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Diversity of the Volatile Organic Compounds Emitted by 55 Species of Tropical Trees: a Survey in French Guiana

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Abstract Volatile organic compounds (VOCs) are produced by a broad range of organisms, from bacteria to mammals, and they represent a vast chemical diversity. In plants, one of the preeminent roles of VOCs is their repellent or cytotoxic activity, which helps the plant deter its predators. Most studies on VOCs emitted by vegetative parts have been conducted in model plant species, and little is known about patterns of VOC emissions in diverse plant communities. We conducted a survey of the VOCs released immediately after mechanical damage of the bark and the leaves of 195 individual trees belonging to 55 tropical tree species in a lowland rainforest of French Guiana. We discovered a remarkably high chemical diversity, with 264 distinct VOCs and a mean of 37 compounds per species. Two monoterpenes (α-pinene and limonene) and two sesquiterpenes (β-caryophyllene and α-copaene), which are known to have cytotoxic and deterrent effects, were the most frequent compounds in the sampled species. As has been established for floral scents, the blend of VOCs is largely species-specific and could be used to discriminate among 43 of the 55 sampled species. The species with the most diverse blends were found in the Sapindales, Laurales, and Magnoliales, indicating that VOC diversity is not uniformly distributed among tropical species. Interspecific variation in chemical diversity was caused mostly by variation in sesquiterpenes. This study emphasizes three aspects of VOC emission by tropical tree species: the species-specificity of the mixtures, the importance of sesquiterpenes, and the wide-ranging complexity of the mixtures.

Keywords VOCs · Chemical diversity · Sesquiterpenes · Tropical · French Guiana

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Introduction

Since the work of Fraenkel in 1959, interest in plant secondary metabolites has increased dramatically (Wink 2003; Hartmann 2007). Within the diverse array of naturally synthesized chemicals, volatile organic compounds (VOCs) are of interest. First, VOCs encompass a broad range of compounds that result from at least three distinct biosynthetic pathways: lipoxygenase, shikimic acid, and terpenoid pathways (Dudareva et al. 2006). Second, they represent one of the most diverse classes of secondary metabolites, with over 2,500 monoterpenes and 5,000 sesquiterpenes described so far (Wink 2006). Third, VOCs

are implicated in a large number of physiological functions, such as plant pollination (Raguso 2008), attraction of seed dispersers (Borges et al. 2008), and defense against biotic and abiotic stresses (Pichersky and Gershenzon 2002; Vickers et al. 2009). VOCs may have a direct deterrent or cytotoxic effect against herbivores and pathogens (De Moraes et al. 2001; Pichersky and Gershenzon 2002; Heil 2004), or indirect effects through the attraction of herbivore enemies as predators or parasitoids (Turlings and Wäckers 2004; Heil 2008). The defensive function of VOCs has been studied intensively in some model plant species (Turlings and Wäckers 2004) but not much is known about importance in complex plant communities such as tropical forests.

Some compounds are common in both reproductive and vegetative plant tissues. In a review on floral scents, Knudsen et al. (2006) emphasized the importance of twelve compounds that occur in over 50% of the 90 plant families investigated. They highlighted six monoterpenes (D-limonene, (E)-β-ocimene, β-myrcene, linalool, α-pinene, β-pinene), one irregular terpene (6-methyl-5-hepten-2-one), four shikimic acid pathway by-products (benzaldehyde, methyl-2-hydroxybenzoate, benzyl alcohol, 2-phenyl ethanol), and one sesquiterpene (β-caryophyllene). Aside from their likely role in plant pollination, most of these have toxic or deterrent activity against microbes and herbivores (De Moraes et al. 2001; Mumm and Hilker 2006; Bakkali et al. 2008). It has been proposed that the widespread distribution of these compounds and their emission by both reproductive and vegetative parts of the plant reflect their importance in chemical defense (Knudsen et al. 2006). We expect that they also will be widespread among tropical tree species, as a response to high predation pressure.

Aside from the common compounds, one of the striking characteristics of plant volatile terpenes is their diversity and the large number of compounds in blends (Gershenzon and Dudareva 2007). The "screening hypothesis" proposes that the ability to produce a large array of defensive compounds increases the probability of possessing active compounds (Jones and Firn 1991; Firn and Jones 2003). A high chemical diversity should help increase the plant's protection against attacks from a wide range of enemies in changing environments (Agrawal and Fishbein 2006; Gershenzon and Dudareva 2007). Moreover, diverse compounds may act synergistically to provide greater toxicity or deterrence (Berenbaum and Neal 1985), or to maintain activity for a longer period of time (Akhtar and Isman 2003). Complex VOC blends also may be involved in making the information sent to parasitoids in tritrophic interactions species-specific (Gershenzon and Dudareva 2007). Thus, the diversity of VOCs in a species may be at least in part related to defensive roles. Studying patterns of VOC diversity and variability across species may shed light on their importance in long-lived species such as tropical trees.

Both the density and diversity of insects are higher in the tropics than in temperate zones (Novotny et al. 2006; Lewinsohn and Roslin 2008). This leads to higher predation pressure on tropical plants compared with their temperate counterparts (Janzen 1970; Coley and Aide 1991). Consequently, tropical plants may have enhanced defenses compared to temperate plants, including more diversity and variability in chemical defenses (Coley and Aide 1991). We postulated that many different VOCs would be released by tropical tree species, reflecting a diverse array of predatory pressure. Moreover, the use of VOCs in defense may not be uniform among species, and this should lead to mixtures of varying complexities.

We sampled the vegetative parts (bark and leaves) of a wide array of tropical plants in natural forests in French Guiana (South America), and identified the blend of constitutive VOCs released immediately in response to physical damage. We used this dataset to determine patterns of VOC diversity among tropical tree species with three objectives: (1) What is the relative frequency of VOCs across species? (2) Are mixtures of VOCs species specific? (3) Is the complexity of the mixtures variable across species?

Methods and Materials

Study Sites Field work was conducted at three sites of old-growth rainforest in French Guiana, namely the Paracou Research Station (5°18′N, 52°53′W), the Nouragues Research Station (4°05′N, 52°40′W), and Montagne Tortue (4°18′N, 52°22′W). Annual rainfall is 2,990 mm for Nouragues, 3,160 mm for Paracou, and ca. 3,500 mm for Montagne Tortue. We worked on five plots of one hectare each: two were at the Paracou site (N=643 and 487 trees), two at Nouragues (N=537 and 567 trees), and one at Montagne Tortue (N = 536 trees). In these plots, each tree with a diameter at breast height (dbh) greater than 10 cm was climbed by professional tree climbers (Baraloto et al. 2009) to collect leaves in order to create a voucher specimen for taxonomic determination. Over 98% of the trees were identified to species or morphospecies, and the species richness ranged from 148 to 210 species per hectare. Sampling was conducted in June 2007 for Paracou, September 2007 for Nouragues, and in November 2007 for Montagne Tortue.

