

## INTERESPECIFIC VARIATION IN THE COMPOSITION OF VOLATILE OILS FROM THE LEAVES OF *Swietenia macrophylla* KING (MELIACEAE)

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The volatile oils from leaves of five Brazilian specimens of *Swietenia macrophylla* King (Meliaceae) collected in three different Amazon Rainforest Conservation Areas in the States of Mato Grosso, Pará, and Rondônia were extracted and analyzed by GC and GC/MS. The oils showed to be composed by terpenoids, majority hydrocarbon sesquiterpenes, being germacrene D (20.5-46.8%) and bicyclogermacrene (8.3-11.1%) the main components. Besides these derivatives, only  $\alpha$ -cubebene,  $\beta$ -caryophyllene,  $\beta$ -gurjunene and  $\gamma$ -cadinene were detected in all of the analyzed samples. This analysis indicated a great diversity of constituents in the oils obtained from specimens collected in these regions, which could be associated to the different susceptibility in the attack of *H. grandella* in *S. macrophylla* cultures.

Keywords: *Swietenia macrophylla* King; Meliaceae; volatile oil composition.

### INTRODUCTION

*Swietenia macrophylla* King (mahogany) is a valuable and intensively exploited neo-tropical timber species whose seedling survival and growth dynamics in natural forests are poorly understood.<sup>1</sup> Chemically, this species is characterized by expressive production of limonoids, which are highly oxygenated degraded triterpenes, characteristics from Meliaceae.<sup>2-4</sup>

Previous investigation of the volatile oils from shoots, mature and senescent leaves from *S. macrophylla*, collected in Igarapé-Açu/PA, indicated the presence of several compounds including germacrene D, hexadecanoic acid, and its respective ethyl ester derivative. Additionally, electroantennograms of both female and male borer moths revealed significant responses to the volatile oil obtained from terminal shoots, mature and senescent leaves from *S. macrophylla*. The GC-EAD of the volatile oil from the mature leaves of *S. macrophylla* suggests  $\beta$ -caryophyllene as the main constituent responsible for antennal response in female *H. grandella* antennae.<sup>5</sup>

Several works describe that the same species grown in different areas could exhibit variations in the composition of its volatile oils,<sup>6,7</sup> but no information about dynamic of volatile derivatives from *S. macrophylla* has previously been described in the literature. Therefore, the purpose of this work was to assess the volatile oil variation from the leaves of different specimens of *S. macrophylla* collected in five different regions of Amazon Rainforest Conservation Areas (Juína/MT, Morada do Sol/PA, Santarém/PA, Vila Progresso/PA, and Ariquemes/RO).

### EXPERIMENTAL

#### Plant material

The leaves of *Swietenia macrophylla* King (Meliaceae) were randomly collected on 14<sup>th</sup> November 1999 (at 9-11 a.m.) from three Amazon Rainforest Conservation Areas in the States of Mato Grosso

(Juína – 1), Pará (Morada do Sol – 2, Santarém – 3, Vila Progresso – 4), and Rondônia (Ariquemes – 5). The plant materials were compared with those previously analyzed<sup>4,5</sup> and voucher specimens were deposited in the Herbarium of the Museum Paraense Emílio Goeldi, Belém, Pará, Brazil.

#### Volatile oil extraction

Immediately after collection, the fresh leaves (400 g) were steam-distilled in a Clevenger-type apparatus for 4 h. After extraction, the crude oils were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and stored at -20 °C in the dark until analyzed. The yield of each extraction was calculated based on the weight of fresh leaves. Quantitative and qualitative analysis of the oils were performed by GC and GC/MS, respectively.

