

## Feeding Selectivity by Mantled Howler Monkeys (*Alouatta palliata*) in Relation to Leaf Secondary Chemistry in *Hymenaea courbaril*

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**Abstract** This study is a quantitative examination of primate feeding selectivity in relation to secondary chemistry within a single plant species, *Hymenaea courbaril*. It provides the first evidence that sesquiterpenes may act as feeding deterrents in mantled howler monkeys. A free-ranging group of mantled howler monkeys at the study site of Sector Santa Rosa, Area de Conservacion Guanacaste, Costa Rica were observed for the 2-month period of *H. courbaril* leaf flush in 1999. Tree characteristic data and leaf specimens were collected from 22 focal trees. Gas chromatography and mass spectrometry were used to estimate relative percentages of sesquiterpenes in leaf specimens. The monkeys fed only on the youngest leaves and only from particular trees. Whereas leaf stage selectivity was likely governed by tannin content and structural carbohydrates in younger and older leaf stages, respectively, differential tree use may be related to variability in sesquiterpene content. There is evidence that  $\alpha$ -copaene may have played a role in interindividual tree use, and that cyperene may also be implicated. However, there is no reported evidence of antiherbivore activity for cyperene.

**Keywords** Primates · Howler monkeys · Feeding selectivity · Secondary chemistry · Sesquiterpenes

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## Introduction

Much research has been conducted in the area of primate feeding selectivity in relation to plant primary and secondary chemistry (see Milton 1998 for an overview). However, in relation to secondary chemistry, research has not advanced much beyond the role of phenolics and alkaloids, and analyses have often been qualitative rather than quantitative (see Calvert 1985 and Berry 1998 for exceptions). The limited focus on these two groups of compounds is puzzling because (1) plants produce a vast array of secondary compounds; (2) many families of secondary compounds have suspected or demonstrated antitherbivore functions; and (3) herbivorous primates feed from a variety of different plant families, many of which differ greatly in their chemistry.

This research is an examination of feeding selectivity in relation to secondary chemistry within a single plant species, *Hymenaea courbaril*, by mantled howler monkeys. The study was not confounded by the many differences between plant species or by other factors involved in primate dietary selection, e.g., food availability and preferences; plant density, location, phenology, production, and abundance; learned local traditions, etc. It is the first time that sesquiterpenes have been considered to play a role in primate dietary choice.

Mantled howler monkeys are generalist herbivores that exhibit pronounced inter- and intraspecific dietary selectivity for plant parts and stages (Milton 1998). They are thought to choose plant parts in a way that maximizes/balances nutritional intake, whereas minimizing intake of secondary compounds (Glander 1978, 1981; Milton 1978; Estrada 1984). In terms of leaf choice, Milton (1979) reports that the protein-to-fiber ratio is the most important determinant. Glander (1981, 1982) has provided evidence that some differential species/tree/leaf stage selectivity is related to the secondary chemistry of leaves (especially the presence/absence of condensed tannins and alkaloids). Milton (1998) suggests that whereas there is little evidence that secondary chemistry plays a role in primate dietary selectivity, detoxification is energetically demanding and animals might be expected to avoid foods that are relatively costly.

Mantled howler monkeys feed on leaves from some trees and not others within particular food species (Milton 1980; Glander 1981; Welker 2004). Glander (1981) demonstrated that leaves from unused trees contained condensed tannins. Welker (2004) reported pronounced differential tree use within the tree species, *H. courbaril*, by a group of mantled howler monkeys (the same group used in the present study). Her subjects fed exclusively on the first two to three pairs of young leaves, fresh out of the terminal bud, and only from particular trees during the 1997 and 1998 periods of leaf flush. Observed differential tree use was not related to tree size, degree of accessibility from surrounding trees, phenology (in terms of possessing the utilized plant part), leaf crop size, primary chemistry, and/or tree location in relation to group movements.

Here, we examined whether the previously observed differential tree and leaf stage use in Welker's study group was in response to differences in secondary chemistry and leaf stages within trees. Of interest was not whether the animals completely avoided particular compounds, because *H. courbaril* leaves are fed on, but whether there are maximum tolerance thresholds, either physiological or gustatory, that might explain selectivity within the species. Trees chosen for analysis were (1) differentially fed from during the previous two field seasons (Welker 2004), (2) located in a fairly continuous grove, and (3) predictable in terms of the period of leaf drop and flush with many trees reported to simultaneously possess young leaves during this time (Welker 2004).

