JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

SPME Applied to the Study of Volatile Organic Compounds Emitted by Three Species of *Eucalyptus* in Situ

CLÁUDIA A. ZINI,[†] FABIO AUGUSTO,[‡] EVA CHRISTENSEN,[§] ELINA B. CARAMÃO,[†] AND JANUSZ PAWLISZYN[||,*]

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1, Institute of Chemistry, Federal University of Rio Grande do Sul (UFRGS), 90040-060 Porto Alegre, Rio Grande do Sul, Brazil, Institute of Chemistry, State University of Campinas (Unicamp), 13083-970 Campinas, São Paulo, Brazil, and MILJØ-KEMI, Danish Environmental Center, LilleTornbjerg Vej 24a, DK-5220, Odense SØ, Denmark

Headspace solid-phase microextraction coupled to gas chromatography/ion trap mass spectrometry−65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) was used to identify and monitor the emission patterns of biogenic volatile organic compounds from leaves of *Eucalyptus dunnii*, *Eucalyptus saligna*, and *Eucalyptus citriodora* in situ. Short extractions (1 min) were performed every 30 min for periods of 8−10 h during 24 days taking advantage of the high capacity of this porous polymer coating. Forty-two compounds were detected and 20 identified in the headspace of *E. saligna* leaves, and 19 of 27 compounds were identified in the headspace of *E. dunnii* leaves. The emission pattern of (*E*)-β-ocimene and rose oxide suggests that they may play a bioactive role in *Eucalyptus*.

KEYWORDS: Solid-phase micro extraction (SPME); *Eucalyptus citriodora*; *Eucalyptus dunnii*; *Eucalyptus saligna*, sampling chamber; biogenic volatile organic compounds (BVOC); rose oxide; *(E)*-β-ocimene

INTRODUCTION

The biogenic volatile organic compounds (BVOC) emitted by plants play an important role in the chemistry of atmosphere (*1*) and in the interorganism interaction. (*2*) Their study is also of potential interest to the pesticide and perfumery industries. (*3*, *4*) Therefore, the composition of BVOC blends is valuable information regarding behavior and possible economical applications of plants.

The methodologies most usually used for extraction of BVOC are based on static or dynamic headspace analyses (*5*), which often involve detached parts of the assessed plant and sometimes maceration or chopping of plant tissues (*3*, *4*). However, such techniques allow enzymatic and chemical reactions, which can change the composition of extracts, and results may not reflect the real BVOC profile of the living plant (*6*). Moreover, BVOC are not emitted continuously and in a uniform rate by live specimens; frequently time-dependent release profiles related to the physiological state of the organism are observed and some compounds are only produced and/or emitted during very short windows of time (*5*). Most of the aforementioned sample-preparation techniques used for monitoring plant emissions require relatively long collection periods (*7*), and consequently, are not suitable to provide information on release of compounds emitted over short periods of time.

Solid-phase microextraction (SPME) is a sample preparation technique (*8*) that can overcome some of the inconvenience of other techniques when applied to plant BVOC analysis. In general, SPME, a fused silica fiber coated with a thin sorbent phase, is exposed to the sample or its headspace, when the analytes present in the sample are sorbed in the fiber coating; in sequence, the fiber is directly introduced in the injection port of a gas or liquid chromatograph and the extracted materials are directly desorbed in the chromatographic system. (*9*)

Most of the application of SPME to living plants involves cut fresh or dry parts of the plant, or plants in vitro, and mainly the use of fibers coated with liquid polymeric phases (*10*−*13*). A few deal with living plants in situ, such as volatiles from Fraser fir branches (*Abies fraseri*) (*14*) and juniper (*15*). With the exception of the last one, the other research work used liquid polymeric coatings. Recently, a porous polymer-coated fiber was used to extract BVOC from a *Eucalyptus citriodora* branch in situ (*16*). Shorter extraction times (1 min) were required by these porous polymer-coated fibers, which in combination with its minute dimensions provided sampling with almost no disturbance of the plant.

In this work, SPME was applied to study BVOC released by three species of *Eucalyptus* trees. Although essential oils from these species have been widely studied (*17*−*23*), investigations on their BVOC have not been reported as often (*1*, *24*).

The main objective of the present investigation was to use SPME coupled to GC/ion-trap mass spectrometry (ITMS) to identify the BVOC emitted by branches of *Eucalyptus* and also follow the changes in the chromatographic profiles obtained for

* Corresponding author. E-mail: janusz@uwaterloo.ca.
[†] Federal University of Rio Grande do Sul.
[‡] State University of Campinas (Unicamp).
[§] Danish Environmental Center.
[||] University of Waterloo.

10.1021/jf025666m CCC: $22.00 © 2002 American Chemical Society
Published on Web 11/07/2002

periods of time spanning from 8 to 10 h. This short-term monitoring provided a picture of the trends on some of the BVOC emission profiles.

MATERIALS AND METHODS

Plants. One tree of *Eucalyptus citriodora* Hook, two of *Eucalyptus dunnii* Maiden (trees A and B) and one of *Eucalyptus saligna* Smith originated from the nursery farm Barba Negra (Guaíba, RS, Brazil) were cultivated in the greenhouse of Department of Biology of University of Waterloo at 19−35 °C. BVOC from juvenile leaves (1.5-year-old specimens) were sampled during May and June 2000.

SPME Materials. The SPME holder and fibers recovered with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) were supplied by Supelco (Oakville, ON, Canada). Before their use, fibers were conditioned according to the supplier specifications.