#### Qualitative and quantitative analysis

GC analysis were performed in a Hewlett-Packard 5890 Series II gas chromatograph (using helium as carrier gas) equipped with an FID detector, and automatic injector (HP 7673) and electronic integrator (HP3396A). The GC was fitted with a capillary column (HP-5, 30 m 0.32 mm, I.D., 0.25  $\mu$ m film thickness) with a stationary phase of 5% cross-linked phenyl in 95% silicone, temperature programming was performed as follows: constant 100 °C for 2 min, a linear ramp from 100-240 °C at 5 °C min<sup>-1</sup>, then held at constant 240 °C for 5 min. Injector (FID) and detector temperatures were 180 and 260 °C, respectively. Relative component concentrations were calculated from GC peak areas in the order of DB-5 column elution.<sup>8</sup>

GC/MS analysis was carried out in an Hewlett-Packard HP-5973 EIMS (70 eV) coupled with a Hewlett-Packard HP-6890 with HP-5 column (30 m 0.25 mm, I.D., 0.25  $\mu$ m film thickness) using the same temperature-programming conditions described above. The identification of the components was based on the comparison of their EI mass spectra with those in the system's spectral library (Willey 229) and confirmed by calculation of their Kovats retention indexes (RI), which were determined relatively to the retention times of a series of *n*-alkanes (C<sub>8</sub> to C<sub>20</sub>) as described in the literature.<sup>9,10</sup>

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**Table 1.** Data of each collection from leaves of *Swietenia macrophylla*, yield, content (in percentage) and composition of respective volatile oils

		1	2	3	4	5
Collection site		Juína / MT	Morada do Sol / PA	Santarém / PA	Vila Progresso / PA	Ariquemes / RO
Volatile oil yield (%)		0.14	0.12	0.13	0.17	0.21
Compounds	KI					
Tricyclene	926	-	-	-	0.19	-
Benzaldehyde	961	-	-	-	1.10	-
Sabinene	976	-	-	0.24	0.16	0.53
NI (MM 136)	964	0.58	-	0.05	-	-
NI (MM 138)	989	-	-	0.35	3.51	-
Isosylvestrene	1007	-	-	-	0.97	-
$\Delta^3$ -Carene	1011	-	0.48	-	-	-
Limonene	1031	-	-	0.43	2.99	2.21
(Z)- $\beta$ -Ocimene	1040	-	-	0.11	0.27	0.22
Benzene acetaldehyde	1043	-	-	-	0.10	-
(E)- $\beta$ -Ocimene	1050	-	-	0.31	1.20	-
$\gamma$ -Terpinene	1062	-	-	-	-	0.24
Acetophenone	1065	-	-	-	0.89	-
NI (MM 136)	1080	-	0.51	0.01	-	-
Pinocampheol	1170	-	-	-	0.90	0.48
Terpin-4-ol	1177	-	-	-	0.63	0.47
NI (MM 152)	1215	-	0.97	0.04	-	-
NI (MM 152)	1219	-	-	-	0.36	0.42
NI (MM 152)	1222	-	-	0.04	-	0.34
(E,Z)-2,4-Decadienal	1291	-	-	0.29	-	-
(E,E)-2,4-Decadienal	1314	0.34	0.48	-	0.95	0.29
$\delta$ -Elemene	1339	0.19	-	-	-	-
$\alpha$ -Cubebene	1351	0.44	0.60	0.98	0.92	0.96
Eugenol	1356	-	-	-	-	0.21
Cyclosativene	1368	-	-	1.23	-	-
$\alpha$ -Ylangene	1372	4.73	-	-	2.90	1.69
$\alpha$ -Copaene	1376	-	5.09	3.16	-	-
$\beta$ -Bourbonene	1384	0.51	1.23	-	1.45	1.23
$\beta$ -Cubebene	1390	-	1.88	4.91	3.10	1.62
$\beta$ -Elemene	1390	1.52	-	-	-	-
$\alpha$ -Gurjunene	1409	-	-	2.40	1.32	-
$\beta$ -Caryophyllene	1418	1.40	0.27	2.23	0.15	1.83
NI (MM 202)	1420	0.29	2.79	-	0.88	1.04
$\beta$ -Gurjunene	1432	0.74	2.29	2.15	2.55	1.67
NI (MM 204)	1434	2.54	-	-	-	2.22
NI (MM 204)	1449	1.07	-	-	-	1.34
$\alpha$ -Humulene	1454	-	0.58	-	-	0.33
Cis-Muurolo-4(14),5-diene	1460	-	-	1.32	-	-
Allo-Aromadendrene	1461	1.79	-	-	-	0.92
NI (MM 202)	1462	1.33	-	0.92	-	1.88
(E)-9- <i>epi</i> -Caryophyllene	1467	-	-	3.39	-	-
$\gamma$ -Gurjunene	1477	-	-	-	1.58	-
Germacrene-D	1480	46.83	20.47	23.06	45.66	43.61
$\beta$ -Ionone	1485	-	-	-	1.65	-
Valence	1491	-	-	-	1.46	-
Bicyclogermacrene	1494	8.26	9.23	11.11	8.54	8.34
$\alpha$ -Muurolole	1499	-	-	-	0.65	1.46
Germacrene-A	1503	1.29	3.75	3.57	2.80	-
$\gamma$ -Cadinene	1513	3.32	4.15	3.98	0.62	1.57
Cis-Calamenene	1521	-	1.74	-	-	-
$\delta$ -Cadinene	1524	4.70	-	5.22	1.24	2.52
Cadina-1,4-diene	1532	-	-	0.83	-	0.81