Field Sampling We sampled species belonging to the most common tree families across sites and for which our plots had more than one individual per species. Overall, 2–6 individuals of the most common species were chosen,

depending on the individuals available, totaling 195 individuals from 55 species. Table 1 lists the sampled species and sampling size. Sampling a large number of species requires a rapid technique and appropriate storage for subsequent analysis (Wajs et al. 2006). For each tree, we cut about 20 mg of tissue from a young leaf. Young leaves were chosen because they are better chemically defended than older ones (McKey 1979). We also sampled a 1-cm^2 piece of bark at 1 m above ground, using a leather punch. Each sample was put immediately into a glass vial (10 ml) and sealed with a screw cap containing a Teflon-lined septum (Varian Instruments, Sunnyvale, CA, USA). In the field, sealed vials were maintained at −4°C in a portable freezer. Samples were transferred to the laboratory where they were stored at −20°C until analysis.

By using four taxa representative of our dataset (*Protium sp.* Burseraceae, *Inga sp.* Mimosaceae, *Guarea sp*. Meliaceae, *Spondias mombin* Anacardiaceae), we verified that the VOC composition as measured with our protocol was consistent with the blend released following mechanical damage of the tissue (either bark or leaves). For 3 individuals per species, we trapped VOCs directly in the field by sealing mechanically damaged tissue in a Teflon bag, and then introducing a Solid Phase Micro Extraction (SPME) fiber into the bag for 5 to 15 min (Bouvier-Brown et al. 2007). For the same tissue, we collected VOCs from frozen samples as described below.

Laboratory Analyses VOCs from the headspace of the samples were adsorbed onto SPME fibers, allowing analysis without solvent extraction (Lord and Pawliszyn 2000). Originally developed for the analysis of pollutants, this technique is suitable for detecting VOCs emitted by plants (Tholl et al. 2006; Mayer et al. 2008). We used fused silica fibers coated with PDMS/DVB (Polydimethylsiloxane/Divinylbenzene) 65 µm (Supelco, Bellefonte, PA, USA), since this coating is an effective trap for plant VOCs (Guo et al. 2006; Bouvier-Brown et al. 2007). Fibers were conditioned before the first use for 30 min at 250°C, following instructions from the manufacturer. Before extraction, the glass vials containing the tissue samples were maintained at room temperature for at least 1 h. The SPME fiber was placed into the vial with the tissue sample (bark or leaf) for 5 to 60 min at ambient temperature (25° C). The exposure time was optimized for each species to maximize the extraction without saturating the analytical column (Appendix 1). The fiber was inserted immediately into the 250°C inlet of a Varian 3800 Gas Chromatograph (GC) fitted with a Saturn 2000 ion-trap Mass Spectrometer (MS; Varian Instruments, Sunnyvale, CA, USA). The GC was run with a non-polar Varian DB-5 column (30 m× 0.25 mm ID, 0.25 µm film) commonly used for the analysis of VOCs (Tholl et al. 2006). Helium was the carrier gas at a constant flow of 1 ml/min. The oven temperature program of the GC started at 50°C, with 6°C/min temperature increase up to 140°C, and then with 4°C/min increments up to 160°C. This temperature was held for 1 min and increased finally to 200°C at 10°C/min. The MS was operated in electronimpact (EI) mode at 70 eV, with a scan range of 30–450 m/z. After each analysis, the fibers were cleaned in the injector port for 10 min at 250°C, and each fiber was reused no more than 100 times, as recommended by Tholl et al. (2006). Control analyses (blanks) were performed every ten analyses to check for contamination of the fiber.

Post-Processing of the GC-MS data Since the SPME technique does not ensure quantitative recovery, only presence/absence data are reported. VOC presence/absence was inferred by using a novel statistical approach (Nicole et al., unpublished). This procedure is both more efficient and more accurate than visual classification procedures for a large number of analyses. All routines were developed in the R statistical software (http://cran.r-project.org/). Here, we review briefly the major steps in the procedure.

A typical GC-MS output is represented by two components. The chromatogram displays the mixture as separated by the GC, and each peak corresponds to the elution of a distinct molecule, characterized by retention time. For each point on the GC chromatogram (N=1,500), a mass spectrum is obtained by fragmentation in the MS chamber. A mass spectrum is represented by a histogram displaying the intensity of each fragment (the mass-to-charge ratio, m/z).

The GC-MS output was saved into a raw text file by using the *GC and GCMS File Translator*™ (ChemSW, http://www.chemsw.com/12149.htm). Peaks were identified automatically in each chromatogram as a 3-point increase followed by a 3-point decrease around a peak intensity of at least 5 kcounts. Each detected peak in the course of the analysis was then defined by the mass spectrum that corresponded to the apex of the peak. The mass spectra corresponding to all peaks in all analysis were grouped into a single matrix in which each row represented the mass spectrum associated with a selected GC peak. A distance matrix was computed by using the pairwise Euclidian distance between any two spectra. An agglomerative clustering algorithm was used to cluster the spectra based on this distance matrix (Ward 1963), and the optimal number of clusters was inferred from a variance analysis (Rousseeuw 1987).

The Kováts Retention Index (RI, Kovats 1958) for each peak were defined as follows

$$RI(i) = 100 \times \left(\frac{\ln(RT(i)) - \ln(RT_{\inf}(i))}{\ln(RT_{\sup}(i)) - \ln(RT_{\inf}(i))} \right) + 100n$$

Table 1 Number of individuals analyzed sorted by order, family, genus, and species