Gas Chromatography/Ion Trap Mass Spectrometry (GC/ITMS). Chromatographic analysis was performed in a Saturn 4D GC−ITMS system (Varian Associates, Sunnyvale, CA) fitted with a 30 m × 0.25 mm × 0.25 μm HP-5MS column (Hewlett-Packard, Avondale, PA) and a septum-equipped programmable injector (SPI). Helium TAG grade (Praxair, Kitchener, ON, Canada) was used as carrier gas. Mass spectra recorded from ITMS were generally used as total ion chromatograms (TIC), except in the cases of isoprene and citronellal where ions with *m*/z 53 and 67, and 67, 95, and 121, respectively, were used. Operational conditions for the chromatographic separation and detection were: 50 °C, then heated at 1 °C/min up to 60 °C, then heated at 30 °C/min up to 280 °C; temperature of SPI, 210 °C; carrier gas helium at 15 psi (1.5 mL/min); ion trap temperature, 150 °C; transfer line at 240 °C; electron multiplier voltage, 1710−1860 V (optimized daily). Identification of the eluates was performed using NIST98 MS spectra base. Whenever necessary, these results were also confirmed by comparison either with retention data obtained in an HP−Innowax 30 m × 0.25 mm × 0.50 μm or/and a HP-1MS 60 m × 0.25 mm × 0.25 μm columns (Hewlett-Packard), as well as with a lab-built MS spectra base previously collected from chromatographic runs of pure compounds (Sigma Chemical Co., St. Louis, MO; Aldrich Chemical Co., Milwakee, WI; and Fluka Chemie AG, Buchs, Switzerland) performed in the same equipment and conditions. When pure compounds were not available, comparison with retention data reported in the literature was also used to tentatively identify some of the compounds (*25*).

Sampling Chamber. To assist the extraction of the BVOC emitted by living *Eucalyptus* leaves a glass chamber was designed and assembled (*16*). It consists of a Pyrex glass cylinder (120 mm wide, ϕ = 60 mm), where leaves can be inserted through a hole in one of its sides. After introduction of the leaf, this hole can be sealed using Teflon tape. A round glass lid secured by clamps closes the other side of the chamber. This lid has several 5-mm holes sealed by Thermogreen LB-1 predrilled septa (Supelco), were a SPME fiber can be introduced to sample the air inside.

Experimental Procedure. The extraction time was 1 min and desorption time 7 min according to previous investigation (*16*). A small branch of *Eucalyptus* was sealed in the glass-sampling chamber, and the air inside the chamber extracted using a PDMS/DVB fiber. This procedure was performed every 30 min for continuous periods between 8 and 10 h, with the sampling container being kept sealed. In addition to essays using intact leaves, the same procedure was used to measure emission profiles from mechanically damaged leaves. In these cases, immediately before sealing the leaf inside the sampling container, six small holes were made in the leaf surface with a stainless steel syringe needle to simulate lesions resulting from the action of insects. Extraction procedures were performed either in the greenhouse (where plants were subject to sunlight and natural temperature variations) or in the laboratory, where plants were illuminated by GE F34T12 (34 W) cool white fluorescent lamps and kept in a controlled-temperature room. Watering was done on a daily basis as required. Radiation in the greenhouse is roughly estimated to be 250−330 times higher than in the laboratory. In the greenhouse, experiments were performed using three fully and three partially expanded undamaged leaves of *E. citriodora*, and three fully expanded leaves of *E. saligna* and *E. dunnii* A and B. In the laboratory, seven totally expanded *E. saligna* leaves and three partially and three totally expanded *E. citriodora* leaves were sampled. Temperature in the greenhouse during sampling varied between 19 and 36 °C, and in the laboratory from 24 to 27 °C. Experiments with each one of the *Eucalyptus* species were performed during three consecutive days in the greenhouse and during 6 consecutive days in the laboratory. In the laboratory, the leaves sampled during the first 3 days were undamaged and in the last 3 days were mechanically damaged. Blank runs of the fiber and of the glass chamber were run before starting each set of daily sampling. During the transportation of the fibers to the laboratory after extractions done in the greenhouse, they were kept under dry ice. Time between sampling and analysis never exceeded 5 min. In these conditions, the loss of extracted analytes is expected to be insignificant(*26*).

Table 1. Identification and relative abundances (RA) for BVOC emitted by *E. citriodora*, *E. dunni*, and *E. saligna* leaves and identified after 1-min extractions with PDMS/DVB SPME fibers

		RA[a]		
no.	compound	E. citriodora	E. dunni	E. saligna
1	isoprene[b]	++/+	*x*/+	++/+
2	hexenal	nd	x[e]	x[e]
3	3-(*Z*)-hexen-1-o l	nd	x[e]	nd
4	α-thujene	x	nd	nd
5	α-pinene	+	++	++
6	camphene	nd	x[e]	x
7	sabinene[c]	x	nd	nd
8	β-pinene[c]	+	x	x[e]
9	β-myrcene	x	x[e]	nd
10	α-phellandrene	nd	x[e]	x[e]
11	3-(*Z*)-hexenol acetate	nd	x[e]	x[e]
12	p-cymene	nd	++/+	++
13/14	limonene + 1,8-cineole[d]	+	++	+
15	(*Z*)-β-ocimene	x	x[e]	x[e]
16	(E)-β-ocimene[b]	+/*x*	*x*/++	+/++
17	γ-terpinene	nd	+/*x*	+
18	terpinolene	nd	nd	x[f]
19	linalool	+	nd	nd
20	*cis*-rose oxide	+	nd	nd
21	campholenal	nd	nd	+/*x*[b,f]
22	*trans*-rose oxide	+	nd	nd
23	isopulegol	+/*x*[b]	nd	nd
24	citronellal	++	nd	nd
25	α-terpineol	nd	x[e]	x[f]
26	*trans*-carveol	nd	nd	x[f]
27	citronellol	++	nd	nd
28	3,7-dimethyl-2,6-octadienal	x	nd	nd
29	citronellyl acetate	+	nd	nd
30	3,7-dimethyl-2,6-octadienal acetate	x	nd	nd
31	β-caryophyllene	++	+[g]	x
32	aromadendrene	nd	+	x[e]
33	α-caryophyllene	x	nd	nd
34	globulol	nd	+/*x*[e]	x[e]

[a] ++, peak area usually 10% or more of the total area of identified peaks; +, peak area usually between 1% and 10% of the total area of identified peaks; *x*, peak area usually less than 1% of total area of identified peaks; nd, non detected; compounds written in bold letters were only tentatively identified. [b] Relative abundance extremely variable from sample to sample. [c] Separation incomplete. [d] Coeluting substances. [e] Presence of the compound is not constant during the sampling period. [f] Present only in the laboratory. [g] Not present in *E. dunnii* B.