Table 1. Continuation

		1	2	3	4	5
Collection site		Juína / MT	Morada do Sol / PA	Santarém / PA	Vila Progresso / PA	Ariquemes / RO
Volatile oil yield (%)		0.14	0.12	0.13	0.17	0.21
Compounds	KI					
<i>Trans</i> -Calamenene	1532	-	1.30	-	-	-
$\alpha$ -Cadinene	1538	0.12	-	3.94	1.35	1.27
NI (MM 204)	1568	-	0.85	0.34	-	0.32
Spathulenol	1576	-	4.07	-	-	1.49
NI (MM 222)	1581	-	0.56	-	-	0.61
NI (MM 222)	1589	1.18	-	0.71	-	0.82
Humulene Epoxide II	1606	-	1.38	-	-	-
NI (MM 222)	1615	2.12	-	-	-	2.95
1- <i>epi</i> -Cubenol	1627	1.01	1.88	1.39	-	-
<i>Epi</i> - $\alpha$ -Cadinol	1641	2.04	7.79	4.93	-	-
NI (MM 222)	1654	-	6.98	2.39	-	1.43
<i>Cis</i> -14-Murolool-5-en-4-one	1682	-	3.96	-	-	-
NI (MM 220)	1686	0.36	-	0.11	-	0.22
NI (MM 220)	1763	-	-	0.09	-	0.35
Methyl hexadecanoate	1927	1.74	5.33	2.52	-	0.59
Ethyl Hexadecanoate	1993	-	-	1.23	0.43	0.72
NI (MM 272)	2035	0.10	-	0.04	-	0.09
Methyl linoleate	2092	0.50	-	-	-	0.68
Methyl octadecanoate	2129	0.11	-	-	0.85	-
<i>E</i> -Phytol Acetate	2221	-	-	-	-	0.41
Monoterpene hydrocarbons		-	0.48	1.09	5.78	3.20
Oxygenated monoterpenes		-	-	-	1.56	0.95
Sesquiterpene hydrocarbons		75.84	52.58	73.48	75.37	69.83
Oxygenated sesquiterpenos		3.05	19.08	6.32	1.65	1.49
Other compounds		2.69	5.81	4.04	3.27	2.90
<b>Total identified</b>		<b>81.58</b>	<b>77.95</b>	<b>84.93</b>	<b>89.57</b>	<b>78.37</b>
<b>Total not identified</b>		<b>9.57</b>	<b>12.66</b>	<b>5.09</b>	<b>4.75</b>	<b>12.69</b>
<b>TOTAL</b>		<b>91.15</b>	<b>90.61</b>	<b>90.02</b>	<b>94.32</b>	<b>91.06</b>

