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A N I N T E R N A T I O N A L J O U R N A L

Seasonal Influence on the Essential Oil Production of *Nectandra megapotamica* (Spreng.) Mez

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ABSTRACT

This study evaluated the seasonal influence on the yield and chemical composition of the essential oil (EO) of Nectandra megapotamica. *Fresh young (YL) and old leaves (OL) obtained from three trees in each season (Nov/2010 to Sep/2011) collected in Santa Maria-RS were hydrodistilled in triplicate. The chemical composition was determined by the gas chromatography coupled to mass spectrometry (GC-MS) and the yield on dry basis was evaluated by two-way ANOVA (seasons, development stage). Spring (Sp) and summer (Su) showed higher average incomes (0.45 and 0.33%), which occurred when flowering, fruiting, and growth of YL and senescence of OL took place, while autumn (Au) presented the lowest yield (0.25%) during the rustification of OL. The highest yield was obtained for the YL in Sp (0.59%) and the lowest for the OL in Au (0.21%). The major constituents of the EO were independent from the season and were identified as α-pinene, bicyclogermacrene, β-pinene, germacrene D, and limonene. Seasonality and phenology influenced the production of EO probably due to morphological and metabolic alterations in the leaves as well as due to the needs of the tree, such as attraction and/or protection.*

Key words: Lauraceae, phenology, variability of volatile oils, terpenes

INTRODUCTION

Approximately 390 species of the family Lauraceae Lindley are found in Brazil (Barroso 1978). *Nectandra megapotamica* (Spreng.) Mez is important due to its wide distribution not only in Brazil, but also in other countries in South America such as Argentina, Paraguay and Uruguay (Baitello 2003). In Brazil, it can be found in the states of Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP), Mato Grosso do Sul (MS), Minas Gerais (MG), Rio de Janeiro (RJ), and Espirito Santo (ES) (Lorenzi 2002; Carvalho et al. 2006). In RS, the species is widely distributed in native forests (Tonini et al. 2003), participating in the composition of the Dense Ombrophilous Forest, Mixed Ombrophilous Forest, Seasonal Deciduous Forest and Seasonal Semideciduous Forest (Marchiori 1997).

The various biological activities described to essential oil (EO) of *N. megapotamica* and/ or its components have aroused interest of Latin American researchers working in the area of its occurrence. Among them are noteworthy the anti-inflammatory activity described by Apel et al. (2006) for EO obtained from specimens in

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Cananéia, SP, the anesthetic action on fat snook (*Centropomus parallelus*) obtained with EO of specimens occurring in Santa Maria, RS (Tondolo et al. 2013), the inhibition of hemolytic activity of jararaca (*Bothrops diporus*) venom with EO obtained from plant material collected in Corrientes, Argentina (Torres et al. 2014), and the insecticidal activity against larvae of Coenagrionidae (Silva et al. 2014). All the described effects are directly influenced by the chemical composition of EO, and knowledge about the income of this extractive is fundamental to the planning and execution of further studies aiming practical applications.

Marked differences have been detected for the yield of EO in the fresh leaves of this species in different environments, ranging from 0.11 - 0.18% in São Paulo, SP (Romoff 2010) to 0.20-0.30% in Santa Maria, RS (Tondolo et al. 2013). However, Apel et al. (2006) reported a yield of 0.1% for the EO extracted from dried leaves collected from specimens occurring on the coast of São Paulo State, in Cananéia. Chemical variability has also been described, with bicyclogermacrene, α-pinene and germacrene D as major compounds in the EO obtained by Tondolo et al. (2013) in RS, while Romoff (2010) reported the highest yields of α-bisabolol and δ-elemene in the EO of leaves of this species obtained from trees in SP.

The production of secondary metabolites, which include the constituents of the EO, may be influenced by the seasonality, rainfall, circadian rhythm, ultraviolet radiation, atmospheric composition, herbivory and pathogen attack, plant age, availability of water and nutrients in the soil, temperature, and altitude (Gobbo-Neto and Lopes 2007). However, phenology and genetic or developmental stage of plant organs should also be considered (Lamien-Meda et al. 2010).