DISCUSSION

***Eucalyptus* leaves BVOC. Table 1** lists some of the identified BVOC emitted by the three *Eucalyptus* species in these experiments. They are listed by elution order in a HP-5MS column. The majority of the compounds listed were identified by comparison of mass spectra and retention data of pure compounds with the unknown ones according to Materials and Methods. Some compounds appearing in **bold** were only tentatively identified by mass spectrum and comparison with literature retention data (*25*). Most of the identified substances are mono- or sesquiterpenoid hydrocarbons and esters that are

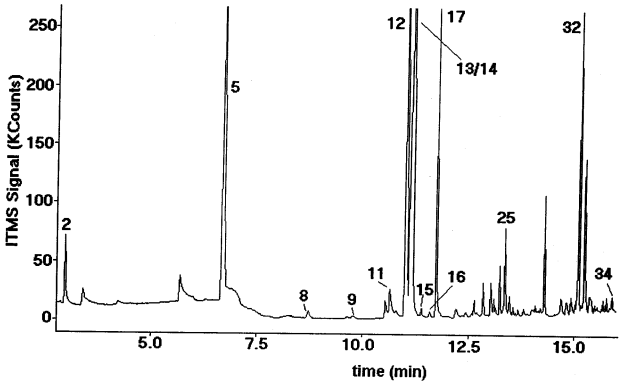

Figure 1. Typical GC–ITMS profile of *E. dunnii* BVOC after 1 min extraction with PDMS/DVB fiber (damaged leaf, extraction performed in the greenhouse) using the glass chamber. Peak identification: see **Table 1**.

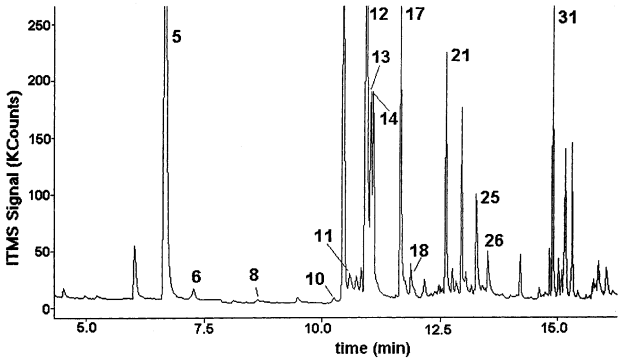

Figure 2. GC–ITMS profile of *E. saligna* BVOC after 1 min extraction with PDMS/DVB fiber (undamaged leaf, extraction performed in the laboratory) using the glass chamber. Peak identification: see **Table 1**.

also frequently found in the essential oil of *Eucalyptus* (*21*). **Figures 1** and **2** show a representative chromatogram of *E. dunnii* and *E. saligna*, respectively, and **Table 1** addresses the identification of the numbered peaks. Similar data have already been published for *E. citriodora* (*16*). The use of PDMS/DVB allowed the detection of 42 compounds and the identification of 20 in the headspace of a living branch of *E. saligna*. The major compounds were α-pinene, *(E)*-*β*-ocimene, and p-cymene. Other components could not be identified and/or were tentatively identified: an ester of propanoic acid, a terpenoid compound present only in the experiments made in the greenhouse, and two hydrocarbon sesquiterpenes The following compounds where found as traces: fenchol, terpinen-4-ol, p-cymen-8-ol, four esters of butanoic or propanoic acid, three oxygenated monoterpenes, one aromatic compound, three hydrocarbon sesquiterpenes and four oxygenated sesquiterpenes.

Considering results obtained from the leaves of both trees of *E. dunnii*, a total of 27 compounds were detected in the headspace of their leaves. In this volatile mixture, 19 components were identified: isoprene, hexenal, 3-(*Z*)-hexen-1-ol, α-pinene, camphene, *β*-pinene, *β*-myrcene, α-phellandrene, 3-(*Z*)-hexenol acetate, p-cymene, limonene, 1,8-cineole, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, *γ*-terpinene, α-tepineol, *β*-caryophyllene, aromadendrene, and globulol. The other components were tentatively identified or remained unidentified. The major compounds were 1,8-cineole, limonene, (*E*)-*β*-ocimene, and p-cymene.

Compounds found in the headspace of *E. citriodora* leaves in situ were already reported in a recent publication (*16*), and are similar to those found in the distilled oil of this species,