Order	Family	Genus	Species	Species code	#
Magnoliales	Annonaceae	*Duguetia*	*surinamensis*	*D. surinamensis*	3
Magnoliales	Annonaceae	*Oxandra*	*asbeckii*	*O. asbeckii*	4
Magnoliales	Annonaceae	*Unonopsis*	*perrottetii*	*U. perrottetii*	5
Magnoliales	Annonaceae	*Unonopsis*	*rufescens*	*U. rufescens*	3
Magnoliales	Annonaceae	*Xylopia*	*nitida*	*X. nitida*	4
Magnoliales	Myristicaceae	*Iryanthera*	*hostmannii*	*I. hostmannii*	2
Magnoliales	Myristicaceae	*Iryanthera*	*sagotiana*	*I. sagotiana*	2
Magnoliales	Myristicaceae	*Virola*	*michelii*	*V. michelii*	2
Laurales	Lauraceae	*Aniba*	*panurensis*	*A. panurensis*	2
Laurales	Lauraceae	*Ocotea*	*argyrophylla*	*O. argyrophylla*	2
Laurales	Lauraceae	*Ocotea*	*percurrens*	*O. percurrens*	2
Laurales	Lauraceae	*Sextonia*	*rubra*	*S. rubra*	4
Myrtales	Myrtaceae	*Myrcia*	*decorticans*	*M. decorticans*	3
Myrtales	Vochysiaceae	*Ruizterania*	*albiflora*	*R. albiflora*	3
Malpighiales	Caryocaraceae	*Caryocar*	*glabrum*	*C. glabrum*	3
Malpighiales	Chrysobalanaceae	*Hirtella*	*glandulosa*	*H. glandulosa*	4
Malpighiales	Chrysobalanaceae	*Licania*	*membranacea*	*L. membranacea*	5
Malpighiales	Chrysobalanaceae	*Parinari*	*campestris*	*P. campestris*	2
Malpighiales	Clusiaceae	*Rheedia*	*madruno*	*R. madruno*	3
Malpighiales	Clusiaceae	*Tovomita*	*spB1*	*T. spB1*	3
Malpighiales	Euphorbiaceae	*Conceveiba*	*guianensis*	*C. guianensis*	5
Fabales	Caesalpiniaceae	*Tachigali*	*melinonii*	*T. melinonii*	2
Fabales	Caesalpiniaceae	*Vouacapoua*	*americana*	*V. americana*	3
Fabales	Papilionaceae	*Bocoa*	*prouacensis*	*B. prouacensis*	3
Rosales	Cecropiaceae	*Pourouma*	*villosa*	*P. villosa*	3
Rosales	Moraceae	*Brosimum*	*guianense*	*B. guianense*	6
Sapindales	Anacardiaceae	*Anacardium*	*spruceanum*	*A. spruceanum*	5
Sapindales	Anacardiaceae	*Thyrsodium*	*guianense*	*T. guianense*	4
Sapindales	Anacardiaceae	*Thyrsodium*	*puberulum*	*T. puberulum*	4
Sapindales	Burseraceae	*Dacryodes*	*nitens*	*D. nitens*	4
Sapindales	Burseraceae	*Protium*	*decandrum*	*P. decandrum*	5
Sapindales	Burseraceae	*Protium*	*opacum*	*P. opacum*	4
Sapindales	Burseraceae	*Protium*	*sagotianum*	*P. sagotianum*	4
Sapindales	Burseraceae	*Tetragastris*	*altissima*	*T. altissima*	4
Sapindales	Burseraceae	*Tetragastris*	*panamensis*	*T. panamensis*	4
Sapindales	Meliaceae	*Carapa*	*procera*	*C. procera*	3
Sapindales	Sapindaceae	*Cupania*	*scrobiculata*	*C. scrobiculata*	5
Sapindales	Simaroubaceae	*Simaba*	*cedron*	*S. cedron*	5
Malvales	Bombacaceae	*Pachira*	*dolichocalyx*	*P. dolichocalyx*	3
Malvales	Sterculiaceae	*Sterculia*	*pruriens*	*S. pruriens*	4
Malvales	Sterculiaceae	*Theobroma*	*subincanum*	*T. subincanum*	4
Malvales	Tiliaceae	*Apeiba*	*glabra*	*A. glabra*	4
Ericales	Lecythidaceae	*Eschweilera*	*congestiflora*	*E. congestiflora*	3
Ericales	Lecythidaceae	*Eschweilera*	*coriacea*	*E. coriacea*	3
Ericales	Lecythidaceae	*Lecythis*	*persistens*	*L. persistens*	4
Ericales	Lecythidaceae	*Lecythis*	*poiteaui*	*L. poiteaui*	4
Ericales	Sapotaceae	*Chrysophyllum*	*argenteum*	*C. argenteum*	3
Ericales	Sapotaceae	*Micropholis*	*egensis*	*M. egensis*	3
Ericales	Sapotaceae	*Micropholis*	*guyanensis*	*M. guyanensis*	5

Table 1 (continued)

Order	Family	Genus	Species	Species code	#
Ericales	Sapotaceae	*Pouteria*	*gonggrijpii*	*P. gonggrijpii*	3
near Gentianales	Icacinaceae	*Poraqueiba*	*guianensis*	*P. guianensis*	4
Gentianales	Apocynaceae	*Aspidosperma*	*cruentum*	*A. cruentum*	3
Gentianales	Apocynaceae	*Aspidosperma*	*marcgravianum*	*A. marcgravianum*	4
Gentianales	Rubiaceae	*Chimarrhis*	*turbinata*	*C. turbinata*	4
Gentianales	Rubiaceae	*Posoqueria*	*latifolia*	*P. latifolia*	3
					195

where $RT(i)$ is the retention time of the i^{th} peak (in minutes), $RT_{inf}(i)$ is the retention time of a reference n-alkane that eluted immediately before the i^{th} peak, and $RT_{sup}(i)$, the retention time of (n+1)-alkane that eluted immediately after the i^{th} peak. In the above equation, n is the number of carbon atoms in the n-alkane that eluted immediately before the i^{th} peak. Together with the mass spectrum, RI is used for the identification. The consistency of the clusters generated automatically was tested by computing the intra-cluster variance of RI. Ambiguous clusters were examined separately and found to correspond either to very similar compounds that can be distinguished with RI or to several co-eluted compounds. Clusters were identified based on comparison to authentic mass spectral standards or to the NIST 98 MS library, the ADAMS library, and to RI reported in the literature (ADAMS). Using these techniques we were able to assign 78% of the compounds as known molecular structures (Appendix 2). For unidentified molecules, we defined them as unknown molecules characterized by their mass spectra and their RI.

Validation of the Protocol We verified our methods by comparing the VOCs obtained from SPME fibers exposed to freshly harvested tissue in the field to those obtained with our lab techniques that used frozen tissue. The qualitative blend of VOCs was the same with the two methods for four different species tested (Fig. 1). During the period of analysis, we confirmed that storage of tissue samples at −20°C did not alter VOC composition by repeating the analyses on three samples at monthly intervals (data not shown). We concluded that the methods were an effective way to sample VOCs from the large number of samples collected in the field study.