Leaves of *H. courbaril* contain three types of secondary compounds, sesquiterpenes (Martin et al. 1972), tannins (Crankshaw and Langenheim 1981; McCloskey 1984), and flavonoids (Lopez 1976; McCloskey 1984; Artavia et al. 1995). Sesquiterpenes are the

primary component of the leaf resin (Martin et al. 1972). Langenheim (1981) believes that the resins/sesquiterpenes are an evolved defense mechanism. Several constituent sesquiterpenes,  $\beta$ -caryophyllene, caryophyllene oxide,  $\alpha$ -humulene, humulene epoxide,  $\alpha$ -selinene, and  $\beta$ -selinene, in *H. courbaril* have demonstrated herbivore deterrent capabilities (Langenheim et al. 1980; Barnola et al. 1994). Several others,  $\beta$ -cadinene,  $\alpha$ -copaene,  $\alpha$ -cubebene, and germacrene D, are inducible in response to herbivore damage (Barnola et al. 1994; Agelopoulos et al. 1999; Vrkočová et al. 2000). One of those,  $\alpha$ -copaene, plus a variant isomer,  $\delta$ -cadinene, may play a deterrent role in oviposition rates by two butterfly species (Ling et al. 2003), and  $\delta$ - and  $\gamma$ -cadinene have suspected bactericidal capabilities (Haznedaroglu et al. 2001). Differential herbivore damage in a congener, *Hymenaea stigonocarpa*, has been attributed to variation in the concentrations of several sesquiterpenes, caryophyllene, humulene, selinenes,  $\gamma$ -cadinene,  $\alpha$ -copaene, and  $\alpha$ -cubebene (Langenheim et al. 1986).  $\gamma$ -Cadinene is one of the primary components of the defensive secretions of soldier termites of the species, *Constrictotermes cyphergaster* (de Asevedo et al. 2006; Quintana et al. 2003).

Whereas Glander (1981) reported that mantled howler monkeys, at his site of Hacienda La Pacifica, Costa Rica, chose foods that are low in tannins; he believes they are avoiding condensed vs. hydrolyzable tannins. Whereas *H. courbaril* leaves contain condensed tannins (Crankshaw and Langenheim 1981; McCloskey 1984), they are primarily found in the terminal bud where they account for more than 16% dry weight (DW). They decrease rapidly so that by the second pair of young leaves, they account for less than 1% DW (Crankshaw and Langenheim 1981). Glander (1981) states that young *H. courbaril* leaves at his study site, which is in the same region of Costa Rica and also classified as dry forest, do not contain condensed tannins. Langenheim (personal communication) claims that there is little variability in tannin content among trees within a microhabitat. Whereas monkeys may have been avoiding condensed tannins by not consuming the terminal buds, it appears unlikely that condensed tannins played an important role in the observed differential tree use because they occur at such low concentrations in the youngest leaves. Because tannins have not been suspected to play a role in differential tree use, and because there is no research to indicate that those flavonoid compounds found in *H. courbaril* act as feeding deterrents, we examined only sesquiterpene content of leaves in the present study.

## Methods and Materials

**Study Site** The site is characterized as dry tropical deciduous forest (Janzen 1983) and is located in the Santa Rosa Sector of the Area de Conservacion Guanacaste, Costa Rica. Santa Rosa is located 35 km northwest of the town of Liberia, Guanacaste Province, in northwest Costa Rica.

**Subjects** The focal group was a free-ranging group of mantled howler monkeys. Animals included one male, three females, and one juvenile. All animals were recognizable as individuals in that they were collared or exhibited unique throat morphology or genital pigmentation.

**Data Collection** Two-minute point time samples (Altmann 1974) were used to record all instances of individual animals feeding on *H. courbaril* leaves. Data were collected during the 1999 period of leaf flush (14/I/99–22/II/99), yielding 136 observations (scans) of *H. courbaril* consumption.

**Trees** All 22 trees (1) possessed young leaves during the period of data collection, (2) were accessible from surrounding trees (as per Welker 2004), and (3) were located within a fairly

continuous grove that the monkeys passed through every few days during 3 yr of data collection (Welker 2004 and current study). During the 2 yr before the current study, Welker observed the monkeys' differential use of grove trees (Welker 2004). Whereas no data are available for which trees were fed from in 1997, in 1998 the monkeys fed from 9 of the aforementioned 22 trees (Welker 2004). The decision to use only grove trees eliminated the possible confound of the few nongrove trees being located in different microhabitats and thus possibly being exposed to different abiotic conditions.

Weekly phenological data were collected from all focal trees before, during, and after the period of leaf flush. Binoculars were used to estimate the volume of the crown (in 10% increments) made up of (1) leaves at the stage of development that the monkeys were observed to eat, and (2) mature leaves beyond the stage at which the monkeys fed. These two stages will hereafter be referred to as "young" vs. "mature" leaves. These data were used to determine whether trees possessed young leaves and hence were hypothetically "available" to the animals for consumption. Weekly estimates of herbivore damage by insects were collected. These were represented as the total percentage of leaf blade missing (0, <25, 25–50, 50–75, and >75%). Binoculars were used to scan the tree crown for evidence of missing leaf blade area, as evidenced by holes in the leaves. No tree exhibited more than 25% leaf blade loss. Equal numbers of trees exhibited either no damage or less than 25% damage and were thus categorized as "undamaged" ( $N=11$ ) and "damaged" ( $N=11$ ), respectively.

