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Differences in volatile terpene composition between the bark and leaves of tropical tree species

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ABSTRACT

Volatile terpenes are among the most diverse class of defensive compounds in plants, and they are implicated in both direct and indirect defense against herbivores. In terpenes, both the quantity and the diversity of compounds appear to increase the efficiency of defense as a diverse blend of compounds provides a more efficient protection against a broader range of herbivores and limits the chances that an enemy evolves resistance. Theory predicts that plant defensive compounds should be allocated differentially among tissues according to the value of the tissue, its cost of construction and the herbivore pressure on it. We collected volatile terpenes from bark and leaves of 178 individual tree belonging to 55 angiosperm species in French Guiana and compare the kind, amount, and diversity of compounds in these tissues. We hypothesized that in woody plants, the outermost part of the trunk should hold a more diverse blend of volatile terpenes. Additionally, as herbivore communities associated with the leaves is different to the one associated with the bark, we also hypothesized that terpene blends should be distinct in the bark vs. the leaves of a given species. We found that the mixture of volatile terpenes released by bark is different and more diverse than that released by leaves, both in monoterpenes and sesquiterpenes. This supports our hypothesis and further suggests that the emission of terpenes by the bark should be more important for trunk defense than previously thought.

1. Introduction

Biogenic volatile organic compounds (VOCs) are implicated in direct plant defense through herbivore repulsion, cytotoxic activity against fungi and pathogens and indirect defense through the attraction of herbivore predators or parasitoids (Pichersky and Gershenzon, 2002; Unsicker et al., 2009). Although the defensive role of VOCs in leaves is well documented (Unsicker et al., 2009), other vegetative tissues such as roots and bark are also known to synthesize volatile compounds, especially when damaged (Mumm and Hilker, 2006; Rasmann and Turlings, 2007). A large proportion of these VOCs are terpenes, either monoterpenes (with 10 carbon atoms), or sesquiterpenes (with 15 carbon atoms) and these compounds are highly diverse both within and among species (Dudareva et al., 2004).

Why do organisms produce an enormous diversity of secondary metabolites instead of just one or two compounds? The many hypotheses posited to explain the possible value of complex terpenes mixtures were reviewed by Gershenzon and Dudareva (2007). First, a diverse combination of terpenes may help provide protection against a diversity of herbivores, parasites and competitors (Pimentel and Bellotti, 1976) and reduce the potential number of herbivore species (Kursar et al., 2009). Second, having a diverse mixture allows each plant to play a slightly different defense strategy than its conspecific neighbors, reducing the likelihood for an enemy to evolve resistance (Price et al., 1980). Moreover, compounds may act synergistically to provide greater toxicity or deterrence than the equivalent amount of a single substance, for instance if some chemicals increase the persistence of others by inhibiting detoxification or excretion in enemies (Berenbaum and Neal, 1985). Finally, individual chemicals may not be useful under normal environmental conditions but become critical in extreme environments (Gershenzon and Dudareva, 2007). They may also indicate the "ghost of herbivory past" (Jones and Firn, 1991; Firn and Jones, 2003), i.e. compounds that do not currently play a

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defensive role may have been effective against now-extinct herbivores.

There is, however, a cost to maintaining a diverse set of VOCs. The biosynthesis of volatile terpenes is especially costly, due to a high demand in ATP (adenosine triphosphate) and NADPH and the need for highly specific enzymes (terpene synthases) that are unique to these biosynthetic pathways (Gershenzon, 1994). Most enzymes of terpenoid biosynthesis that catalyze general types of reactions have high substrate specificities (Gershenzon, 1994). Therefore, synthesizing more diverse mixtures increases the cost of production due the cost of the synthesis of multiple specific enzymes. From a biochemical point of view, the production of a diverse set of VOCs in a given plant tissue therefore indicates a significant investment of resources into the biochemical pathways leading to these compounds (Gershenzon, 1994).