Statistical Analyses For each individual tree, the VOC composition was defined as the composite of all compounds found in the leaves, in the bark or in both tissues. The resulting dataset was characterized by a presence/absence matrix, where each row represented the compounds found in an individual tree, and each column represented a single compound. We calculated the chemical richness (number of distinct compounds) for each individual, each species, and each plant order. The significance of differences in mean VOC diversity (the number of compounds)

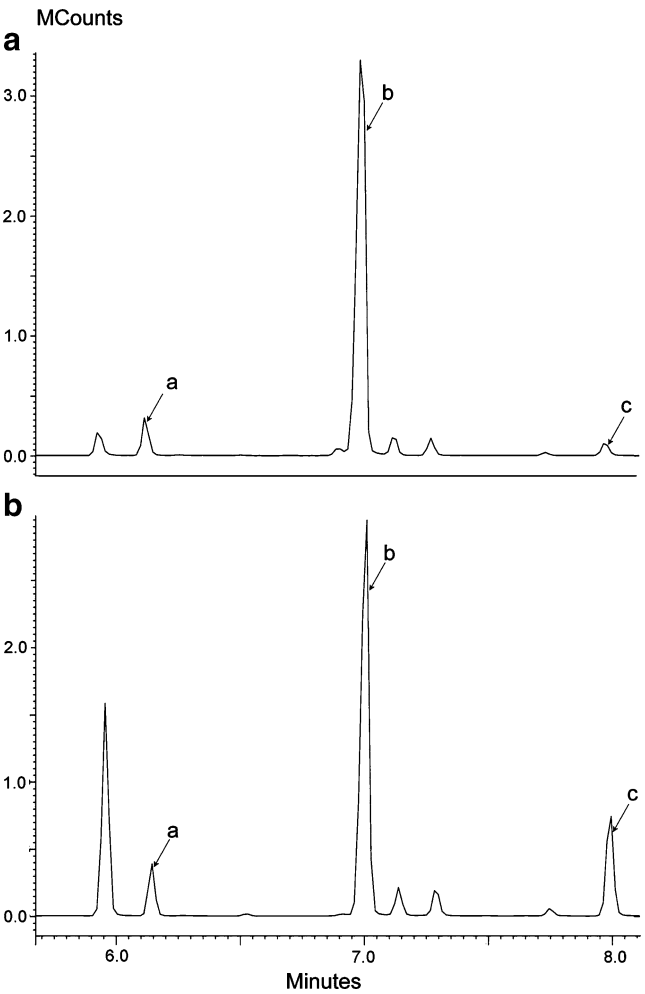

Fig. 1 Comparison of the composition in monoterpenes for the leaves of one individual of *Protium sp.*, for **a** extraction in the field and **b** extraction in glass vials from frozen samples. Similar results were obtained for the other species. Compounds shown are: **a** α-pinene, **b** sabinene, **c** γ-terpinene

among species and orders were tested with the non parametric *Kruskal-Wallis* test.

To assess whether our dataset could be used to discriminate among species, we constructed a matrix of chemical dissimilarity between pairs of individuals using the Manhattan distance $D_{x,y} = \sum_{i=1}^{N_m} |x_i - y_i|$, where x and y are two distinct individuals, and N_m is the total number of compounds in our dataset. We then constructed a hierarchical dendrogram with the Ward clustering algorithm (Ward 1963). Dendrogam node support was assessed based on approximately unbiased (AU) p-values computed from 10,000 bootstraps (pvclust package; Suzuki and Shimodaira 2006).

Results

A total of 264 compounds was found in the 55 sampled species. This included one nitrogen-containing compound, three compounds from the shikimic acid pathway, 34 compounds from the lipoxygenase pathway (or green leaf volatiles), 57 monoterpenes, and 169 sesquiterpenes. We were able to assign names to 206 of the 264 compounds (78%). The 58 unidentified compounds are mostly sesquiterpenes, and many of them may be previously unreported.

Four terpenes were released by over 90% of the species (Fig. 2): α-pinene (all species) and D-limonene (96% of the species) in the monoterpene group, and β-caryophyllene (94% of species) and α-copaene (92% of species) in the sesquiterpene group. About 23% of the compounds were present in the VOC blend of only a single species (Fig. 2).

For 43 of the 55 sampled species, the clustering analysis grouped all individuals of the same species in well-supported clusters (AU *P*-value greater than 0.80; Fig. 3). Above the species level, this clustering analysis did not show any pattern of chemical similarities within genera or families (Fig. 3). Similar results were obtained by considering only monoterpenes and sesquiterpenes.

The complexity of the VOC profile varied significantly both across all species and within each plant order