**Leaf Specimens** Young leaf specimens (3 g/tree) were collected. Specimens approximated the stage of development the animals were observed to eat, i.e., the first, second, and/or third pairs of leaves out of the terminal leaf bud that were ~2 cm in length and very tender. In addition, for six trees that the monkeys fed from, one specimen each of a younger and older leaf stage were collected. The younger stage consisted of 1 g of terminal leaf buds, and the older stage consisted of 1 g of the sixth or seventh pair of leaves out of the leaf bud. The monkeys were not observed to feed on either of these two stages.

All leaf specimens were field-dried in a dry-box by using a light bulb to reduce humidity. Langenheim (personal communication) and Adams (personal communication) recommended the air-drying procedure for field-processing *H. courbaril* samples for subsequent sesquiterpene extraction and analyses. Because sesquiterpenes exhibit only moderate volatility among the terpenoids, Langenheim and Adams have determined that they do not degrade with this technique. Samples were weighed before and after drying.

**Laboratory Analyses** The sesquiterpenes under examination are contained in the essential oils of the leaf resin. These essential oils were extracted at the Institut für Organische Chemie, Universität Hamburg, Germany. Samples were finely ground in a mortar and pestle with liquid  $N_2$ . One gram of ground young leaves was used for each individual tree analysis. Leaf samples were weighed with an Ohaus Analytical Standard Scale, accurate to 0.001 g. Samples were placed in 100 ml of deionized water and hydrodistilled for 3 hr in a Likens–Nickerson apparatus, from boiling point to heat source removal, using *n*-hexane as a collection solvent. Extracts were reduced to uniform volumes under a nitrogen gas jet.

Extracts were analyzed for sesquiterpene content with a Carlo Erba Strumentazione HRGC equipped with a Chrompak CPSil 5 fused silica capillary column (25 m×0.25 mm with a film thickness of 0.25  $\mu$ m). The oven began at 50°C, after which temperature increased by 3°C/min until 230°C was reached. Hydrogen, with an inlet pressure of 70 kPa, was used as the carrier gas. Chromatograms were recorded with a Merck Hitachi model 2500 with FID electronic integrator. Some compounds were not detected in some specimens, either because they occurred at extremely low concentrations or because they lacked an individual peak.

Because of the resulting truncated data sets (values were missing for some compounds for some specimens), a maximum likelihood estimate (MLE) was substituted at the advice of Dr. Paul Kostyniak (toxicology consultant, SUNY Buffalo, Buffalo, NY, USA).

Mass spectrometric analyses (EI 70 eV) were conducted on a sample of specimens to ensure accurate identification of the gas chromatography peaks. Equipment consisted of a Hewlett-Packard HP 5890 gas chromatograph (CPSil 5 fused silica capillary column, 25 m × 0.25 mm, 0.25 μm film thickness) coupled to a VG Analytical 70-250S mass spectrometer.

All mass spectra were cross-referenced with the sesquiterpene mass spectral library (Joulain and König 1998). In addition, two samples were analyzed independently by Adams by using a Hewlett Packard MSD and his library of essential oils.

Gas chromatography results yielded the relative percentages of component sesquiterpenes in a given sample of essential oil, collectively summing to 100%. Values for individual compounds are relative to one another and not translatable into actual concentrations.

*Data Analyses* Some sesquiterpenes were combined for data analysis purposes as follows. Each of three oxidized analogs of caryophyllene, i.e., caryophyllene oxides 1, 2, and 3, were examined individually and in combination. In addition, the oxidized analogs were also included with β-caryophyllene, under the heading “total caryophyllene”. Whereas caryophyllene oxide occurs naturally in *H. courbaril*, β-caryophyllene is readily converted into these oxidized analogs upon exposure to air (König, personal communication). Thus, some portion of the oxidized forms may have resulted from the rearrangement of β-caryophyllene. Langenheim et al. (1986) grouped caryophyllene and caryophyllene oxide together. α-Humulene and its oxidized analog, humulene epoxide, were also examined separately and together as “total humulene”. Finally, α- and β-selinene were examined individually and in combination because Martin et al. (1974) reported that there does not appear to be any significance as to the relative concentrations of the two isomers and there is high variability within *Hymenaea*.