Optimal defense theory predicts that plants allocate chemical defenses to different tissues relative to the intrinsic risk of herbivory faced by the plant tissue, the tissue's value to plant fitness, and the cost of the chemical defense (McKey, 1974). There is substantial experimental evidence in support of optimal defense theory (Zangerl and Rutledge, 1996; Ohnmeiss and Baldwin, 2000; Kaplan et al., 2008; Radhika et al., 2008) but most of the previous studies deal with roots, leaves and flowers (McCall and Fordyce, 2010). In woody plant species, chemical defense can be allocated differentially in bark (for wood protection) and in leaf tissues. For most of the species, wood and leaves have a similar construction cost (Poorter and Villar, 1997) and both attack on wood and leaves have an impact on plant fitness. High defoliation rates reduce growth rate and seed production as found for the tropical shrub *Piper arieaianum*, Piperaceae (Marquis, 1984). Nonetheless, studies show that most woody species can compensate for a relatively high level of defoliation, up to 25% for the tropical species *Casearia nitida* (Boege, 2005). Nonetheless, attacks on wood by pathogens and xylophagous insects may have a stronger impact on plant fitness as they compromise the mechanical, hydraulic, and physiological integrity of a tree. Herbivory on phloem dramatically increases the risk of secondary infection (Pearce, 1996; Romero and Bolker, 2008) and exposes the plant to an increased risk of breakage (Franklin et al., 1987), whereas leaves can be more easily shed and replaced following damage (Schowalter et al., 1986). In the mangrove forests of coastal Belize, primary consumption by woodborers may be responsible for greater losses from the canopy than consumption by folivores (Feller and Mathis, 1997): by girdling, pruning, and hollowing, woodborers killed over 50% of the *Rhizophora mangle* canopy in experimental plots and leaf-feeding herbivores removed less than 6% of the canopy (Feller, 2002). Thus, for long-lived trees, preventing damage to the living tissues of the trunk is essential and we predict that VOCs should be more diverse in bark, the outmost part of the stems of woody plants, than in the leaves.

Moreover, in a given species, wood and leaves are usually attacked by different communities of insect herbivores (Novotny et al., 2003). Leaf-eating insects mostly belong to the Lepidoptera, Coleoptera and Orthoptera orders (Novotny et al., 2003), while wood-specialized insects belong to the Auchenorrhyncha (Novotny and Wilson, 1997) or Cerambycidae family (Tavakilian et al., 1997). Moreover, wood pathogens are largely non-overlapping with leaf pathogens (Gilbert and Hubbell, 1996). Thus, the distinct repertoire of herbivore and pathogen pressures on bark and leaves should favor a distinct allocation of defensive compounds in the tree, such that some terpenes may be specialized to non-overlapping communities of herbivores. In a study concerning the phytoalexins from the leaves, cortex and xylem of mulberry (*Morus alba*) it has been found that the compounds accumulating in the different tissues were chemically distinct (Pearce, 1996). Moreover, previous studies on the essential oil constituents of bark and leaves already pointed out that even if overlaps exist between these two tissues there are also differences in terpene composition (Lago et al., 2004; Singh et al., 2007).

Here we test two hypotheses related to the allocation of volatile terpenes in tropical trees: (1) leaves should emit a less diverse array of volatile terpenes than bark, as expected under the optimal defense theory, (2) bark and leaves of the same species should hold a distinct array of compounds. We tested these hypotheses by collecting terpenes from bark and leaves of 55 species of trees in French Guiana and then comparing the kind, amount, and diversity of compounds in these tissues.

2. Results and discussion

2.1. Effect of the plant organ on the diversity of the terpenes blend

Across the 55 species with more than two individuals analyzed, bark tends to emit more compounds than leaves (Wilcoxon one-sided test, $W = 11221.5$, $P < 0.001$) with a mean of 25 VOCs per individual in bark samples and a mean of 19 VOCs per individual in leaves. More specifically, 40 species of the 55 sampled emitted more compounds in bark than in leaves (Fig. 1). This pattern was similar for all terpenes and also when monoterpenes and sesquiterpenes were examined separately (Fig. 1).