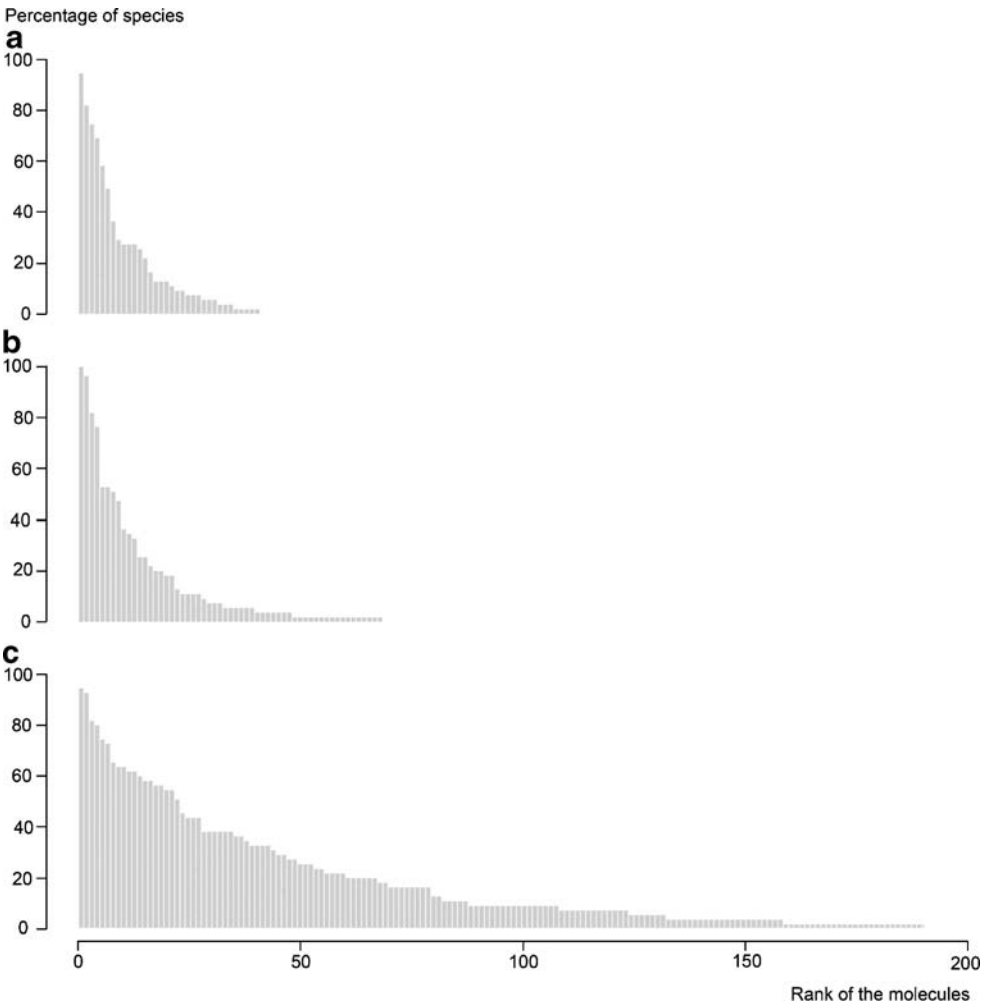

Fig. 2 Distribution of the VOCs isolated in the dataset with the percentage of species emitting each molecule for the three most common groups: **a** green leaf volatiles, **b** monoterpenes, and **c** sesquiterpenes

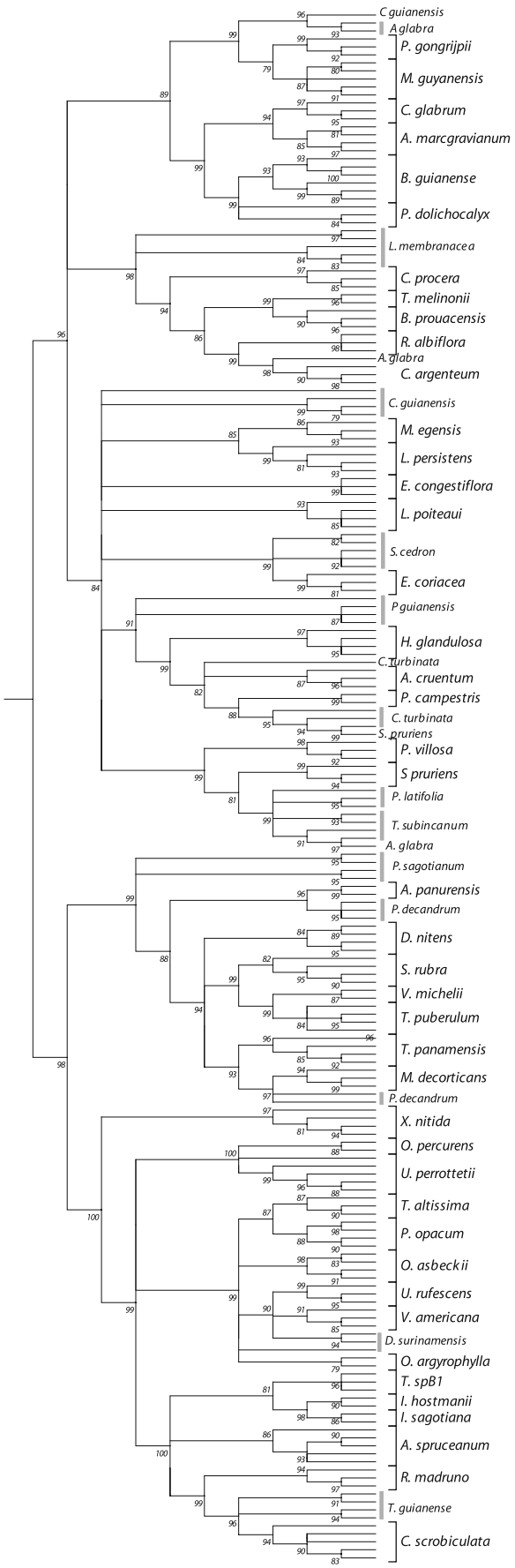

Fig. 3 Dendrogram displaying the dissimilarities in VOC composition among individuals, based on Ward clustering and Manhattan distance. Bootstrap support values greater than 80% on the nodes are reported on the tree. Species with names indicated in *grey* did not group into single clusters with a bootstrap value greater than 80%

(Kruskal-Wallis test, $P<0.001$). The mean number of VOCs per individual was 36.8 (Fig. 4). Chemical diversity was higher for species belonging to the Laurales, Sapindales, or Magnoliales (Fig. 4a), mean number of compounds per species in each order 43.8, 48.7, and 58.5, respectively). Two exceptions to this general trend were *Simaba cedron,* a member of the Simaroubaceae, and *Sextonia rubra*, in the Lauraceae. Both species had lower chemical diversity relative to other members of their families (Fig. 4a), mean number of compounds 17.4 and 33.5, respectively). Species belonging to the Ericales, Gentianales, and Malvales emitted less diverse mixtures of VOCs (21.3, 22.6, and 24.6, respectively). These differences were mainly due to differences in the number of sesquiterpenes (Fig. 4b).

Discussion

The most prevalent compounds that were identified in this study have defensive roles in model plant species, and they are among the most common compounds identified in floral scents (Knudsen et al. 2006). Within the monoterpenes, both α-pinene and limonene are toxic to fungi, bacteria, and insects (Miresmailli et al. 2006; Bakkali et al. 2008). These two compounds are known to be emitted in large quantities by tropical trees, as atmospheric chemists have previously detected them in high concentration in the atmosphere above Amazonian forests (Greenberg et al. 2004). Here, we demonstrated that a large array of species emit them. The other monoterpenes ((E)-β-ocimene, β-myrcene, linalool, β-pinene) and the irregular terpene (6-methyl-5-hepten-2-one), common in floral scents, are also found in vegetative emissions (Appendix 1). The sesquiterpenes caryophyllene and α-copaene have been implicated in direct and indirect responses against herbivores and pathogens (Heil 2004; Gols et al. 2008; Köllner et al. 2008), and they were distributed widely in the studied tree species.

The blend of VOCs is largely species-specific, as most species formed single clusters based on their VOC profiles. Taxa above the species level (genus, family, or order) usually were not characterized by a distinct profile. This result is in agreement with the pattern observed in floral scents where VOCs are consistent within a species but usually differ among closely related ones (Knudsen et al. 2006).