## Results

*Used vs. Unused Trees* Table 1 shows mean relative percentages of the various sesquiterpenes identified in leaf specimens from used and unused trees along with *t*-test results. Specimens from unused trees contained significantly higher values for α-copaene and cyperene. For those trees that were fed from, frequency of use was not correlated with relative percentages of α-copaene ( $R=-0.341$ ,  $P=0.304$ ,  $N=11$ ) or cyperene ( $R=0.113$ ,  $P=0.741$ ,  $N=11$ ). However, when all trees were divided into three categories of use/nonuse (Fig. 1), there were significant or near significant differences between the groups for both α-copaene ( $F=3.406$ ,  $df=2$ ,  $P=.054$ ,  $N=22$ ) and cyperene ( $F=3.826$ ,  $df=2$ ,  $P=.040$ ,  $N=22$ ). Whereas there is an obvious pattern of use associated with α-copaene that may be indicative of a maximum consumption threshold (Fig. 1), cyperene shows no such pattern. The range of values for α-copaene were 0.31–4.16. No feeding scans were recorded for any tree whose leaves had >3% of the compound, and only four scans with >2.5% (three scans for one tree with a value of 2.53% and one scan with 2.98%). Only four unused trees had values of <2.5% vs. seven with values of >2.5%. In the case of cyperene, in all but four trees, values for cyperene were lower in used than unused trees. However, 11 feeding scans were recorded for the tree with the overall second highest relative percentage of cyperene, i.e., 1.74%.

*Leaf Stage in Relation to Sesquiterpene Chemistry* Young leaves were compared with buds and a more mature stage of development. No significant differences were found between

**Table 1** Comparison of mean sesquiterpene values in used and unused trees

Compounds	MLE Values	Mean Score for Used Trees (N=11)	Range for Used Trees	Mean Score for Unused Trees (N=11)	Range for Unused Trees	t Score	P Value
δ-Cadinene		0.80±0.1	0.34–1.38	0.92±0.0	0.47–1.38	0.95	0.356
γ-Cadinene	2	0.47±0.0	0.02–1.02	0.54±0.0	0.35–0.77	0.70	0.499
β-Caryophylline		12.32±1.8	3.29–19.99	7.40±1.5	1.34–16.67	<b>-2.12</b>	<b>0.047</b>
Caryophyllene oxide 1		1.16±0.1	0.75–1.85	1.48±0.1	0.80–2.22	1.87	0.076
Caryophyllene oxide 2	2	1.53±0.6	0.02–5.63	1.06±0.1	0.45–1.92	-0.78	0.446
Caryophyllene oxide 3		26.06±2.1	15.36–34.75	29.27±2.1	18.48–43.68	1.10	0.283
Total caryophyllene oxide		28.74±1.8	17.76–36.72	31.82±2.1	20.09–46.31	1.10	0.286
Total caryophylline		41.06±2.1	27.92–50.46	39.22±2.3	26.83–49.66	-0.59	0.560
α-Copaene		1.84±0.2	0.31–2.98	2.89±0.3	1.39–4.16	<b>2.61</b>	<b>0.017</b>
β-Copaene	16	0.08±0.0	0.02–0.28	0.02±0.0	0.02–0.05	-2.11	0.061
α-Cubebene	4	0.18±0.0	0.02–0.40	0.30±0.0	0.02–0.47	1.92	0.069
Cyperene	2	0.55±0.1	0.02–1.74	1.01±0.1	0.57–2.04	<b>2.30</b>	<b>0.033</b>
β-Elementene		0.91±0.1	0.38–1.70	1.00±0.1	0.30–1.93	0.54	0.597
Germacrene A	7	0.30±0.1	0.02–0.62	0.29±0.1	0.02–1.06	-0.08	0.940
Germacrene D	1	1.76±0.3	0.12–3.42	1.21±0.4	0.02–4.28	-1.10	0.285
α-Guaiene	3	0.35±0.1	0.02–0.82	0.40±0.0	0.15–0.66	0.44	0.670
α-Humulene		2.12±0.3	0.63–3.36	1.32±0.3	0.40–3.13	-2.04	0.055
Humulene epoxide		3.70±0.2	2.65–4.92	4.23±0.3	2.99–6.07	1.61	0.123
Total humulene		5.81±0.2	4.87–7.21	5.55±0.3	4.21–6.86	-0.74	0.466
α-Selinene		2.23±0.4	0.57–4.52	1.38±0.2	0.49–2.51	-2.00	0.066
β-Selinene		4.02±0.6	1.88–7.55	3.14±0.2	2.29–3.82	-1.51	0.146
Selinenes		6.25±0.2	2.52–10.46	4.52±0.3	2.78–6.00	-1.83	0.093
δ-Selinene	1	2.06±0.5	0.02–5.27	2.90±0.3	1.52–4.20	1.44	0.171
Selena 3,7-diene	6	0.16±0.0	0.02–0.29	0.22±0.0	0.02–0.38	1.14	0.266