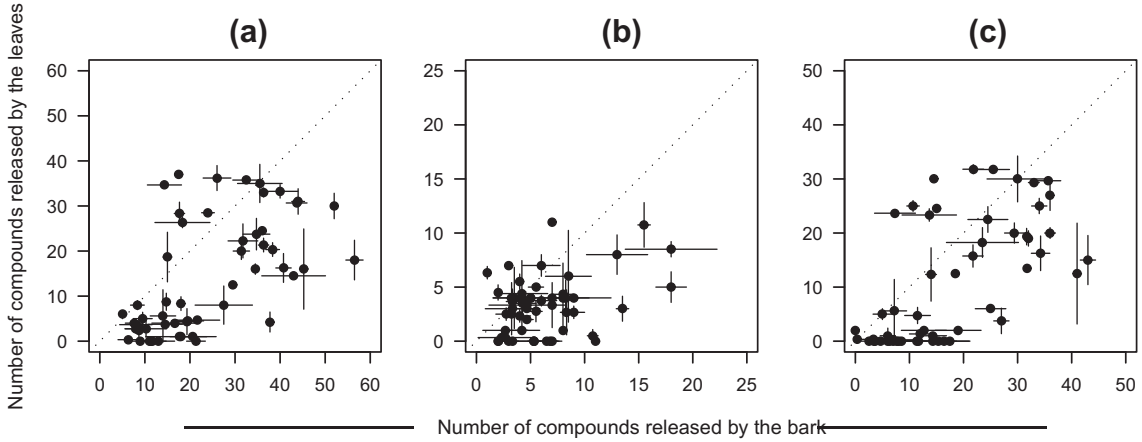

Fig. 1. Relationship between the numbers of terpenes released by the leaves versus number of terpenes released by the bark for 55 species: (a) total number, (b) number of monoterpenes, and (c) number of sesquiterpenes. In the panels, each dot represents a tree species. Both the mean number of VOC across sampled individuals and the standard deviation (SD) around the mean value are reported. The dotted line represents the 1:1 line.

Overall, and for both monoterpenes and sesquiterpenes considered separately, we found that bark samples emitted more volatile terpenes than leaves in 40 of the 55 species tested. Considering that a more diverse mixture of compounds allows better protection against herbivores (Gershenzon and Dudareva, 2007), bark tissues tend to be more broadly defended by volatile terpenes than leaf tissues. This result supports the prediction from the optimal defense theory (McKey, 1974): bark tissue protects the wood which is more difficult to replace than leaves (Franklin et al., 1987). Investment into defense should therefore be biased towards the bark as a more valuable and more vulnerable tissue. Some studies are available for conifer species and they found that the increases in needles were of the same magnitude as those seen in stem bark upon Methyl Jasmonate treatment (Martin et al., 2003) indicating no differences in terpenes allocation between the bark and the needles of this species. But they also found out that induced terpene accumulation in needles is much lower than in wood (Martin et al., 2003) providing support for a higher level of defense in woody part than in needles for this species.

One of the main challenges for testing this hypothesis is the difficulty in precisely assessing the vulnerability and fitness value of each plant tissue and to measure plant investment in defense (Stamp, 2003). Defense of sapwood is one of the primary functions of bark (Paine et al., 2010), and the outer part of the stem that includes resource-rich sapwood is much sought after by many insect species, and should therefore be well protected. For trees, wood tissue turns over more slowly than leaf tissue and thus represents a higher overall resource allocation (Franklin et al., 1987). Our result emphasizes the defensive role of volatile terpenes in the bark of angiosperms and should encourage more studies about defensive mechanisms through volatile terpenes in woody plants (Unsicker et al., 2009).

Our study is based on the terpenes blend detected for each individual sample (bark or leaves) of each individual. A potential source of error in the determination of volatile terpenes composition is the effect of symbiotic organisms. Indeed, in some studies, it has been shown that in the presence of endophytic fungi, the relative amount of compounds can changes in leaves (Mucciarelli et al., 2007). Nonetheless, it seems that the changes induced by endophytic fungi are mostly quantitative and do not affect the qualitative composition of the blend. As we worked only in presence/absence, effects of endophytic fungus in our results are likely to be low.

2.2. Effect of the plant organ on the composition of the terpenes blend

A total of 57 monoterpenes and 169 sesquiterpenes were found (Table 1). We first explored the overlap in composition of VOCs between leaves and bark. A total of 97 VOCs were released only by bark (38%), 26 only by leaves (10%), and 131 by both tissues (52%). The bark released more unique monoterpenes and sesquiterpenes than leaves (Fig. 2).

The Multiple Analysis of Variance (MANOVA) showed that plant tissue type, plant species identity, and the interaction between the two factors all had a significant effect on terpene composition (Adonis MANOVA, $P = 0.001$ for all effects, Table 2). Plant tissue type explained 8% of the variance in VOC composition, and plant species identity, 44%. Finally, 31% of the variance was explained by the interaction between tissue type and species (Table 2).

A substantial proportion (31%) of the variability in VOC composition was explained by the interaction between species and tissue type meaning that, within a given species, the bark and the leaves possess a distinct array of terpenes. Such differences in terpene composition of bark and leaves have already been pointed out (Lago et al., 2004; Singh et al., 2007) and our result show that this may be a more general pattern than previously thought. There is an

Table 1
Isolated compounds with their presence (1) or their absence (0) in the bark and in the leaves. The compounds authenticated with standards are indicated by ∗.