## RESULTS AND DISCUSSION

Data of each collection of leaves of *S. macrophylla* as well as the volatile oils yields are shown in Table 1. These data indicate that the specimens from Ariquemes (5) and Vila Progresso (4) regions present the greatest oils content (0.21 and 0.17% w/w, respectively) followed by the oils from Juína (1), Morada do Sol (2), and Santarém (3), which showed similar yields (0.12-0.14% w/w). Comparatively, the yields of these five analyzed specimens were higher of those detected previously to *S. macrophylla* from Igarapé-Açú, which were calculated as 0.048% to mature and 0.047% to senescent leaves.<sup>5</sup>

Chemically, the crude oils were analyzed by CG and CG/MS which lead to the identification of fifty-four constituents for which together account for 77.9-89.6% of the total oil content. The relative amounts of each component in the crude oils are presented in Table 1. In the five analyzed oil samples, the predominant components were hydrocarbon sesquiterpene derivatives, including  $\alpha$ -cubebene,  $\beta$ -caryophyllene,  $\beta$ -gurjunene, germacrene D, bicyclogermacrene and  $\gamma$ -cadinene, all of which were detected in the five studied specimens. These data also showed the predominance of germacrene-D in the five analyzed oils, with its relative abundance being higher in specimens 1 (46.83%), 4 (45.66%) and 5 (43.61%), similar to the oils previously analyzed.<sup>5</sup> A biogenetically related compound from germacrene D,<sup>11</sup> the sesquiterpene bicyclogermacrene, was detected as the second most abundant derivative with a similar composition

in specimens 1 (8.26%), 2 (9.23%), 4 (8.54%), and 5 (8.34%), but with a higher relative amount in specimen 3 (11.11%).

The combined relative amounts of hydrocarbon sesquiterpenes were shown to be similar (69.83-75.84%) in the oils from leaves collected in regions 1, 3, 4, and 5. Comparatively, the oil from region 2 showed a lower relative amount of non-oxygenated C<sub>15</sub> derivatives (52.58%). In contrast, the relative proportion of oxygenated derivatives in the oil from region 2 was approximately 20%, with *epi*- $\alpha$ -cadinol being the major compound (7.79%). Despite of the monoterpenes have been detected as minor derivatives in the crude oils, a higher proportion of these compounds was observed in specimens 4 (7.34%) and 5 (4.15%), in which limonene was the major component in both oils (2.99% and 2.21%, respectively). Otherwise, the occurrences of these C<sub>10</sub> derivatives was not observed in the oil from specimen 1, but were detected only in very low amounts in the oils from specimens 2 and 3, similar to what had been observed in other Meliaceae specimens.<sup>12-14</sup>

Finally, observations in the field showed that trees from distinct geographic origin exhibited a differentiation in their susceptibility to attack by *H. grandella*.<sup>15</sup> As determined previously by GC/EAD measurements,<sup>5</sup> the sesquiterpene  $\beta$ -caryophyllene could play a role in attracting *H. grandella* to lay its eggs on the leaves of *S. macrophylla*. In the present work, the distinctive abundance of  $\beta$ -caryophyllene in each one of the five analyzed oils could partially explain the different behavior of the attack of this lepidopterous in *S. macrophylla*

cultures.<sup>15</sup> In summary, the results presented here provide additional information concerning the chemical variation in volatile oils from *S. macrophylla*, indicating that the ecological relationship of these volatile oils and *H. grandella* require further detailed investigation.

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#### SUPPLEMENTARY MATERIAL

Available in <http://quimicanova.sbq.org.br>, in format PDF, with free access.

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**Figure 1S.** Brazilian Amazon Forest conservation areas in which the five specimens of *S. macrophylla* were collected – Juína/MT (1), Morada do Sol/PA (2), Santarém/PA (3), Vila Progresso/PA (4), and Ariquemes/RO (5)

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