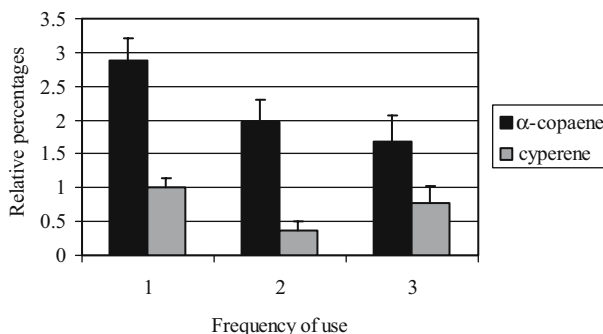
df=20, significant values are in boldface and numbers of substituted MLE values for missing values are indicated

young and older leaves. However, values for cyperene ( $t=2.496$ ,  $df=10$ ,  $P=0.032$ ) and selena 3,7-diene ( $t=3.034$ ,  $df=9$ ,  $P=0.014$ ) were greater in buds relative to young leaves and those for γ-cadinene were near significant ( $t=2.348$ ,  $df=10$ ,  $P=0.056$ ).

*Leaf Damage in Relation to Sesquiterpene Chemistry and Tree Use* Undamaged trees contained higher relative percentages of α-copaene, δ-cadinene, and γ-cadinene, and results for α-cubebene are nearly significant (Table 2). Whereas a chi-square test indicated that monkeys did not preferentially feed from undamaged trees ( $\chi^2=1.636$ ,  $df=1$ ,  $P=0.201$ ), only four trees categorized as undamaged were fed from vs. seven damaged trees.

*Sesquiterpenes with Suspected Deterrent Function* A discriminant function analysis was conducted to determine how well relative percentages of α-copaene and cyperene predicted tree

**Fig. 1** Relative percentages of  $\alpha$ -copaene and cyperene in three categories of use: 1=no scans, 2=1–5 scans, 3=>5 scans



use. Results were significant (Wilks  $\Lambda=0.642$ ,  $\chi^2=8.434$ ,  $df=2$ ,  $P=0.015$ ). The discriminant function coefficients were 0.729 for  $\alpha$ -copaene and 0.627 for cyperene based upon their degree of correlation with the discriminant function (0.781 and 0.687, respectively). The frequency distribution of the canonical variable for used and unused trees is presented in Fig. 2.

With the exception of  $\alpha$ -copaene and  $\gamma$ -cadinene, all compounds implicated in differential insect leaf predation in *H. stigonocarpa* (Langenheim et al. 1986) are intercorrelated in leaf samples from used and unused trees in the present study (see Table 3). As noted,  $\beta$ -cadinene,  $\alpha$ -copaene, and  $\alpha$ -cubebene have been shown to be inducible in response to herbivorous damage (Barnola et al. 1994). Cyperene was not correlated with any of those compounds.

## Discussion

Seventeen sesquiterpenes and several oxidized analogs were identified in young leaf specimens from the 22 study trees. Many are widespread in plant essential oils ( $\delta$ -cadinene,  $\gamma$ -cadinene,  $\beta$ -caryophyllene,  $\alpha$ -copaene,  $\beta$ -copaene,  $\alpha$ -cubebene,  $\beta$ -elemene, germacrene D,  $\alpha$ -guaiene,  $\alpha$ -humulene,  $\alpha$ -selinene, and  $\beta$ -selinene) whereas some are known from only one or two genera (cyperene) (Joulain and König 1998). Unused trees contained higher

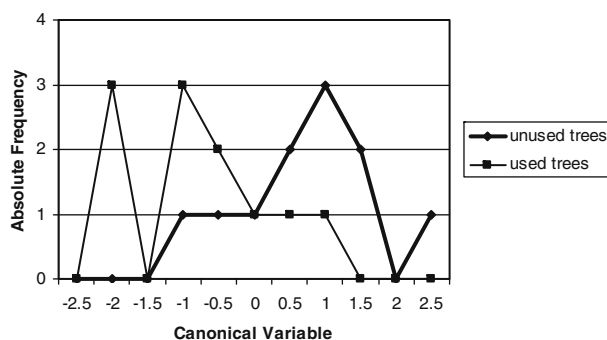
**Table 2** Comparison of mean values in damaged and undamaged trees

Compounds	Mean for Undamaged	Mean for Damaged	<i>t</i> Score	<i>P</i> Value
Possible deterrent function				
$\beta$ -Caryophyllene	10.23±2.2	9.49±1.4	0.29	0.773
Total caryophyllene oxides	28.46±1.9	32.15±2.6	-1.16	0.261
$\alpha$ -Humulene	1.70±0.4	1.74±0.2	-0.08	0.936
Humulene epoxide	3.85±0.2	4.08±0.3	-0.67	0.508
$\alpha$ -Selinene	1.89±0.4	1.72±0.3	0.36	0.721
Inducible compounds				
$\delta$ -Cadinene	1.00±0.0	0.72±0.0	<b>2.35</b>	<b>0.029</b>
$\gamma$ -Cadinene	0.61±0.1	0.40±0.1	<b>2.46</b>	<b>0.023</b>
$\alpha$ -Copaene	2.87±0.4	1.86±0.2	<b>2.50</b>	<b>0.021</b>
$\alpha$ -Cubebene	0.30±0.0	0.18±0.0	<b>2.00</b>	0.059
Other				
Cyperene	0.79±0.1	0.78±0.2	0.04	0.966