	Molecules	Bark	Leaves
Monoterpene	(4*E*,6*Z*)-allo-Ocimene	1	0
	1-8-Cineole	1	1
	3-Carene	1	1
	3*p*-Menthene	1	0
	6-Methyl-5-hepten-2-one	0	1
	allo-Ocimene	0	1
	alpha-Phellandrene	1	1
	alpha-Pinene∗	1	1
	alpha-Terpinene	1	1
	alpha-Terpineol	0	1
	alpha-Terpinolene	1	0
	alpha-Thujene	1	1
	beta-Myrcene∗	1	1
	beta-Ocimene	1	1
	beta-Phellandrene	1	1
	beta-Pinene	1	1
	beta-Terpinolene	1	1
	Camphene	1	1
	Campholenal	0	1
	Camphor	1	0
	Carvacrol-methyl-ether	1	0
	Cymen-8-ol	1	0
	delta-2-Carene	1	1
	E-beta-Ocimene	0	1
	gamma-Terpinene	1	1
	iso-Methoxy-thymol	1	0
	Limonenen∗	1	1
	Linalool∗	1	1
	Linalool-oxide-cis	1	0
	Linalool-oxide-dihydroxy	1	0
	linalool-oxide-trans	1	1
	Mentha-1-7(8)-diene	1	1
	Mentha-2-8-diene	1	0
	Mentha-2-8-dienol	1	0
	Myrtenal	0	1
	o-Cymene	1	0
	o-Xylene	1	0
	p-Cymene	1	1
	p-Cymenene	1	0
	p-Xylene	1	0
	Perilene	1	1
	Pinocarvone	0	1
	Rose-furan-oxide	1	0
	Sabinene	1	1
	Sabinene-hydrate-cis	1	1
	Sabinene-hydrate-trans	1	0
	Sylvestrene	1	0
	Terpinene-4-ol	1	1
	Thuja-2-4(10)-diene	1	1
	Thymol-methyl-ether	1	0
	trans-Pinocarveol	0	1
	trans-Verbenol	0	1
	Tricyclene	1	0
	Verbenene	1	0
	Z-beta-Ocimene	0	1
	Unidentified monoterpene 1	1	0
	Unidentified monoterpene 2	1	0
sesquiterpene	(3*E*,6*Z*)-alpha-Farnesene	0	1
	(*E*)-beta-Farnesene	1	1
	(*Z*)-beta-Farnesene	1	1
	(*Z*,*E*)-alpha-Farnesene	1	1
	1-*epi*-*a*-Pinguisene	1	0
	5-*epi*-Aristolochene	1	0
	7-*epi*-alpha-Cedrene	1	1
	7-*epi*-alpha-Selinene	1	1
	African-2(6)-ene	1	1
	African-2,6-diene	1	0
	allo-Aromadendra-4(15),10(14)-diene	1	1
	allo-Aromadendrene	1	1
	alpha-Alaskene	1	0
	alpha-Cadinene	1	1
	alpha-Cadinol	1	1
	alpha-Cedrene	1	1

(*continued on next page*)

Table 1 (*continued*)