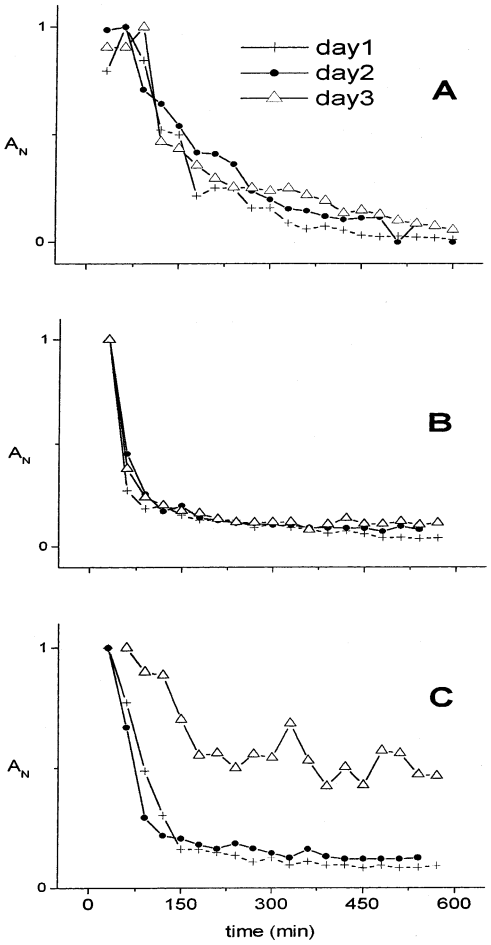

Figure 3. Circadian profiles for *β*-caryophyllene emitted by *E. citriodora* leaves: (A) greenhouse sampling, undamaged leaves; (B) laboratory sampling, undamaged leaves; (C) laboratory sampling, damaged leaves.

presenting citronellal as the major component (*17*−*21*). Preliminary experiments and method development were also reported in this former publication.

Using the porous polymer-coated fiber PDMS/DVB, it was also possible to follow the different patterns of behavior of BVOC emission of three species of *Eucalyptus* during a total of 24 days every 30 min for continuous periods of 8 to 10 h (**Table 1**). To assist and simplify the evaluation of the chromatographic profiles obtained of BVOC emitted by the *Eucalyptus* branch, for each compound and sampling series, the peak areas were normalized according to eq 1.

$$A_{\mathrm{N}} = \frac{A_X}{A_{\mathrm{MAX}}} \tag{1}$$

where A_{N} is the normalized area, A_X is the peak area x min after enclosing the leaves inside the glass chamber, and A_{MAX} is the maximum peak area for the compound in the set of measurements in a day. Normalized areas were corrected in the case in which environmental temperatures changed during sampling (*27*). The same general behaviors observed for some *E. citriodora* BVOC (*16*) were also seen for other *E. dunnii* and *E. saligna* BVOC emissions when A_{N} was plotted against the leaf enclosure time, for example: (1) The area under the chromatographic peak is maximum in the moment of enclosure of the leaf inside the chamber, decreasing during the experiment, as it is shown in **Figure 3** for *β*-caryophyllene in *E. citriodora* emissions. (2) The (*E*)-*β*-ocimene circadian profiles in the

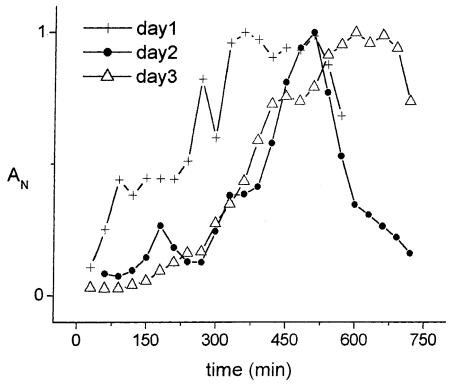

Figure 4. Circadian profiles for (*E*)-*β*-ocimene emitted by *E. dunnii* A leaves: greenhouse sampling; undamaged leaves in day 1 and day 2; damaged leaves in day 3. Extracted ion monitored: *m*/*z* 91.

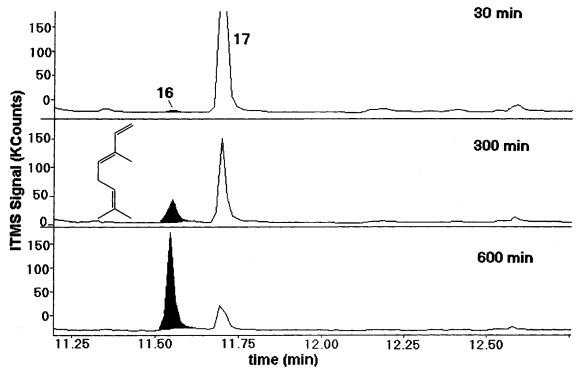

Figure 5. Increasing amount of (*E*)-*β*-ocimene shown after 30, 300, and 600 min of sampling *E.dunnii* A BVOC. Peak identification: see **Table 1**.

headspace of the assessed *Eucalyptus* leaves show a gradual increase of the area of the chromatographic peak until it reaches a maximum, decreasing after that or remaining nearly constant, as is shown in **Figure 4**. **Figure 5** shows the (*E*)-*β*-ocimene chromatographic peak in different sampling times.

(*E*)-*β*-Ocimene. The three species *E. citriodora*, *E. dunnii*, and *E. saligna* emitted (*E*)-*β*-ocimene in a way similar to pattern 2 described above. Both isomers of *β*-ocimene were detected only while sampling in the greenhouse environment, which may be indicating that natural light would be an important factor for its production and/or emission. The only exception was traces of the compound in the *E. citriodora* leaves headspace in the first day of sampling in the laboratory. Investigations of pine needles emissions (*28*) show that (*E*)-*β*-ocimene is light dependent and was not emitted under nonphotosynthetic conditions. The same is confirmed by Halitschke et al. (*29*), where (*E*)-*β*-ocimene was not detected in the headspace of *Nicotiana attenuata* leaves during dark periods, even though temperature was raised to match that of the light period. Residual traces emitted by *E. citriodora* leaves in the first sampling day in the laboratory may have been a residual amount produced by the leaves before having been transported to the laboratory. For *E. dunnii* A, maximum emission of (*E*)-*β*-ocimene ranged from 350 to 700 min of sampling time; for *E. dunnii* B, from 200 to 400 min; for *E. saligna*, from 420 to 540 min; for *E. citriodora*, from 300 to 400 min. In the circadian profile of (*E*)-*β*-ocimene emitted by *E. dunnii* A (**Figure 4**) it is possible to see variations in the line curve while it goes up and down, as in day 1 at 120, 300, and 420 min, for example. These variations occur also in the circadian profiles of (*E*)-*β*-ocimene emitted by the other *Eucalyptus* species under study and, in several cases, short periods of emission drop would happen at the same time as temperature drop and/or presence of cloudy sky.