$df=20$ , significant results are in boldface



**Fig. 2** Absolute frequency distribution of the canonical variable in the discriminant function analysis to determine how well relative percentages of  $\alpha$ -copaene and cyperene predicted tree use



relative percentages of  $\alpha$ -copaene and cyperene. For those trees that were fed from, no pattern was discernable between relative percentages of cyperene and frequency of tree use. However, in the case of  $\alpha$ -copaene, those trees for which few feeding scans were observed ( $N=1-5$  scans/tree) yielded intermediate values when compared with unused trees and more frequently used ( $N=6-44$  scans) trees. These results, along with the relative percentage above which no consumption was observed, suggest the possibility of a dose-dependent effect on tree choice and frequency/duration of use. Whereas young leaves had significantly lower values for cyperene and selenane 3,7-diene, when compared with terminal buds, it is difficult to ascertain whether those compounds played a role in bud avoidance as buds contain high concentrations of condensed tannins, and there is evidence of their avoidance by howler monkeys. As mentioned, the occurrence of cyperene is rare relative to other sesquiterpenes, and there are no known reports of a deterrent function. Selenane 3,7-diene has not been reported to occur naturally but rather is the result of a rearrangement of  $\beta$ -selinene (Joulain and König 1998). Undamaged trees yielded higher values for  $\alpha$ -copaene,  $\delta$ -cadinene, and  $\gamma$ -cadinene. Finally,  $\alpha$ -copaene and cyperene values were found to be predictive of tree use with  $\alpha$ -copaene being the strongest predictor.

Langenheim and colleagues have established that populations and subpopulations within *Hymenaea* congeners can be categorized as different chemical “types” based upon the profile of their sesquiterpenes (see, for example, Crankshaw and Langenheim 1981, for a discussion of types). Little intrapopulational variation in sesquiterpene composition, in terms of being classified as one type or another, has been observed in Central American and dry forest *Hymenaea* populations (Martin et al. 1974). Whereas the amount of chemical variation observed in the present study may not be sufficient to qualify them as different

**Table 3** Correlation matrix for possible insect deterrent compounds

Compounds	$\alpha$ -Copaene ( <i>R</i> )	$\alpha$ -Cubebene ( <i>R</i> )	$\delta$ -Cadinene ( <i>R</i> )	$\gamma$ -Cadinene ( <i>R</i> )	Cyperene ( <i>R</i> )
$\alpha$ -Copaene	1				
$\alpha$ -Cubebene	0.642**	1			
$\delta$ -Cadinene	0.716**	0.769**	1		
$\gamma$ -Cadinene	0.233	0.602*	0.650**	1	
Cyperene	0.294	0.019	0.115	-0.012	1

$N=22$

\*  $P < .01$

\*\*  $P < .001$



types, between-tree differences could render leaves from some trees more palatable to the monkeys than others.

There are three reported lines of evidence in support of an antiherbivore function for  $\alpha$ -copaene: (1) the facultative production of  $\alpha$ -copaene in response to defoliation (Barnola et al. 1994; Vrkočová et al. 2000), (2) differential insect predation in *H. stigonocarpa* trees (Langenheim et al. 1986), and (3) differential oviposition rates by two butterfly species (Ling et al. 2003). Whereas a facultative response is not conclusive evidence of a deterrent function, in that levels of  $\alpha$ -copaene likely covary with other compounds that may play a deterrent or maintenance function, the differential leaf consumption by insects and monkeys and differential oviposition rates by butterflies lend support to a possible antiherbivore function for this compound. Interestingly, other compounds that were also implicated in the aforementioned inducibility experiments and differential insect predation and oviposition rates (i.e.,  $\alpha$ -cubebene,  $\delta$ -cadinene, and  $\gamma$ -cadinene) covaried in the present study. Whereas these other compounds did not appear to influence leaf choice by the monkeys, relative percentages were greater in undamaged trees, reinforcing a possible deterrent role for insects.

These results must be treated with caution. Observed differences between young leaf specimens and leaf stages were the result of comparing the relative percentages of compounds in essential oil extracts. Nothing can be concluded about the actual concentrations of those compounds in a given area of leaf blade. A second potential problem arises from the fact that Glander (unpublished data in Glander 1982) reports within-tree variability in leaf quality in relation to location within the canopy for some tree species. Thus, one leaf specimen per tree may not be representative of the average sesquiterpene chemistry of all young leaves in the crown. Whereas, Langenheim and colleagues found little experimental evidence of phenotypic plasticity in resin composition in response to variable abiotic conditions, e.g., light, photoperiod, temperature, and moisture (Stubblebine, unpub.; Langenheim et al. 1975, 1979), there were quantitative differences in resin production and hence the possibility of differing concentrations of the constituent sesquiterpenes.