Molecules	Bark	Leaves
alpha-Copaene	1	1
alpha-Cubebene	1	1
alpha-Cuprenene	1	0
alpha-Curcumene	1	1
alpha-Duprezzianene	1	0
alpha-Guaiene	1	1
alpha-Gurjunene	1	1
alpha-Humulene	1	1
alpha-Longipinene	1	1
alpha-Muurolene	1	1
alpha-Santalene	1	1
alpha-Selinene	1	1
alpha-Ylangene	1	1
Anastreptene	1	0
Aromadendra-4,10(14)-diene	1	0
Aromadendrene	1	1
beta-Acoradiene	1	0
beta-Barbatene	1	0
beta-Bazzanene	1	0
beta-Bisabolene	1	1
beta-Bourbonene	1	1
beta-Calacorene	1	0
beta-Chamigrene	0	1
beta-Cubebene	1	1
beta-Curcumene	1	1
beta-Elemene	1	1
beta-Humulene*	1	0
beta-Maaliene	1	1
beta-Selinene	1	0
beta-Vetivene	1	0
beta-Ylangene	1	1
Bicyclo-elemene	1	1
Bicyclogermacrene	1	1
Bourbon-11-ene	1	1
Bourboneral	1	0
Brasila-1(6),5(10)-diene	1	0
Brasiladiene	1	0
Cadalene	1	0
Cadina-1(10),6-diene	1	0
Cadina-3,5-diene	1	1
Cadinene-ether	1	1
Calamenol	0	1
Calameren-9-ol	1	0
Calarene	1	1
Caryophyllene-oxide	1	1
cis-Cadina-1,4-diene	1	1
cis-Calamenene	1	1
Cubenol	1	0
Cuparene	1	0
cyclo-Bazzanene	1	0
Cymene-2,5-dimethoxy-para	1	0
Cyperadiene	1	0
Cyperene	1	1
delta-Cadinene	1	1
delta-Elemene	1	1
delta-Selinene	1	1
Dendrolasine	1	1
E-Caryophyllene*	1	1
E-gamma-Bisabolene	1	0
epi-alpha-Cadinol	1	0
epi-alpha-Muurolol	1	1
Epistolene	1	0
Eremophyladiene	1	1
Erythrodiene	0	1
Funebrene-2-*epi*-alpha	1	0
gamma-Cadinene	1	1
gamma-Curcumene	1	1
gamma-Elemene	1	1
gamma-Guaiene	1	0
gamma-Muurolene	1	1
gamma-Selinene	1	1
Germacrene-B	0	1
Germacrene-D	1	1
Gorgonene	1	0
Guaiadiene	1	1

Table 1 (*continued*)

Molecules	Bark	Leaves
Hinesene	1	1
iso-Bicyclogermacrene	1	0
iso-Carryophyllene	1	1
Isoledene	1	1
Maali-1,3-diene	1	0
Muurolol	1	1
Oppositadiene	1	0
Pacifigorgia-1(9),10-diene	1	0
Pacifigorgia-2,10-diene	1	0
Palustrol	0	1
Presilphiperfolene	1	0
Rotundene	1	0
Selina-4,11-diene	1	0
Selina-4,7-diene	1	1
Sesquicineole	0	1
Sesquiphellandrene	1	1
Sesquithujene	1	1
Sesquithujene-7-*epi*	1	1
Spathulenol	1	1
Striatene	1	0
tau-Cadinol-di-exo	1	1
trans-Cadina-1,4-diene	1	1
trans-Calamenene	1	1
trans-Cubebol	1	1
Veltonal	1	0
Viridiflorene	1	1
Widrene	1	0
Z-alpha-Bisabolene	1	1
Unidentified sesquiterpene 1	1	1
Unidentified sesquiterpene 2	1	0
Unidentified sesquiterpene 3	1	0
Unidentified sesquiterpene 4	1	0
Unidentified sesquiterpene 5	1	0
Unidentified sesquiterpene 6	1	1
Unidentified sesquiterpene 7	1	1
Unidentified sesquiterpene 8	1	0
Unidentified sesquiterpene 9	1	0
Unidentified sesquiterpene 10	1	0
Unidentified sesquiterpene 11	1	1
Unidentified sesquiterpene 12	0	1
Unidentified sesquiterpene 13	1	1
Unidentified sesquiterpene 14	0	1
Unidentified sesquiterpene 15	1	0
Unidentified sesquiterpene 16	1	0
Unidentified sesquiterpene 17	1	0
Unidentified sesquiterpene 18	1	0
Unidentified sesquiterpene 19	1	0
Unidentified sesquiterpene 20	1	0
Unidentified sesquiterpene 21	1	0
Unidentified sesquiterpene 22	0	1
Unidentified sesquiterpene 23	1	1
Unidentified sesquiterpene 24	1	0
Unidentified sesquiterpene 25	1	1
Unidentified sesquiterpene 26	1	1
Unidentified sesquiterpene 27	1	1
Unidentified sesquiterpene 28	1	1
Unidentified sesquiterpene 29	1	1
Unidentified sesquiterpene 30	1	1
Unidentified sesquiterpene 31	1	1
Unidentified sesquiterpene 32	1	1
Unidentified sesquiterpene 33	1	1
Unidentified sesquiterpene 34	1	1
Unidentified sesquiterpene 35	1	0
Unidentified sesquiterpene 36	1	1
Unidentified sesquiterpene 37	1	1
Unidentified sesquiterpene 38	1	1
Unidentified sesquiterpene 39	1	0
Unidentified sesquiterpene 40	1	1
Unidentified sesquiterpene 41	1	0
Unidentified sesquiterpene 42	1	1
Unidentified sesquiterpene 43	1	1
Unidentified sesquiterpene 44	1	0
Unidentified sesquiterpene 45	1	0
Unidentified sesquiterpene 46	1	1
Unidentified sesquiterpene 47	1	0

