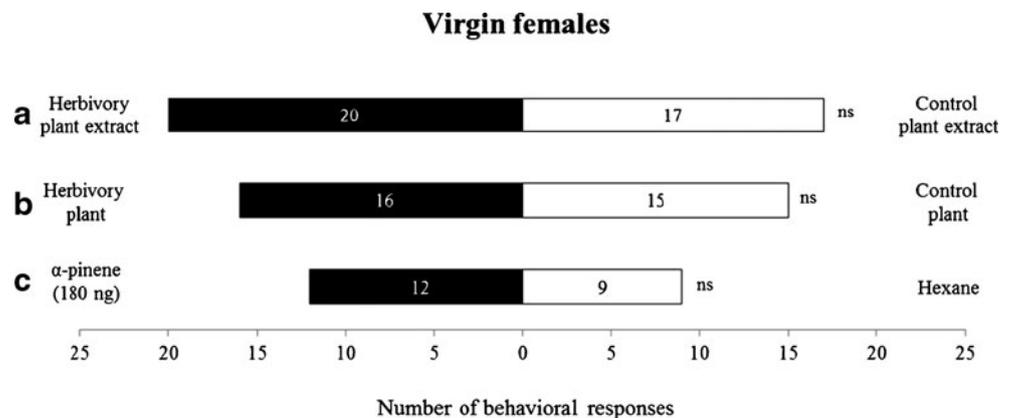
(Winters et al., 2009). Temperature was kept constant in our experiments.

Although C6 aldehydes, alcohols, and their esters (green leaf volatiles; GLVs) are commonly emitted by plants after disruption of their tissues (Matsui, 2006), we did not detect any of these compounds in our study. Bouwer (2010) tentatively identified the GLVs (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate, as well as the monoterpenes α -pinene, β -pinene, and limonene, in headspace extracts of *E. globulus*, *E. viminalis*, and *E. citriodora* leaves.

Plants synthesize and emit volatiles in response to herbivory (Turlings et al., 1990), with blends of VOCs emitted by healthy plants being modified after wounding or herbivore feeding (Walling, 2000). In our study, we detected more compounds in the blend of VOCs emitted by plants in response to herbivory than in the blend emitted by control plants. Although the herbivore-damaged and mechanically damaged plants emitted the same VOCs, the total amount of VOCs emitted by the former was nearly 10-fold higher than that emitted by mechanically damaged plants.

Although it is known that volatiles released after herbivory selectively attract parasitoids (De Moraes et al., 1998), the responses of herbivores to these volatiles is less well studied. To date, there appears to be no general response of herbivores

Fig. 8 Number of responses of individual virgin females of *Thaumastocoris peregrinus* (Heteroptera, Thaumastocoridae) to (a) volatile collections of plants, and (b) plants, of *Eucalyptus benthamii* with herbivore damage or no damage (control), and (c) to α -pinene at 180 ng vs. a hexane control, in a Y-tube olfactometer. * = Statistically different, ns = not different at $P \leq 0.05$



to plant conspecific-damaged VOCs: they can be attracted to (e.g., Schültz et al., 1997; Piesik et al., 2011) or repelled from plants attacked by conspecifics (e.g., Delphia et al., 2007; De Moraes et al., 2001; Hegde et al., 2011), with the amount of compounds released influencing responses (Piesik et al., 2011). Different insect orders respond differently to plants that are attacked or not attacked by conspecifics. For example, beetles are usually attracted to VOCs emitted by attacked plants, whereas moths and aphids avoid infested plants (Turlings and Wäckers, 2004). Little is known about the responses of Heteropterans, and more specifically of Thaumastocorids, to herbivore-damaged plants.

In our study, we focused on the responses of mated and virgin females of *T. peregrinus* to VOCs emitted by *E. benthamii* plants undamaged or damaged by conspecifics. Results indicated that virgin females did not prefer any treatment over another, while mated females preferred VOCs from undamaged over damaged plants. In accord with these results, mated females preferred hexane over α -pinene at amounts similar to that released following herbivory. When we tested this compound at a lower amount, equivalent to that released by undamaged plants, mated females showed no preference to hexane over α -pinene, suggesting that only higher amounts of α -pinene are repellent.

This preference of mated females for undamaged plants may represent a preference of these insects to an environment with fewer competitors and natural enemies for their offspring (Dicke and van Loon, 2000). Supporting this idea, Thaler (1999) found a higher percentage of parasitism on caterpillars of *S. exigua* associated with herbivore damage by tomato plants (*Lycopersicon esculentum*) when compared with control plants. Thus, the preference of mated females to control plants in our study could be due to a higher emission of VOCs after herbivory, especially of α -pinene, a known repellent present in *Eucalyptus* spp. essential oil (Batish et al., 2008; Liu et al., 2011).

Virgin females, did not prefer VOCs from control over damaged plants. Our bioassays with α -pinene revealed that they didn't exhibit a preference for hexane over α -pinene, even at the high amount (180 ng). A lack of preference by virgin females has also been noted for the onion fly, *Delia antiqua* (Meigen) (Diptera, Anthomyiidae) to *n*-dipropyl disulfide, an attractant emitted from bacterial metabolism of onion root exudates and decomposition of plant material (Romeis et al., 2003), for *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) to tomato leaves (Proffitt et al., 2011), and for the parasitoid *Cotesia vestalis* (Haliday) (Hymenoptera, Braconidae) to host-infested plants over intact plants (Kugimiya et al., 2010). Thus, in these species at least, it appears that foraging for an oviposition site may be associated with mating status.

Understanding the mechanisms of how plants, herbivores, and their natural enemies interact may be useful for developing

new pest management strategies. Future studies should focus on studying the blends of VOCs emitted by other *Eucalyptus* species, and on testing the responses of *T. peregrinus* to them.

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