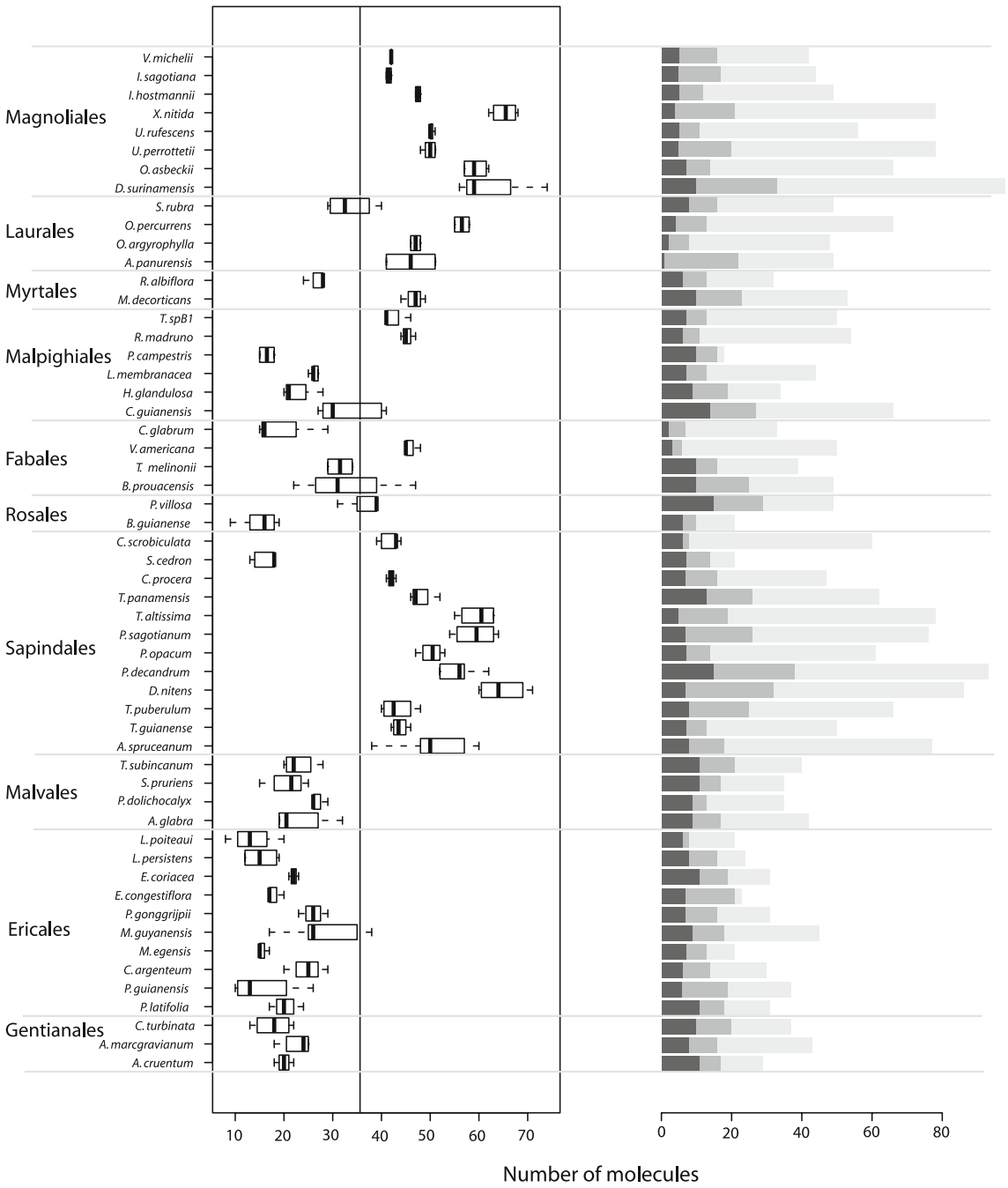

Fig. 4 **a** Mean number of compounds and the variation observed among conspecific individuals. For each box, *leftmost point* represents the 5% quantile and the *rightmost point*, the 95% quantile. The *rectangular box* represents the 25% quantile to 75% quantile ranges. The *dark line* shows the median of the distribution. The *vertical line* represents the mean number of compounds in the dataset (36.8) **b** contribution of the three more common class of compounds to the mean number of compounds for each species with green leaf volatiles in black, monoterpenes in *dark grey* and sesquiterpenes in *light grey*. *Horizontal lines* separate the distinct order in the dataset

The total diversity of VOCs varied greatly across species. Species in the Sapindales, Magnoliales, and Laurales generally released a more diverse blend than species in other orders. Exceptions were *Simaba cedron* (Simaroubaceae, Sapindales), and *Sextonia rubra* (Lauraceae, Laurales), which emit simpler mixtures of VOCs than other species in their respective families. *Simaba cedron* is actively defended by quassinoids, oxygenated triterpenes that are not detected by GC-MS (Ozeki et al. 1998). A non-volatile defensive compound has been isolated in great quantity from *S. rubra* wood (D. Stien, unpublished results). These specific examples suggest that plants

defended by other compounds may have simpler VOC profiles than plants that rely mainly on VOC defense.

Terpenoids made up the majority of the observed chemical diversity, consistent with our current knowledge on plant VOC synthesis (Wink 2003). Sesquiterpenes were more diverse than monoterpenes in tropical trees, with many compounds produced by only a few species. Diverse sesquiterpene composition is not the rule for all tree species: conifers emit mostly monoterpenes and only a few sesquiterpenes (Keeling and Bohlmann 2006). Our data suggest that production of sesquiterpenes may be more important than previously thought for tropical plants. In previous studies, VOC emissions from tropical forests have been quantified mostly in atmospheric studies of air pollutants (Guenther 2002). Generally, sesquiterpenes are difficult to detect in the air, due to their short lifetime in the atmosphere (Kesselmeier and Staudt 1999; Guenther 2002). Understanding the importance of sesquiterpene emissions by tropical forests remains an important challenge (Bouvier-Brown et al. 2007), and the present work provides a reference database for future investigations in this direction.

We hypothesize that plant defense by VOCs depends not only on the abundance of the various emitted VOCs, but also on the diversity of the blend. Fine et al. (2006) recently compared the investment in the production of monoterpenes and sesquiterpenes in tropical tree species to test the hypothesis that species under a higher herbivory pressure are better defended chemically. This hypothesis was not supported for the genus *Oxandra* (Annonaceae, Magnoliales). Our analysis may provides an explanation for their result: Fine et al. (2006) only compared overall concentration of terpenes and did not look at the diversity of the blend.

An assumption of our study is that, to measure the chemical diversity of VOC blends, we considered each compound as an independent unit. However, VOCs are linked by their biosynthetic pathways, especially in the sesquiterpene and monoterpene groups where terpene synthases often are involved in the synthesis of multiple products. An important challenge is to determine whether biosynthetic pathways may be partially elucidated by data from large-scale surveys.