Studies related to the production of EO with seasonality have not been conducted for *Nectandra megapotamica* growing in RS until now. Additionaly, knowing the ideal time to collect plant material with the aim of optimizing the production of active substances is essential in the management of natural populations of aromatic plants. Therefore, the aim of this study was to evaluate the performance of the production of EO and content of their major constituents, common to all the samples in the leaves of two stages of development from different individuals in a population over the seasons in a year.

MATERIALS AND METHODS

Extraction and characterization of the essential oil

Leaves were collected from nine adult trees of *Nectandra megapotamica*. Three of these trees were resampled at different seasons, i.e., three trees were sampled per season, resulting in 12 observations (Tables 2 and 3). This procedure was used in order to analyze the seasonal influence on the yield and major chemical compositions of the EO. The collections were conducted in Seasonal Deciduous Forest (IBGE 2012) at the base of *Morro do Elefante*, Santa Maria, RS, Brazil. The trees were located with GPS navigation, between 29º 40' 43" and 29º 40' 51" S latitude, and 53º 43' 08" and 53º 43' 16" W longitude, with altitude of 114-172 m. The climate in this region is classified as *Cfa* subtropical humid (EMBRAPA 2011), with an annual temperature average between 17.9 and 19.2°C, containing rainfall evenly distributed throughout the year, reaching 1691 mm. Soils of the class Regolithic Neosol eutrophic leptic, Charrua unit predominate onsite (Streck et al. 2008). The species was identified by Prof. Dr. Solon Jonas Longhi and a voucher specimen (SMDB in 13107) was deposited in the official Herbarium of University Federal of Santa Maria (UFSM). The collections of the leaves were individual and in triplicate for each season, held between 9:00 and 11:00 a.m. from November 2010 to September 2011. The leaves were classified into young (YL) and old (OL) according to their position and insertion on the branches, separation in nodes and internodes, color, size, texture, and particle deposition on the adaxial surface. Leaves presenting necrosis, yellowing, insect attack, signs of nutritional deficiency, among others were discarded. Further field observations were made on the phenology of the trees sampled.

Essential oils were obtained by hydrodistillation in triplicate for 3 h (European Pharmacopoeia 2007) using Clevenger type apparatus, a heater blanket and glass balloons of 5000 mL containing 500 g of fractionated fresh leaves and 3000 mL of distilled water. For each extracted sample, the moisture content of the leaves was obtained by drying the samples of plant material in an oven at 50°C until the stabilization of the wheigt, determined on analytical balance. The volume of EO was determined in graduated cylinder (0.1 mL) and the mass was obtained on analytical balance aiming to calculate the dry basis yield (% w/w). Essential oils were stored in amber glass bottles at - 4°C in the absence of light. The chemical composition was determined by the gas chromatography coupled with mass spectrometry (GC/MS) on Agilent 6890N gas chromatograph with a 5973 mass detector. Prior to injection, samples of EO were diluted in hexane pesticide grade (1:1000). The split used was 1:100, and the ionization technique was electron impact at 70 eV. The analysis parameters were: silica capillary column HP-5MS (30 m x 0.25 mm x 0.25 μm), volume of injection 1.0 µL, helium as carrier gas, with flow of 1.3 ml/min; injector and detector temperatures, 250 and 320°C, respectively, analysis programm: 40 to 320°C at 4°C/min, and delay of four minutes. The identification of the constituents was performed by comparison of the linear retention indices with literature data, and the fragmentation pattern of the mass spectra with the system database (Adams 2001; NIST 2005). For quantitative analysis, the percentage composition of the samples was calculated by area integration of the peaks in the chromatograms.