Table 1 (*continued*)

	Molecules	Bark	Leaves
	Unidentified sesquiterpene 48	1	0
	Unidentified sesquiterpene 49	1	0
	Unidentified sesquiterpene 50	1	1

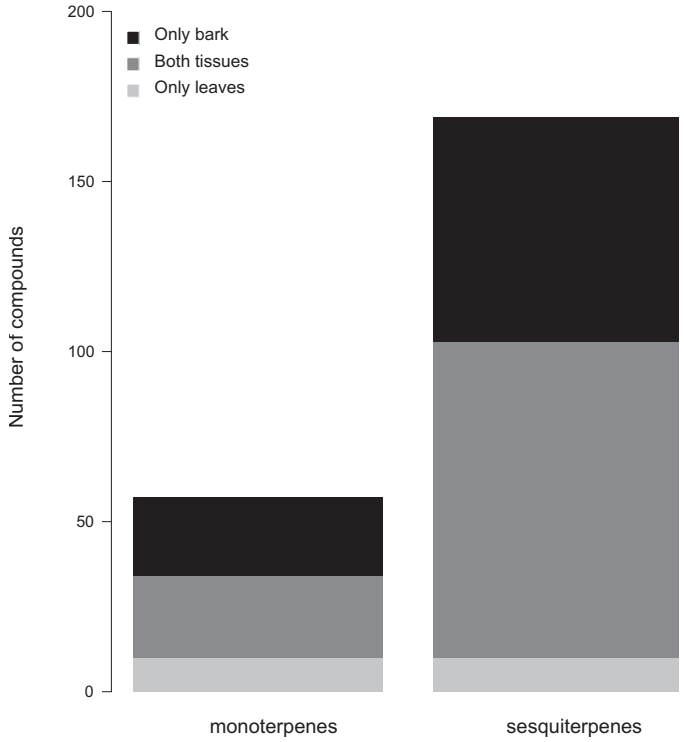

Fig. 2. Number of mono- and sesquiterpenes in bark only (black), leaves only (light grey) or found in both tissues (dark grey).

Table 2
Non-parametric MANOVA of the effect of tissue and species identity on VOC composition of the samples included in this study. *Df* indicates the degrees of freedom of the factor, *F* the value of the statistic, R^2 the percentage of variance explained by the factor and *P* the *P* value associated with the factor.

	Df	*F*	R^2	*P*
Tissue	1	137.89	0.08	0.001
Species	54	14.54	0.46	0.001
Tissue × species	54	9.99	0.32	0.001
Residuals	246		0.14	
Total	355		1.00	

overlap in the terpene composition of the bark and the leaves of a given individual but our result show that some compounds may only be expressed in one tissue. One hypothesis to explain this is the fact that the herbivore and pathogen communities associated with the bark and the leaves are different (Novotny et al., 2003). A distinct herbivory and pathogen pressure could therefore favor in plants a distinct allocation of defensive compounds.

We further found that some compounds seem to be restricted to one tissue (either bark or leaves) but are shared among species. This is highlighted by the fact that 8% of the variability in VOC composition is explained by tissue type only. There are therefore some compounds that seem to be more expressed in one of the two tissues independently of the species. This is confirmed by the fact that we found 38% of the compounds were found only in bark analysis and 10% only in leaf analysis. This finding may be explained by the low host specificity among herbivores of tropical plants (Novotny et al., 2003): most tropical herbivores and pathogens are able to feed on multiple tree species and a shared herbivore pressure across tree species on the same tree part may result in a shared chemical composition. More unique compounds are found in the bark (38% vs. 10%) and future studies are needed to link the presence of these compounds with the associated herbivore and pathogen species.

3. Conclusions

In this study, we found that bark of tropical tree species tend hold a distinct and more diverse blend of volatile terpenes than leaves. Future studies should be conducted to precise the effect of terpenes diversity on the diversity and density of herbivores. Moreover, we focused only on volatile defensive compounds and non-volatiles can also control the distribution of insect herbivores (Meurer-Grimes and Tavakilian, 1997). Such a negative correlation between chemical diversity and the number of herbivore species has been reported for Nothofagus trees in South America and New Zealand (Lavandero et al., 2009). In that study, non-volatile chemical uniqueness and chemical diversity of leaves limited the number of species of specialist leaf-feeding insects. A logical next step in studies of volatile defenses in tropical plants would therefore be to link the terpene diversity and composition that we report here to the associated herbivore guilds and to explore the relationship with other defensive compounds in the bark and the leaves of tree species.