Regarding possible storage of this compound in the leaves oil glands, several studies about *E. citriodora* leaf oil do not report (*Z*)- and (*E*)-*β*-ocimene in the leaves oil (*18*−*21*), with three exceptions (*17*). (*Z*)-*β*-Ocimene was only detected as a trace in the headspace of the three experimental species while sampling in the greenhouse. Both isomers were not included in the volatile emission of an *E. citriodora* seedling (*24*), neither in the headspace of its leaves (*12*).

(*E*)-*β*-Ocimene and also (*Z*)-*β*-ocimene are minor components of *Eucalyptus* leaves oils. In the study made by Boland et al. (*21*), from the 111 species studied, 44 presented *β*-ocimene as a minor component in the oil. *Eucalyptus sphaerocarpa* presents the highest percentage (2.84%) for (*E*)-*β*-ocimene and *E. dunnii* the highest for the (*Z*) isomer (3.63%). Data about *E. dunnii* (*19*, *21*) and *E. saligna* leaves oils (*17*, *21*, *23*) are not abundant and among the published research, only one paper shows the presence of (*E*,*Z*)-*β*-ocimenes in *E. dunnii* leaf oil (*21*) and another in *E. saligna*. (*23*) However, this compound is reported in the emissions of *Eucalyptus camaldulensis* and *Eucalyptus maculata*. (*24*)

The occurrence of ocimene is widespread as a BVOC of plants. It is known for its multiple ecological interactions, such as indirect plant defense (attractant to predators and parasitoids of herbivores) or attractant to pollinators (*2*). Some plants release (*E*)-*β*-ocimene after being attacked by herbivores (*30*). In the case of infested lima bean detached leaves, the mixture (*E*,*Z*)-*β*-ocimene was attractive to the predator *P. persimilis.*

In the case of *E. saligna* and *E. dunnii*, a branch distal to the sampling branch was mildly infested by spidermite (*Tetranychus urticae*), and *E citriodora* had also a distal branch mildly infested by the insect longtailed mealybug *(Pseudococcus longispinus)*. Therefore, the unique emission pattern of (*E*)-*β*-ocimene may be suggesting an infochemical role for (*E*)-*β*-ocimene in *Eucalyptus*, although nothing can be stated, because such a complex subject would deserve further research. Moreover, because the leaves were only inspected visually, it cannot be concluded that the leaves were the only one responsible for volatile emissions or if the plant offered a substrate for spidermite enzymes that subsequently produced BVOC (*30*).

Recently, an investigation on nonhost cue compounds for the *Eucalyptus* woodbohrer presents (*E*)-*β*-ocimene as a possible candidate (*31*).

***β*-Caryophyllene and Others.** *β*-Caryophyllene showed an emission profile close to pattern 1 for *E. saligna* and *E. citriodora* in the 18 days of BVOC monitoring. *E. dunnii* B showed also the presence of this sesquiterpene, although with an emission pattern similar to pattern 2, and the same compound was not detected in the volatile emissions of *E. dunnii* A.

β-Caryophyllene has been reported in the essential oil of *E. dunnii* leaves in small amounts expressed as area percentage in a range of 0.19−2.8% (*21*, *22*), although it was not detected in the essential oil of the two experimental trees of this work. However, it was detected in the hydrodistilled oils of *E. saligna* and *E. citriodora* (data not shown). In *E. saligna* oil it is eventually found (*17*, *21*, *23*), and in *E. citriodora* it is a common component of the oil. (*17*−*21*).

Its role as an infochemical has been suggested by Langenheim et al. (*32*), where it showed toxicity toward the generalist herbivore *Spodoptera exigua* Hübn. Other biological roles are mentioned further on by the same researcher (*2*) and suggested in the literature, such as elicitor of a neurone response in the Egyptian cotton leaf worm (*Spodoptera littoralis* B.) (*33*), and

of antennal responses in female codling moth *Cydia pomonella* L. (*13*) In the view of these biological roles of β-caryophyllene and also of its different behavior in *E. dunnii* A and B versus *E. saligna* and *E. citriodora* volatile emissions, it is plausible to hypothesize that this sesquiterpene may eventually have an infochemical role in *Eucalyptus*, although this subject deserves further investigation.

Similar behavior as the one presented in **Figure 3** was also seen for other compounds emitted by *E. saligna* leaves in the laboratory experiments: α-pinene, p-cymene, limonene, 1,8-cineole, campholenal, α-terpineol, γ-terpinene, campholenal, α-terpineol, *trans*-carveol. For *E. dunnii* A in the greenhouse, this pattern was observed for α-pinene, p-cymene, limonene, 1,8-cineole, and γ-terpinene. In the volatile emissions of *E. dunnii* B no clear pattern was observed, except for β-caryophyllene.

A rapid decrease in monoterpene emissions was also observed in wounded pine needles (*28*), and in wounded conifers (*34*). According to Loreto et al. (*28*), even though the causes for this decrease are unclear, two hypotheses may be raised to explain this fast monoterpene emission decrease of damaged vegetation: (1) wound sealing through terpene solidification while they reach the air; (2) emptied monoterpene reservoirs. In the present case, these hypotheses might be applied to damaged *Eucalyptus* leaves emission profiles, but not for the undamaged. Other hypotheses might be raised to help explain the decreasing emission profile, such as oxidative degradation and/or plant stress (even though minimum) because of branch enclosure inside the chamber, and sorption by the plant. (*35*)

The greem leaf volatiles (GLV) hexenal, 3-(*Z*)-hexenol, and 3-(*Z*)-hexenol acetate were detected in *E. dunnii* headspace after mechanical damage. 3-(*Z*)-hexenol was detected 30 min after mechanical damage and 3-(*Z*)-hexenol acetate was still present in the headspace after 1.5 h. These compounds are products of the lipoxygenase pathway and are released from freshly cut or damaged tissue (*36*).