One important limitation of our analysis is that we characterized the blend of VOCs released immediately after inflicting mechanical damage and not after an herbivory event. We assumed that this blend is representative of the constitutive VOCs released immediately after herbivore attack (see also Banchio et al. 2005; Mithöfer et al. 2005). However, VOC emissions are often modulated by factors such as elicitors released by herbivores (Mattiacci et al. 1995). In some species, VOCs are stored in specialized cells and volatilized immediately after wounding, whereas in others, synthesis is triggered by herbivory (Turlings and Wäckers 2004). Our study was designed to detect general pattern of constitutive VOCs among numerous long-lived species. Detailed studies will be necessary to understand fully the relative role of constitutive and induced VOCs in the defensive strategies of tropical trees.

In summary, our analysis highlights three major aspects of VOC emissions by tropical tree species. First, for most species, the intraspecific variation of the VOC blend is low, suggesting that VOC composition is species-specific. Second, sesquiterpenes are a key constituent of the VOC blend. Future studies should link pathogens and herbivore occurrence as well as herbivory rates with the quantity and diversity of sesquiterpenes in leaves and bark. Third, the complexity of the VOC mixtures varies significantly among species, indicating large differences in VOCs production among species. Future studies will need to relate this observation to our understanding of the evolution of plant defenses and to the herbivores and pathogens community associated with each species.

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Appendix 1

SPME fiber exposure time for leaves and bark of each species

species code	Bark extraction (min)	Leaf extraction (min)
D. surinamensis	15	15
O. asbeckii	15	15
U. perrottetii	15	15
U. rufescens	15	15
X. nitida	15	15
I. hostmannii	30	30
I. sagotiana	30	30
V. michelii	30	30
A. panurensis	5	15
O. argyrophylla	5	15
O. percurrens	5	15
S. rubra	5	15
M. decorticans	30	15
R. albiflora	30	30
C. glabrum	30	30
H. glandulosa	30	30

L. membranacea	60	30
P. campestris	60	30
R. madruno	30	30
T. spB1	15	15
C. guianensis	30	30
T. melinonii	15	30
V. americana	5	5
B. prouacensis	30	30
P. villosa	30	30
B. guianense	30	30
A. spruceanum	15	30
T. guianense	15	15
T. puberulum	15	15
D. nitens	5	15
P. decandrum	5	15
P. opacum	5	15
P. sagotianum	5	15
T. altissima	5	15
T. panamensis	5	15
C. procera	30	30
C. scrobiculata	15	15
S. cedron	60	15
P. dolichocalyx	30	30
S. pruriens	30	30
T. subincanum	30	30
A. glabra	30	30
E. congestiflora	30	15
E. coriacea	30	15
L. persistens	30	15
L. poiteaui	30	30
C. argenteum	30	30
M. egensis	30	30
M. guyanensis	30	30
P. gonggrijpii	30	30
P. guianensis	30	15
A. cruentum	30	30
A. marcgravianum	30	30
C. turbinata	30	30
P. latifolia	30	30

Appendix 2

Compounds tentatively identified. For each compound, the Kovats RI and the number of species that emits the compound is indicated. The compounds authenticated with standards are indicated by *.

classe	compound	RI	# species
N_compound	2-isopropyl-3-methoxypyrazine	1091	1
shikimic pathway	unknown S 1	1206	15
	methyl salicylate	1206	5
	1,4-dimethoxybenzene	1249	1
lipidic pathway (LP)	hexanal	813	52
	n-hexanol	872	45
	(*E*)-2-hexenal	863	41
	penten-3-ol	741	38
	2-ethyl furan	749	32
	(*Z*)-3-hexenol	864	27
	unknown LP 1	733	20
	isopentyl alcohol	768	16
	unknown LP 2	735	15
	octen-3-ol	984	15
	3-octanone	989	15
	unknown LP 3	854	14
	2-pentanone	743	12
	penten-1-ol	791	9
	3-pentanone	749	7
	heptanal	905	7
	hexyl hexanoate	1386	7
	(*E*)-3-hexenol	857	6
	1-pentanol	769	5
	hexenyl acetate	1014	5
	3-methyl-3-buten-1-ol	757	4
	3-octanol	1000	4
	hexenyl-3-methylbutanoate	1231	4
	(*Z*)-2-hexenol	872	3
	octanone	989	3
	hexenyl butanoate	1186	3
	octene	800	2
	(E)-2-octen-1-al	1066	2
	hexyl butanoate	1192	2
	unknown LP 4	805	1
	unknown LP 5	909	1
	unknown LP 6	970	1
	hexenyl isobutanoate	1142	1
	hexenyl isovalerate	1236	1
irregular terpene	6-methyl-5-hepten-2-one	989	1
monoterpene	α-pinene *	940	55
	limonene *	1034	53
	p-cymene	1030	45
	β-pinene	985	42
	3-carene	1014	29
	β-myrcene *	992	29
	α-phellandrene	1011	28
	camphene	958	26
	linalool *	1103	20
	p-xylene	881	19
	α-thujene	932	18
	β-phellandrene	1036	14
	β-terpinolene	1089	14
	1,8-cineole	1038	12