4. Experimental

4.1. Study sites

This study was conducted at three sites in the Neotropical forest of French Guiana: Paracou Research Station (5°15′ N; 52°55′ W), Nouragues Research Station (4°05′ N, 52°40′ W) and Montagne Tortue (4°18′ N; 52°22′ W). All three sites are covered with pristine tropical rain forest. Annual rainfall is 2990 mm for Nouragues, 3160 mm for Paracou and ca. 3500 mm for Montagne Tortue. In five plots of one hectare each, we sampled all trees with a diameter at breast height (dbh) greater than 10 cm (Baraloto et al., 2012). Two of these plots were at the Paracou site (n = 643 and 487 trees), another two plots were at Nouragues (n = 537 and 567 trees) and one plot at Montagne Tortue (n = 536 trees). Sampling was conducted in June 2007 for Paracou, September 2007 for Nouragues and November 2007 for Montagne Tortue. Each tree was individually climbed by a professional tree climber and a voucher specimen was collected for taxonomic determination. Over 98% of the trees were identified to the species or morphospecies, and the tree species richness ranged between 148 and 210 tree species per hectare (Baraloto et al., 2012).

4.2. Field sampling protocol

For 55 species represented by at least two individuals in our plots, two to five individuals were selected, totaling 178 individuals (Courtois et al., 2009). For each individual, we collected a 1–2 cm^2 piece of leaf (~20-mg), and a piece of bark using a punch of 1 cm^2 (20–70 mg). For each tree, recently but fully expanded sunny leaves were collected from the next-to-last shoot along the sampled branch at the crop (Baraloto et al., 2010). Bark samples were collected about 1 m above the ground. Each sample was immediately placed into a glass vial (10 mL) sealed with a screw-capped top containing a Teflon-lined septum (Varian Instruments,

Sunnyvale, CA, USA). Sealed vials were kept in ice for less than 4 h after sampling and they were then stored at −20 °C until analysis. We verified that tissue damaged when still on the plant had the same VOC emission spectrum as the tissue collected using our protocol (see Courtois et al., 2009 for more details).

4.3. Chemical analyses

VOCs were extracted by Solid Phase Micro Extraction (SPME) followed by a GC/MS (Gas Chromatography/Mass spectrometry) analysis. For details about the protocol, see Courtois et al. (2009). Briefly, the SPME fiber (Polydimethylsiloxane/Divinylbenzene 65 μm; Supelco, Bellefonte, Pennsylvania, USA) was exposed to the headspace of the tissue sample (bark or leaf) at ambient temperature (25 °C) for 5–60 min depending on the species (see Courtois et al., 2009 for details on the exposure time per species) and inserted immediately into the inlet of a Varian 3800 gas chromatography fitted with a Saturn 2000 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA, USA). GC analyses were conducted with a Varian DB5-ms column (5% phenyl 95% dimethylpolysiloxane). Helium was the carrier gas at a constant flow of 1 ml/min. Un-exposed fibers (blanks) were analyzed every ten samples to check for contamination of the fiber. Since the SPME technique cannot be used to reliably estimate the abundances of the compounds, only presence/absence of the chemicals are reported here. VOC presence/absence was inferred from the chromatograms using a statistical approach implemented in the package MSeasy (Nicole et al., 2012) developed in the R statistical software (http://cran.r-project.org/) and available on the R CRAN web site (http://cran.r-project.org/web/packages/), based on the mass spectrum of each detected compound, and the corresponding retention index (Kováts, 1958). Compounds were identified based on the comparison of mass spectra with standards or with the NIST 98 MS library, the ADAMS library (Adams, 2001) and with indices reported in the literature (Adams, 2001). Overall, we were able to assign 78% of the VOCs to known molecular structures. Unidentified compounds were defined as morpho-molecules characterized by their mass spectrum and their retention index. VOCs were classified into monoterpenes and sesquiterpenes (Dudareva et al., 2004).