Two other compounds showed up in the volatile emissions of *E. saligna* leaves as traces in the first sampling day in the greenhouse, showing increasing emission in the second and third day of sampling. One of them is a terpene whose mass spectra shows *m*/*z* 69 as base peak and a molecular weight of 150, and the other terpene has a base peak *m*/*z* 69 and molecular weight *m*/*z* 204. Both were tentatively identified as (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMTN) and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), respectively. These same compounds were also found in the volatile kairomone of lima bean plants infested by *T. urticae* (*30*). DMNT is known as a predator attractant in agricultural plants (*37*) and was recently pointed out as an apparent nonhost cue of *Olea europeae* in relation to *Phorocantha semipunctata*, an *Eucalyptus* woodborer (*31*). The unique behavior of these terpenes in the headspace of *E. saligna* leaves, their presence in the kairomone of lima bean plants, the presence of *T. urticae* in both experiments (lima bean plants and *Eucalyptus* leaves) are interesting facts that may or may not be pointing out the same compounds, even though the complete identification of these components of *E. saligna* volatile emissions were not accomplished in this work.

α-Farnesene was detected in the headspace of *E. saligna* and *E. dunnii* B leaves, presenting a emission pattern similar to DMNT. In *E. dunnii* A, it was detected as traces. This terpene also plays infochemical roles in other plants (*13*).

The differences observed in the behavior of some BVOC while in the laboratory or in the greenhouse, or from one tree of the same species to the other may be explained by different abiotic and biotic factors characteristic to these two environments and different individuals. Terpenoid emission rates are known to be temperature dependent and some compounds are also light dependent, as mentioned for isoprene and (*E*)-β-ocimene (*29*). The intensity of light and proper light wavelength range is of crucial importance for photosynthesis, growing, and other plant functions. The intensity of light in the laboratory was estimated to be 250−330 times lower than in the greenhouse, and the wavelength range provided by a cool white fluorescent light does not include red and farred wavelengths, which are important for the plant (*38*). Variability among individual trees is also a well-known fact (*21*) and it can be further extended to variability of volatile emission according to leaf position and age as it was demonstrated by Halitschke et al. (*29*). In this work, variability among individuals is seen in the different emission behavior of *E. dunnii* A and B, both of the same age and reared in the same environmental conditions.

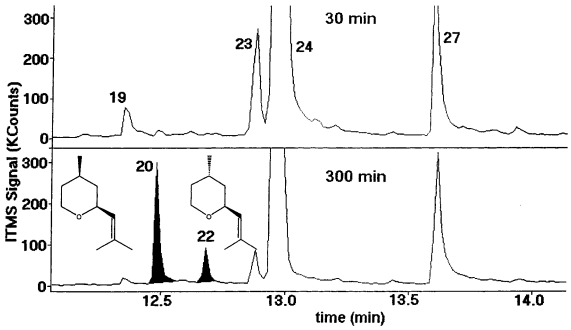

Figure 6. *Cis*- and *trans*-rose oxide shown after 300 min of sampling compared with its absence after 30 min of sampling of *E. citriodora* BVOC. Peak identification: see **Table 1**.

Rose Oxide. Just recently, *cis*- and *trans*-rose oxide were reported as BVOC of an *E. citriodora* branch with an emission pattern similar to pattern 2 (**Figure 6**) (*16*). This publication complements the former discussing the possible role of this compound.

Rose oxide (*cis*- and *trans*-4-methyl-2-(methyl-1-propenyl)-tetrahydropyran (see **Figure 4** for structure) is not a common component of the oil of *E. citriodora* (*17*−*21*) even though it has eventually been reported as a minor component, e.g., 0.4% of *cis*-rose oxide of an Indian oil, 1% of a Maroccan oil (*20*), 0.19% for *cis*-rose oxide and 0.05% for *trans*-rose oxide in a Rwandan oil; 0.05% for *cis*-rose oxide and 0.08% for *trans*-rose oxide in a Bangladeshi oil (*17*). In the study of the oil of the leaves of 111 species of *Eucalyptus* in Australia, rose oxide is not ever reported as present in anyone of the species (*21*), neither is it in the BVOC emitted by an *E. citriodora* seedling (*24*), nor in the headspace of leaves of *E. citriodora* (*12*).

Both isomers of rose oxide were reported as defense substances of the insect *Aromia moschata* L. (*39*) where the *cis*-isomer was present in higher proportion than the *trans*- (22% to 4%) as observed here. The secretion is manufactured in its thoracic glands and is discharged when a predator threatens the insect. Some distal leaves of the same *E. citriodora* experimental tree were mildly infected by the fungus mealy bug (*P. longispinus*), and therefore it is reasonable to hypothesize that the observed release of *cis*- and *trans*-rose oxide may be a response of the plant. However, as it was earlier stated for (*E*)-β-ocimene, emission of BVOC is a complex process and a complete explanation requires further investigation.

CONCLUSIONS

As recent publication (*16*) emphasized that many factors may influence the production of BVOC by plants, such as abiotic factors (light intensity, water, season, etc) or age of the leaves or plant, cultivars, plant species, etc. As SPME is a simple, relatively nonexpensive, noninvasive, fast extraction tool, allowing the monitoring of the BVOC released by the plant over short intervals of time for long periods, it is a convenient tool for the investigation of plant−plant or plant−herbivore−predator or plant−environment interaction in situ. SPME opens a horizon for a simpler and faster investigation of these phenomena.