	α-terpinene	1022	11
	γ-terpinene	1062	11
	β-ocimene	1049	10
	sabinene	978	10
	o-xylene	905	7
	iso-methoxythymol	1241	6
	o-cymene	1025	6
	Perilene	1114	6
	tricyclene	930	6
	delta-2-carene	1002	5
	α-terpinolene	1083	4
	cis-sabinene-hydrate	1075	4
	terpinene-4-ol	1188	4
	allo-ocimene	1119	3
	linalool-oxide-trans	1075	3
	unknown monoterpene 1	1173	3
	mentha-1-7(8)-diene	1010	3
	p-cymenene	1096	3
	verbenene	986	3
	3p-menthene	1000	2
	camphor	1156	2
	carvacrol-methyl-ether	1232	2
	(*E*)-β-ocimene	1047	2
	mentha-2,8-diene	1001	2
	thuja-2,4(10)-diene	961	2
	thymol-methyl-ether	1227	2
	(4*E*,6*Z*)-allo-ocimene	1131	1
	α-terpineol	1202	1
	campholenal	1133	1
	cymen-8-ol	1194	1
	linalool-oxide-cis	1180	1
	linalool-oxide-dihydroxy	1112	1
	unknown monoterpene 2	1047	1
	mentha-2,8-dienol	1123	1
	myrtenal	1204	1
	pinocarvone	1170	1
	rose-furan-oxide	1197	1
	trans-sabinene-hydrate	1108	1
	sylvestrene	1021	1
	trans-pinocarveol	1149	1
	trans-verbenol	1152	1
	(*Z*)-β-ocimene	1035	1
sesquiterpene	β-caryophyllene *	1427	52
	α-copaene	1381	51
	α-humulene	1462	45
	δ-cadinene	1521	44
	germacrene D	1487	41
	cyperene	1412	40
	bicyclogermacrene	1502	36
	allo-aromadendrene	1467	35
	sesquithujene	1393	35
	α-ylangene	1375	34
	di-exo-T-cadinol	1479	34
	γ-cadinene	1518	33
	calarene	1436	32
	unknown sesquiterpene 1	1393	32
	aromadendrene	1445	31
	unknown sesquiterpene 2	1503	31
	α-cubebene	1349	30
	unknown sesquiterpene 3	1502	30
	trans-calamenene	1526	28
	δ-elemene	1338	25
	β-humulene *	1433	24
	bicyclo-elemene	1335	24
	eremophyladiene	1541	24
	α-selinene	1496	21
	caryophyllene-oxide	1592	21
	cis-cadina-1,4-diene	1498	21
	γ-elemene	1432	21
	unknown sesquiterpene 4	1350	21
	unknown sesquiterpene 5	1439	21
	α-cadinene	1536	20
	β-bazzanene	1529	20
	(*Z*)-α-bisabolene	1509	19
	α-muurolene	1507	18
	unknown sesquiterpene 6	1399	18
	unknown sesquiterpene 7	1435	18
	sesquithujene-7-epi	1389	18
	gamma-selinene	1479	17
	allo-aromadendra-4(15),10(14)-diene	1455	16
	sesquiphellandrene	1514	16
	african-2(6)-ene	1361	15
	α-cedrene	1416	15
	7-epi-α-cedrene	1404	14
	α-guaiene	1439	14
	unknown sesquiterpene 8	1450	14
	1-epi-α-pinguisene	1370	13
	β-maaliene	1417	13
	(*Z*)-β-farnesene	1451	12
	β-bisabolene	1503	12
	unknown sesquiterpene 9	1483	12
	viridiflorene	1496	12
	anastreptene	1370	11
	g-muurolene	1480	11
	iso-carryophyllene	1411	11
	rotundene	1469	11
	unknown sesquiterpene 10	1327	11
	unknown sesquiterpene 11	1434	11
	β-calacorene	1547	10
	β-curcumene	1512	10
	α-curcumene	1484	9
	δ-selinene	1492	9
	unknown sesquiterpene 12	1500	9

unknown sesquiterpene 13	1528	9
unknown sesquiterpene 14	1547	9
unknown sesquiterpene 15	1369	9
unknown sesquiterpene 16	1405	9
unknown sesquiterpene 17	1451	9
β-cubebene	1385	7
β-selinene	1490	7
(*Z,E*)-α-farnesene	1488	6
β-elemene	1385	6
unknown sesquiterpene 18	1325	6
spathulenol	1585	6
trans-cadina-1,4-diene	1538	6
7-epi-α-selinene	1534	5
α-longipinene	1358	5
β-barbatene	1458	5
β-bourbonene	1390	5
β-ylangene	1424	5
cadina-3,5-diene	1454	5
calameren-9-ol	1555	5
cis-calamenene	1532	5
cuparene	1517	5
gorgonene	1446	5
hinesene	1528	5
muurolol	1603	5
oppositadiene	1393	5
presilphiperfolene	1312	5
unknown sesquiterpene 19	1507	5
unknown sesquiterpene 20	1443	5
unknown sesquiterpene 21	1425	5
α-gurjunene	1413	4
β-acoradiene	1472	4
bourboneral	1555	4
cadinene-ether	1570	4
γ-curcumene	1480	4
guaiadiene	1407	4
maali-1,3-diene	1351	4
unknown sesquiterpene 22	1530	4
unknown sesquiterpene 23	1420	4
unknown sesquiterpene 24	1449	4
selina-4,7-diene	1513	4
striatene	1461	4
trans-cubebol	1514	4
(*E*)-β-farnesene	1461	3
aromadendra-4,10(14)-diene	1442	3
cadina-1(10),6-diene	1461	3
epi-α-muurolol	1655	3
unknown sesquiterpene 25	1455	3
unknown sesquiterpene 26	1484	3
unknown sesquiterpene 27	1458	3
α-alaskene	1514	2
α-cadinol	1666	2
α-santalene	1423	2
bourbon-11-ene	1429	2
brasiladiene	1336	2
cubenol	1624	2
cyclo-bazzanene	1523	2
cyperadiene	1358	2
dendrolasine	1576	2
epi-α-cadinol	1654	2
gamma-guaiene	1511	2
isoledene	1378	2
unknown sesquiterpene 28	1457	2
unknown sesquiterpene 29	1523	2
unknown sesquiterpene 30	1437	2
unknown sesquiterpene 31	1328	2
unknown sesquiterpene 32	1389	2
unknown sesquiterpene 33	1509	2
unknown sesquiterpene 34	1533	2
unknown sesquiterpene 35	1443	2
unknown sesquiterpene 36	1444	2
selina-4,11-diene	1482	2
(3*E*,6*Z*)-α-farnesene	1481	1
5-epi-aristolochene	1476	1
african-2,6-diene	1345	1
α-cuprenene	1555	1
α-duprezzianene	1389	1
β-chamigrene	1534	1
β-vetivene	1536	1
brasila-1(6),5(10)-diene	1436	1
cadalene	1635	1
calamenol	1550	1
cymene-2,5-dimethoxy-para	1415	1
(*E*)-γ-bisabolene	1529	1
epistolene	1393	1
erythrodiene	1447	1
2-epi-α-funebrene	1419	1
germacrene B	1567	1
iso-bicyclogermacrene	1489	1
pacifigorgia-1(9),10-diene	1385	1
pacifigorgia-2,10-diene	1431	1
palustrol	1581	1
unknown sesquiterpene 37	1462	1
unknown sesquiterpene 38	1493	1
unknown sesquiterpene 39	1577	1
unknown sesquiterpene 40	1585	1
unknown sesquiterpene 41	1422	1
unknown sesquiterpene 42	1334	1
unknown sesquiterpene 43	1343	1
unknown sesquiterpene 44	1496	1
unknown sesquiterpene 45	1424	1
unknown sesquiterpene 46	1448	1
unknown sesquiterpene 47	1329	1
unknown sesquiterpene 48	1506	1
unknown sesquiterpene 49	1420	1

unknown sesquiterpene 50	1423	1
sesquicineole	1516	1
veltonal	1595	1
widrene	1441	1

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