Results for  $\beta$ -caryophyllene,  $\alpha$ -humulene, and  $\alpha$ -selinene are of interest in that they have known insect deterrent qualities and yet did not deter the monkeys. Mantled howler monkeys are of moderate size, and considering the relatively high proportion of leaves and flowers in their diet, it seems likely they possess the ability to detoxify a variety of plant secondary compounds.

With regard to leaf stage selectivity, the high concentration of condensed tannins in leaf buds likely precluded their consumption, regardless of sesquiterpene content. Total phenolics and condensed tannins drop from 16% DW to less than 1% DW by the second leaf pair, whereas individual sesquiterpenes may increase or decrease (Crankshaw and Langenheim 1981). Resin yield decreases as leaves become more mature and sesquiterpene and phenolic compounds level off. Mature leaves are tough and, other than some saturniid moth larvae, no herbivores have been observed to feed on them (Janzen 1983). They do not appear to be better defended chemically relative to young leaves. Crankshaw and Langenheim (1981) report that leaf toughness increases between the third leaf pair and the fourth to sixth pairs. Monkeys were not observed to eat leaves beyond the third pair. Whereas Welker (2004) concluded that leaf toughness did not play a role in differential tree use in *H. courbaril*, she considered only toughness of young leaves. Thus, toughness of mature leaves may have played a role in leaf stage selectivity.

In summary, results for  $\alpha$ -copaene suggest it as a possible factor affecting palatability and consumption in monkeys. Once the range of concentrations of this compound is determined for this tree species, it must be determined if there is a maximum acceptance

threshold for  $\alpha$ -copaene and whether this threshold approximates levels in the leaves of unused trees. With reference to plant part selectivity, the youngest leaves contain lesser amounts of total phenolics, condensed tannins, and certain sesquiterpene compounds, relative to the terminal leaf buds. In addition, they are less tough and contain more protein and moisture than mature leaves (Crankshaw and Langenheim 1981). Thus, the animals were feeding on the leaf stage that was of high nutritional value, low in chemical defenses relative to buds, and low in mechanical defenses relative to mature leaves, and appeared to be choosing between individual plants and parts/stages in relation to their primary and secondary chemistry.

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## References

- AGELOPOULOS, N. G., HOOPER, A. M., MANIAR, S. P., PICKETT, J. A., and WADHAMS, L. J. 1999. A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *J. Chem. Ecol.* 25:1411–1425.
- ALTMANN, J. 1974. Observational study of behavior: Sampling methods. *Behaviour* 49:227–265.
- ARTAVIA, D., BARRIOS, M., and CASTRO, O. 1995. A flavonol rhamnoside from *Hymenaea courbaril* leaves. *Fitoterapia* LXVI:91–92.
- BARNOLA, L. F., HASEGAWA, M., and CEDEÑO, A. 1994. Mono- and sesquiterpene variation in *Pinus caribaea* needles and its relationship to *Atta laevigata* herbivory. *Biochem. Syst. Ecol.* 22:437–445.
- BERRY, J. P. 1998. The chemical ecology of mountain gorillas (*Gorilla gorilla beringei*), with respect to antimicrobial constituents in the diet. Ph.D. dissertation, Cornell University, Ithaca, NY.
- CALVERT, J. J. 1985. Food selection by western gorillas (*Gorilla gorilla*) in relation to food chemistry. *Oecologia* 65:236–246.
- CRANKSHAW, D. R. and LANGENHEIM, J. H. 1981. Variation in terpenes and phenolics through leaf development in *Hymenaea* and its possible significance to herbivory. *Biochem. Syst. Ecol.* 9:115–124.
- DE ASEVEDO, N. R., FERRI, E., SERAPHIN, J. C., and BRANDÃO, D. 2006. Chemical composition of intraspecific variability of the volatile constituents from the defensive secretions of *Constrictotermes cyphergaster*. *Sociobiology* 47:891–902.
- ESTRADA, A. 1984. Resource use by howler monkeys (*Alouatta palliata*) in the rainforest of “Los Tuxtlas”, Veracruz, Mexico. *Intl. J. Primatol.* 5:105–131.
- GLANDER, K. E. 1978. Howler monkey behavior and plant secondary compounds: A study of strategies, pp. 561–574, in G. G. Montgomery (ed.). *The Ecology of Arboreal Folivores*. Smithsonian Institution Press, Washington, DC.
- GLANDER, K. E. 1981. Feeding patterns in mantled howler monkeys, pp. 231–257, in A. C. Kamil and T. D. Sargent (eds.). *Foraging Behavior: Ecological, Ethological, and Psychological Approaches*. Garland Press, New York.
- GLANDER, K. E. 1982. The impact of plant secondary compounds on primate feeding behavior. *Yearb. Phys. Anthropol.* 25:1–18.
- HAZNEDAROGLU, M. Z., KARABAY, N. U., and ZEYBEK, U. 2001. Antibacterial activity of *Salvia tomentosa* essential oil. *Fitoterapia* 72:829–831.
- JANZEN, D. H. 1983. *Hymenaea courbaril*, pp. 253–256, in D. H. Janzen (ed.). *Costa Rican Natural History*. Chicago University Press, Chicago.
- JOULAIN, D. and KÖNIG, W. A. 1998. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. E. B. Verlag, Hamburg.
- LANGENHEIM, J. H. 1981. Terpenoids in the Leguminosae, pp. 627–655, in R. M. Polhill and P. H. Raven (eds.). *Advances in Legume Systematics*. Royal Botanical Gardens, Kew.