Statistical analysis

Yields were evaluated by descriptive statistics, performed in a spreadsheet (Excel ®). Analysis of variance – ANOVA was carried out on the program Assistat version 7.6 Beta on a bifactorial design (4x2): Factor 1 - seasons (spring - Sp, summer - Su, autumn - Au and winter - Wi), and Factor 2 - stage of development of the leaves (YL and OL), totalizing eight treatments with nine replications. Preceding the ANOVA, a Bartlett test was performed to check the homogeneity of variances. Since this is a native untamed species and it was possible to compare the unknown genotypes, the selective accuracy (SA) was used as a measure of experimental precision (Resende and Duarte 2007). This statistical tool replaced the coefficient of variation (CV%) and was classified as very high, high, moderate and low for SA ≥ 0.90, ≥ 0.70 to <0.90, ≥ 0.50 and <0.70, and <0.50, respectively (Cargnelutti Filho and Storck 2009). For the values of F calculated ≥ 1, the SA was given by $\sqrt{1-(1/Fcalculated)}$. The major constituents common to all the samples and their respective classes were plotted for the visualization of their behavior in the seasons, using the general averages and trend curves. For these, the coefficient of determination (R^2) was calculated and when nonlinear trend was observed, the maximum technical efficiency was plotted (MTE), given by = $x^+ = -\hat{b}_1/(2\hat{b}_2)$ e $y^+ = \hat{b}_1 + 2\hat{b}_2 x^+$ (Storck et al. 2011).

RESULTS AND DISCUSSION

Analysis of the essential oil yields

The values of EO yields showed mean and median close to each other, but differed from the mode. This indicated the occurrence of asymmetric distributions for both the development stages of the leaves and seasons, classified as negative because the asymmetry values were less than zero (0), except for the yields of YL in Sp and OL in Au and Wi, which were symmetrical (Table 1).

Table 1 - Descriptive statistics for the yield on dry bases of the EO in leaves of *Nectandra megapotamica* collected in *Morro do Elefante*, Santa Maria, RS, Brazil.

Factors		Yield on dry basis of the essential oil											
		Vmax	Vmin	Mean	SE	Mode	Median	S^2	S	CV%	K	A	ML%
Sp	YL	0.75	0.49	0.59	0.03	Amodal	0.56	0.008	0.090	15.09	-0.56	0.73	60.39
Su	YL	0.45	0.29	0.38	0.02	Amodal	0.38	0.003	0.058	15.33	-1.52	-0.34	50.59
Au	YL	0.34	0.16	0.28	0.02	Amodal	0.29	0.003	0.055	19.77	2.15	-1.31	53.79
Wi	YL	0.40	0.25	0.35	0.02	Amodal	0.37	0.002	0.048	13.82	0.91	-1.17	53.32
Sp	OL	0.40	0.21	0.30	0.02	Amodal	0.30	0.005	0.071	23.57	-1.43	-0.15	51.68
Su	OL	0.34	0.22	0.28	0.01	Amodal	0.28	0.002	0.044	15.53	-1.62	-0.25	48.17
Au	OL	0.27	0.15	0.21	0.01	Amodal	0.20	0.002	0.040	18.82	-0.47	0.06	49.49
Wi	OL	0.32	0.25	0.28	0.01	Amodal	0.28	0.001	0.029	10.06	-1.78	0.09	50.56

Legend: Sp - spring; Su - summer; Au - autumn; Wi - winter; YL - young leaves; OL - old leaves; Vmax - maximum value; Vmin - minimum value; SE - standard error; S^2 - variance; S - standard deviation; CV - coefficient of variation; K - kurtosis; A - asymmetry; ML% - mean moisture content of fresh leaves.

The flattening of the distributions resulted in platykurtic whose distributions were either flat or opened since their Kurtosis values were negative. The exception was for YL in Au and Wi, which presented leptokurtic whose distributions were acute or closed. This indicated some influence throughout the seasons in the synthesis of the EO constituents in the leaves, which were detected by the ANOVA.

The yield on dry basis of the EO of YL and OL showed small and homogeneous variances by Bartlett's Test - $\chi 2$ calculated (13.13) < $\chi 2$ tabulated (14.07, p > 0.05; GL 71). The ANOVA showed a highly significant F test for season (F = 39.30 **, p <0.001; GL = 3; GL Residue = 64; critical F = 4.10), stage of leaf development (F = 91.10 **, p <0.001; GL = 1; GL residue = 64; critical F = 7.05), and also for the interaction between both the factors (F = 17.77 ** p <0.001; GL = 3; GL residue = 64; critical F = 4.10). The experiment showed general mean of 0.33% (CV 16.98%), which corresponded to 0.17% yield on wet basis with medium moisture content in fresh leaves of 52.25%. The average yield (Tukey test p 0.05) was statistically different for the seasons, being 0.45 (a), 0.33 (b), 0.25 (c) and 0.32% (b); with less significant difference (LSD) of 0.05 for Sp, Su, Au and Wi, respectively, as well as for the stages of the leaves, with 0.40 (a) for YL and 0.27% (b) for OL – with LSD of 0.03.