4.4. Statistical analyses

To assess differences in diversity of the VOC mixture in the bark and in the leaves, we calculated the number of compounds in each tissue for a given individual. For each individual, we tested the null hypothesis that the difference between the number of compounds in the bark minus the number of compounds in the leaves was greater than zero (i.e. $N_{\text{compounds in bark}}(i) - N_{\text{compounds in leaves}}(i) > 0$ for each individual tree i). In any given tissue, the number of compounds per individual was not normally distributed (Shapiro test, $W = 0.95$, $P < 0.001$). Hence, the significance of differences in VOCs diversity among tissues of a given individual was tested with a one-tailed Wilcoxon test.

We then tested for differences in the chemical composition in the blend of VOCs between tissues (bark versus leaves). We constructed a distance matrix based on the VOCs composition using the Jaccard index (S) defined as $S = \frac{a}{a+b+c}$, where a is the number of shared compounds between two chemical analyses, b the number of compounds found only in one tissue and c the number of compounds found only in the other. We tested the effect of tissue, species and the interaction between these two factors on the distance matrix by using a non-parametric multivariate analysis of variance or MANOVA (Anderson, 2001). Briefly, this analysis calculates a "pseudo-F" ratio analog to Fisher's F-ratio for each factor and their interactions based on a distance matrix. The partial squared coefficient of correlation R^2 is the percentage of variance in the chemical distance matrix that is explained by the factor, and significance (P values) is calculated by performing multiple permutations on the rows or columns of the matrices (in our case, 10,000 permutations).

All statistical tests were conducted with the *R* statistical software version 2.10.0 (http://cran.r-project.org/) using the package vegan (Oksanen et al., 2010).

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Appendix A. Sampled species with the number of sampled individuals per species (For each individuals, two samples were available, one for the bark and one for the leaves)

Family	Genus	Species	# Sampled individuals
Anacardiaceae	*Anacardium*	*spruceanum*	4
	Thyrsodium	*guianense*	4
		puberulum	4
Annonaceae	*Duguetia*	*surinamensis*	2
	Oxandra	*asbeckii*	4
	Unonopsis	*perrottetii*	5
		rufescens	3
	Xylopia	*nitida*	4
Apocynaceae	*Aspidosperma*	*cruentum*	2
		marcgravianum	4
Bombacaceae	*Pachira*	*dolichocalyx*	3
Burseraceae	*Dacryodes*	*nitens*	2
	Protium	*decandrum*	4
		opacum	4
		sagotianum	4
	Tetragastris	*altissima*	3
		panamensis	4
Caesalpiniaceae	*Tachigali*	*melinonii*	2
	Vouacapoua	*americana*	3
Caryocaraceae	*Caryocar*	*glabrum*	3
Cecropiaceae	*Pourouma*	*villosa*	3
Chrysobalanaceae	*Hirtella*	*glandulosa*	4
	Licania	*membranacea*	5
	Parinari	*campestris*	2
Clusiaceae	*Rheedia*	*madruno*	3
	Tovomita	*spB1*	3
Euphorbiaceae	*Conceveiba*	*guianensis*	3
Icacinaceae	*Poraqueiba*	*guianensis*	3
Lauraceae	*Aniba*	*panurensis*	2

Sampled species with the number of sampled individuals per species (For each individuals, two samples were available, one for the bark and one for the leaves) (*continued*)

Family	Genus	Species	# Sampled individuals
	Ocotea	*argyrophylla*	2
		percurrens	2
	Sextonia	*rubra*	3
Lecythidaceae	*Eschweilera*	*congestiflora*	3
		coriacea	3
	Lecythis	*persistens*	4
		poiteaui	3
Meliaceae	*Carapa*	*procera*	3
Moraceae	*Brosimum*	*guianense*	5
Myristicaceae	*Iryanthera*	*hostmannii*	2
		sagotiana	2
	Virola	*michelii*	2
Myrtaceae	*Myrcia*	*decorticans*	3
Papilionaceae	*Bocoa*	*prouacensis*	2
Rubiaceae	*Chimarrhis*	*turbinata*	3
	Posoqueria	*latifolia*	3
Sapindaceae	*Cupania*	*scrobiculata*	5
Sapotaceae	*Chrysophyllum*	*argenteum*	3
	Micropholis	*egensis*	3
		guyanensis	5
	Pouteria	*gonggrijpii*	3
Simaroubaceae	*Simaba*	*cedron*	5
Sterculiaceae	*Sterculia*	*pruriens*	4
	Theobroma	*subincanum*	3
Tiliaceae	*Apeiba*	*glabra*	3
Vochysiaceae	*Ruizterania*	*albiflora*	3

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