- LANGENHEIM, J. H., STUBBLEBINE, W. H., and LINCOLN, D. E. 1975. Vegetative growth and leaf resin composition in *Hymenaea courbaril* under photoperiodic extremes. *Biochem. Syst. Ecol.* 3:219–228.
- LANGENHEIM, J. H., STUBBLEBINE, W. H., and FOSTER, C. E. 1979. Effect of moisture stress on composition and yield in leaf resin of *Hymenaea courbaril*. *Biochem. Syst. Ecol.* 7:21–28.
- LANGENHEIM, J. H., FOSTER, C. E., and MCGINLEY, R. B. 1980. Inhibitory effects of different quantitative compositions of *Hymenaea* leaf resins on a generalist herbivore *Spodoptera exigua*. *Biochem. Syst. Ecol.* 8:385–396.
- LANGENHEIM, J. H., CONVIS, C. L., MACEDO, C. A., and STUBBLEBINE, W. H. 1986. *Hymenaea* and *Copaifera* leaf sesquiterpenes in relation to lepidopteran herbivory in southeastern Brazil. *Biochem. Syst. Ecol.* 14:41–49.
- LING, B., ZHANG, M., KONG, C., PANG, X., and LIANG, G. 2003. Chemical composition of volatile oil from *Chromolaena odorata* and its effect on plant, fungi, and insect growth. *Chin. J. Appl. Ecol.* 14:744–746.
- LOPEZ, J. A. 1976. The isolation and characterization of funiferine N-oxide. A new alkalide from *Tillacora funifera* Oliver (Menispermaceae) alkaloid N-oxides. Study of the N-oxides of funiferine and pheanthine and chemical constituents of selected medicinal plants from Costa Rica L (Papaveraceae) *Hymenaea courbaril* L (Leguminosae) *Physalis angulata* L (Solanaceae). Ph.D. dissertation, University of Pittsburgh, Pittsburgh.
- MARTIN, S. S., LANGENHEIM, J. H., and ZAVARIN, E. 1972. Sesquiterpenes in leaf pocket resin of *Hymenaea courbaril*. *Phytochemistry* 11:3049–3051.
- MARTIN, S. S., LANGENHEIM, J. H., and ZAVARIN, E. 1974. Quantitative variation in leaf pocket resin composition in *Hymenaea courbaril*. *Biochem. Syst. Ecol.* 2:75–87.
- MCCLOSKEY, L. P. 1984. Leaf phenolic compounds in the tropical tree genera *Hymenaea* and *Copaifera*. Ph. D. dissertation, University of California, Santa Cruz.
- MILTON, K. 1978. Behavioral adaptations to leaf-eating by the mantled howler monkey (*Alouatta palliata*), pp. 535–549, in G. G. Montgomery (ed.). *The Ecology of Arboreal Folivores*. Smithsonian Institution Press, Washington, DC.
- MILTON, K. 1979. Factors influencing leaf choice by howler monkeys: A test of some hypotheses. *Am. Nat.* 114:362–378.
- MILTON, K. 1980. *The Foraging Strategy of Howler Monkeys*. Columbia University Press, New York.
- MILTON, K. 1998. Physiological ecology of howlers (*Alouatta*): Energetic and digestive considerations and comparison with the colobinae. *Intl. J. Primatol.* 19:513–548.
- QUINTANA, A., REINHARD, J., FAURE, R., UVA, P., BAGNÈRES, A.-G., MASSIOT, G., and CLÉMENT, J.-L. 2003. Interspecific variation in terpenoid composition of defensive secretions of European Reticulitermes termites. *J. Chem. Ecol.* 29:639–652.
- VRKOČOVÁ, P., VALTEROVÁ, I., VRKOČ, J., and KOUTEK, B. 2000. Volatiles released from oak, a host tree for the bark beetle *Scolytus intricatus*. *Biochem. Syst. Ecol.* 28:933–947.
- WELKER, B. J. 2004. Proximate mechanisms governing feeding behavior and selectivity in mantled howler monkeys, *Alouatta palliata*. Ph.D. dissertation, State University of New York, Buffalo.