Differences were also observed for the interactions between both the factors, with averages of 0.60 (aA), 0.38 (bA), 0.28 (cA) and 0.35% (bcA) for the interaction of YL with Sp, Su, Au and Wi, and 0.30 (aB), 0.28 (abB), 0.21 (bB) and 0.29% (aB) for the interaction of OL and seasons described above in the same order – being LSD of 0.07 to the stage of leaf development (capital letters), and 0.05 for the seasons (lowercase). The selective accuracy (SA) obtained was 0.99 for the factors 1 and 2, and 0.97 for the interaction between them, being classified as very high, despite the CV% obtained be considered medium.

The average yield of EO on wet weight (0.17%) was similar than those observed to date for this species growing in São Paulo, 0.11 - 0.18% for the samples collected in February and August 2007, respectively (Romoff 2010). The dried yield obtained in this work (Table 1) was superior than that observed by Apel et al. (2006) for trees collected in Cananéia, SP (0.10%). These discrepancies could be attributed to different populations compared and/or to biotic and abiotic factors (Gobo-Neto and Lopes 2007). Considering the yield described by Tondolo et al. (2013) obtained in Sp (November 2010) from trees belonging to the same population of Santa Maria, RS, the average did not differ from the yield observed in this work for the same season (2010/2011). In this period different phenological events occurred for the evaluated specimens, which frequently caused variability in EO observed (Lamien-Meda et al. 2010). However, the avarage reported by Tondolo et al. (2013) was superior to the results obtained in the work for Su, Au and Wi.

Analysis of the major chemical composition of the essential oil

Eight major constituents, common to all the samples of EO of YL and OL, were observed in each season, representing an average of 83% of the total composition (Tables 2 and 3). Constituents that were not found in all the samples of EO, the unidentified compounds, or the trace constituents have not been presented here.

Table 2 - Major constituents common to all samples of EO of young leaves of *Nectandra megapotamica* collected in the four seasons of the year at *Morro do Elefante*, Santa Maria, RS, Brazil.

Constituents	LRI			Content (%)												M
				---- Spring ----			---- Summer ----			---- Autumn ----			---- Winter ----			
	-- Exp.* --		Lit.	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 3	Tr 6	Tr 7	Tr 8	Tr 9	Tr 1	Tr 4	
α-Pinene^M	929	935	932^N	33.0	44.2	22.5	20.9	31.1	33.0	34.7	18.6	11.1	14.5	20.5	17.4	25.1
Bicyclogerm.^{S}	1495	1501	1500^A	7.9	6.0	32.3	38.5	23.8	36.5	20.9	26.3	33.3	25.8	20.2	23.3	24.6
β-Pinene^M	971	975	974^N	18.3	22.1	13.0	14.6	19.6	12.1	22.3	13.4	5.9	7.3	10.0	13.8	14.4
Germ. D^S	1479	1484	1483^N	4.9	2.9	11.4	12.3	10.0	9.0	5.6	12.3	10.3	8.6	10.8	11.0	9.1
Limonene^M	1024	1028	1022^N	8.5	9.8	3.8	3.1	3.7	3.3	5.3	5.2	2.6	3.0	4.0	4.8	4.8
Caryophyllene^S	1417	1421	1420^N	2.1	0.7	1.6	1.3	2.4	1.0	2.0	3.4	2.1	1.8	5.2	3.0	2.2
β-Mircene^M	987	992	991^N	5.9	5.9	1.7	0.4	0.7	1.6	0.4	0.9	1.4	1.8	2.7	0.9	2.0
Eucalyptol^M	1025	1029	1029^N	2.7	1.9	0.5	0.3	0.6	0.3	0.1	0.7	0.3	0.4	0.7	0.5	0.8
Monoterpenes				68.4	83.9	41.5	39.3	55.7	50.3	62.8	38.8	21.3	27.0	37.9	37.4	47.0
Sesuiterpenes				14.9	9.6	45.3	52.1	36.2	46.5	28.5	42.0	45.7	36.2	36.2	37.3	35.9
Total				83.3	93.5	86.8	91.4	91.9	96.8	91.3	80.8	67.0	63.2	74.1	74.7	82.9

Legend: LRI – Linear Retention Index; Exp. – experimental data; * - LRI amplitude obtained for the samples; M - Average percentage of the constituents in all samples; Lit. - literature data; N - NIST (2005); A - Adams (2001); Tr – sampled Tree; Bicyclogerm. – bicyclogermacrene; Germ. D – germacrene D.