The fact that SPME is portable and requires only thermal reconditioning allows this technique combined to a portable GC to be a good alternative for field sampling, specially for remote areas. This technique has already been used in the study of synthetic pathways related to plants *in vitro* (*40*). However, the knowledge of the plant headspace modification provided by adsorbent coated fibers as a preconcentration/extraction technique can be used in vivo and in situ and opens a new door to elucidate biosynthetic pathways or possible communications with other organisms, as well as help protect crops and forests.

ACKNOWLEDGMENT

We thank Ms. Lynn Hoyles for her technical advice. We also gratefully acknowledge Dr. Maarten Posthumus for kindly providing his technical advice and the mass spectra of DMNT and TMTT.

LITERATURE CITED

(1) Kesselmeier, J.; Staudt, M. Biogenic Volatile Organic Compounds (VOC): an Overview on Emission, Physiology and Ecology. *J. Atmos. Chem.* **1999**, *33*, 23−88.

(2) Langenheim, J. H. Higher plant Terpenoids: a Phytocentric Overview of Their Ecological Roles. *J. Chem. Ecol.* **1994**, *20*, 1223−1280.

(3) Dicke, M.; Sabelis, M. W.; Takabayashi, J.; Bruin, J.; Posthumus, M.A. Plant Strategies of Manipulating Predator-prey Interactions Through Allelochemicals: Prospects for Application in Pest Control. *J. Chem. Ecol.* **1990**, *16*, 3091−3118.

(4) Shang, C.; Hu, Y.; Deng, C.; Hu, K. Rapid Determination of Volatile Constituents of *Michelia alba* flowers by Gas Chromatography−Mass Spectrometry with Solid-Phase Microextraction. *J. Chromatogr. A* **2002**, *942*, 283−288.

(5) Agelopoulos, N.G.; Pickett, J.A. Headspace Analysis in Chemical Ecology: Effects of Different Sampling Methods on Ratios of Volatile Compounds Present in Headspace Samples. *J. Chem. Ecol.* **1998**, *24*, 1161−1172.

(6) Tollsten, L.; Bergström, G. Headspace Volatiles of Whole Plants and Macerated Plant Parts of *Brassica* and *Sinapis*. *Phytochemistry* **1988**, *27*, 4013−4018.

(7) Stránsk; Valterová, I. Release of volatiles during the flowering period of *Hydrosme rivieri* (Araceae). *Phytochemistry* **1999**, *52*, 1387−1390.

(8) Arthur, C. L.; Pawliszyn, J. Solid-Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Anal. Chem.* **1990**, *62*, 2145−2148.

(9) Pawliszyn, J. *Solid-Phase Microextraction: Theory and Practice;* Wiley-VCH: New York, 1997.

(10) Maes, K.; Vercammen, J.; Pham-Tuan, H.; Sandra, P.; Debergh, P. C. Critical Aspects for the Reliable Headspace Analysis of Plants Cultivated *in vitro*. *Phytochem. Anal.* **2001**, *12*, 153−158.

(11) Schäfer, B.; Henning, P.; Engewald, W. Analysis of Monoterpenes from Conifer Needles Using Solid-Phase Microextraction. *J. High Resolut. Chromatogr.* **1995**, *18*, 587−592.

(12) Betts, T. J. Solid-Phase Microextraction of Volatile Constituents from Individual Fresh *Eucalyptus* Leaves of Three Species. *Planta Med.* **2000**, *66*, 193−195.

(13) Backman, A.-C.; Bengtsson, M.; Borg-Karlsson, A.-K.; Liblikas, I.; Witzgall, P. Volatiles from Apple (*Malus domestica*) Eliciting Antennal Responses in Female Codling Moth *Cydia pomonella* (L.) (*Lepidoptera: Tortricidae*): Effect of Plant Injury and Sampling Technique. *J. Biosci.* **2001**, *56*, 262−268.

(14) Vereen, D. A.; McCall, J. P.; Butcher, D. J. Solid-Phase Microextraction for the Determination of Volatile Organics in the Foliage of Fraser Fir (*Abies fraseri*). *Microchem. J.* **2000**, *65*, 269−276.

(15) Augusto, F.; Koziel, J.; Pawliszyn, J. Design and Validation of Portable SPME Devices for Rapid Field Air Sampling and Diffusion-Based Calibration. *Anal. Chem.* **2001**, *73*, 481−486.

(16) Zini, C. A.; Augusto, F.; Christensen, E.; Smith, B. P.; Caramão, E. B.; Pawliszyn, J. Monitoring Biogenic Volatile Compounds Emitted by *Eucalyptus citriodora* using SPME. *Anal. Chem.* **2001**, *73*, 4729−4735.

(17) Lawrence, B.M. Progress in Essential Oils: Juniperry Oil, Juniper Leaf, Wood and Branch Oil, *Eucalyptus citriodora* Oil, and Cubeb Oil. *Perfum. Flavor.* **2001**, *26*, 68−81.

(18) Chalchat, J.-C.; Garry, R.-P.; Sidibé, L.; Harama, M. Aromatic plants of Mali (V): chemical composition of essential oils of four *Eucalyptus* species implanted in Mali: *Eucalyptus camaldulensis*, *E. citriodora*, *E. torelliana* and *E. tereticornis*. *J. Essent. Oil Res.* **2000**, *12*, 695−701.

(19) Bignell, C. M.; Dunlop, P.; Brophy, J. J. Volatile Leaf Oils of Some Queensland and Northern Australian Species of the Genus *Eucalyptus* (Series II) Part II. Subgenera (a) Blakella, (b) Corymbia, (c) Unnamed, (d) Idiogenes, (e) Monocalyptus and (f) Shymphyomyrtus. *Flavour Fragrance J.* **1997**, *12*, 277−284.

(20) Lawrence, B. M. Progress in Essential Oils − Savory Oil, Eucalyptus citriodora Oil, Geranium Oil and Siberian Fir Needle Oil. *Perfum. Flavor.* **1996**, *21*, 55−63.

(21) Boland, D. J.; Brophy, J. J.; House, A. P. N. *Eucalyptus Leaf Oils: Use, Chemistry, Distillation and Marketing;* Inkata Press: Melbourne, 1991.

(22) Mizrahi, I.; Traverso, J. R.; Juárez, M. A.; Bandoni, A. L.; Muschietti, L.; Baren, C. V. Composition of the Essential Oil of *Eucalyptus dunnii* Maiden Growing in Argentina. *J. Essent. Oil Res.* **1997**, *9*, 715−717.

(23) Foudil-Cherif, Y.; Meklati, B. Y.; Verzera, A.; Mondello, L.; Dugo, G. Chemical Examination of Essential Oils from the Leaves of Nine *Eucalyptus* Species Growing in Algeria. *J. Essent. Oil Res.* **2000**, *12*, 186−191.

(24) He, C.; Murray, F.; Lyons, T. Monoterpene and isoprene emissions from 15 *Eucalyptus* species in Australia. *Atmos. Environ.* **2000**, *34*, 645−655.

(25) Adams, R. P. *Identification of Essential Oils by Ion Trap Mass Spectroscopy;* Academic Press: New York, 1989.

(26) Müller, L. Field Analysis by SPME. In *Applications of Solid-Phase Microextraction*, Pawliszyn, J., Ed.; RSC: Cambridge, 1999.

(27) Koziel, J.; Jia, M.; Pawliszyn, J. Air Sampling With Porous Solid-Phase Microextraction Fibers. *Anal. Chem.* **2000**, *72*, 5178−5186.

(28) Loreto, F.; Nascetti, P.; Graverini, A.; Mannozzi, M. Emission and Content of Monoterpenes in Intact and Wounded Needles of the Mediterranean Pine, Pinus pinea. *Funct. Ecol.* **2000**, *14*, 589−595.

(29) Halitschke; R.; Kessler, A.; Kahl, J.; Lorenz, A.; Baldwin, I. T. Ecophysiological Comparison of Direct and Indirect Defenses in *Nicotiana attenuata*. *Oecologia* **2000**, *124*, 408−417.

(30) Dicke, M.; Van Beek, T. A.; Posthumus, M. A.; Ben Dom, N.; Van Bokhoven, H.; De Groot, A. E. Isolation and Identification of Volatile Kairomone that Affects Acarine Predator-Prey Interactions − Involvement in Host Plant in its Production. *J. Chem. Ecol.* **1990**, *16*, 381−396.

(31) Barata, E. N.; Pickett, J. A.; Wadhams L. J.; Woodcock, C. M.; Mustaparta, H. Identification of Host and Nonhost Semiochemicals of *Eucalyptus* Woodborer *Phoracantha semipunctata* by Gas Chromatography-Electroantennography. *J. Chem. Ecol.* **2000**, *26*, 1877−1895.

(32) Langenheim, J. H.; Foster, C. E.; McGinley, R. B. Inhibitory Effects of Different Quantitative Compositions of *Hymenaea* Leaf Resins on a Generalist Herbivore *Spodoptera exigua*. *Biochem. Syst.* Ecol. **1980**, *8*, 385−396.

(33) Jonsson, M.; Anderson, P. Electrophysiological Response to Herbivore-Induced Host Plant Volatiles in the Moth *Spodoptera littoralis*. *Physiol. Entomol.* **1999**, *24*, 377−385.

(34) Litvak, M. E.; Monson, R. K. Patterns of Induced and Constitutive Monoterpene Production in Conifer Needles in Relation to Insect Herbivory. *Oecologia* **1998**, *114*, 531−540.

(35) Welke, B.; Ettlinger, K.; Riederer, M. Sorption of Volatile Organic Chemicals in Plant Surfaces. *Environ. Sci. Technol.* **1998**, *1099*, 9−1104.

(36) Paré, P. W.; Tumlinson, J. H. Plant Volatiles as Defense against Insect Herbivores. *Plant Physiol.* **1999**, *121*, 325−331.

(37) Azuma, H.; Thien, L.B.; Toyota, M.; Asakawa, Y.; Kawano, S. Distribution and Diferential Expression of (*E*)-4,8-Dimethyl-1,3,7-Nonatriene in Leaf and Floral Volatiles of *Magnolia* and *Liriodendron* Taxa. *J. Chem. Ecol.* **1997**, *23*, 2467−2478.

(38) Sager, J. C.; McFarlane, J. C. Radiation. In *Plant Growth Chamber Handbook*; Laughans, F. R.W., Tibbitts, T. W., Eds.; Iowa, 1997.

(39) Vidari, G.; Bernardi, M. D.; Pavan, M.; Ragozzino, L. Rose Oxide and Iridodial from *Aromia moschata* L. (Coleoptera: Cerambycidae). *Tetrahedron Lett.* **1973**, *41*, 4065−4068.

(40) Koch, T.; Bandemer, K.; Boland, W. Biosynthesis of *Cis*-Jasmone: A Pathway for the Inactivation and the Disposal of the Plant Stress Hormone Jasmonic Acid to the Gas Phase. *Helv. Chim. Acta* **1997**, *80*, 838−850.

Received for review May 13, 2002. Revised manuscript received September 14, 2002. Accepted September 14, 2002. This work was funded by NSERC (Natural Sciences and Engineering Research Council), Canada. The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, for scholarships to C. A. Zini and F. Augusto, respectively. We also thank Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for financial support, and KLABIN RIOCELL S. A. for providing the trees.

JF025666M