Table 3 - Major constituents, common to all samples of EO of old leaves of *Nectandra megapotamica* collected in the four seasons of the year at *Morro do Elefante*, Santa Maria, RS, Brazil.

Constituents	LRI			Content (%)												M
				---- Spring ----			---- Summer ----			---- Autumn ----			---- Winter ----			
	-- Exp.* --		Lit.	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 3	Tr 6	Tr 7	Tr 8	Tr 9	Tr 1	Tr 4	
α-Pinene^{M}	929	935	932^{N}	37.8	42.4	30.4	29.3	36.2	39.1	41.6	21.5	11.5	15.1	15.6	15.8	28.0
Bicyclogerm.^{S}	1495	1502	1500^{A}	5.2	7.4	41.3	21.4	23.9	31.2	15.8	24.5	26.0	24.9	20.8	25.1	22.3
β-Pinene^{M}	972	975	974^{N}	20.3	23.0	13.0	20.6	24.9	15.2	26.0	15.4	6.5	7.8	9.4	13.0	16.3
Germ. D^{S}	1479	1484	1483^{N}	2.6	2.9	5.1	7.6	5.7	5.8	3.3	8.8	11.4	7.2	10.9	11.3	6.9
Limonene^{M}	1024	1028	1022^{N}	9.2	10.0	2.4	4.8	3.8	3.8	5.3	5.7	2.9	3.1	4.4	4.6	5.0
Caryophyllene^{S}	1417	1421	1420^{N}	1.4	0.8	0.6	1.8	1.2	0.8	1.3	3.0	3.0	1.7	5.3	3.0	2.0
β-Mircene^{M}	988	992	988^{N}	6.1	5.4	0.7	0.8	0.5	1.8	0.5	1.0	1.5	1.8	2.7	0.8	2.0
Eucalyptol^{M}	1027	1029	1029^{N}	1.9	0.8	0.2	0.7	0.3	0.1	0.1	0.7	0.3	0.3	0.5	0.4	0.6
Monoterpenes				75.3	81.6	46.7	56.2	65.7	60.0	73.5	44.3	22.7	28.1	32.6	34.6	51.8
Sesquiterpenes				9.2	11.1	47.0	30.8	30.8	37.8	20.4	36.3	40.4	33.8	37.0	39.4	31.2
Total				84.5	92.7	93.7	87.0	96.5	97.8	93.9	80.6	63.1	61.9	69.6	74.0	83.0

Legend: LRI – Linear Retention Index; Exp. – experimental data; * - LRI amplitude obtained for the samples; M - Average percentage of the constituents in all samples; Lit. - literature data; N - NIST (2005); A - Adams (2001); Tr – sampled Tree; Bicyclogerm. – bicyclogermacrene; Germ. D – germacrene D.

A decrease in the percentage of monoterpenes, represented by α-pinene, β-pinene, and limonene was observed due to the change of the season from Sp toward Wi, while the opposite behavior was detected for the sesquiterpenes bicyclogermacrene and germacrene D in the EO of YL (Fig. 1A and 2A) and OL (Fig. 1B and 2B). The behavior of the monoterpenes was described by the linear equations, which presented high coefficients of determination (R^2) in most cases, showing the seasonal influence on the chemical composition of the EO of both YL and OL. Noteworthy was the fact that the behavior of the sesquiterpenes bicyclogermacrene and germacrene D was described by the polynomial equations, since they showed elevated percentages in Sp, reaching a peak (MTE) between Su and Au and decreased with the arrival of Wi.

The chemical composition of EO analyzed differed in relation to the works already performed with the EO of this species (Apel et al. 2006; Romoff et al. 2010). The proportion of different constituents could be related to the phenology and environmental conditions (attracting pollinators and seed dispersers, defense and protection against herbivores and microorganisms, among others).

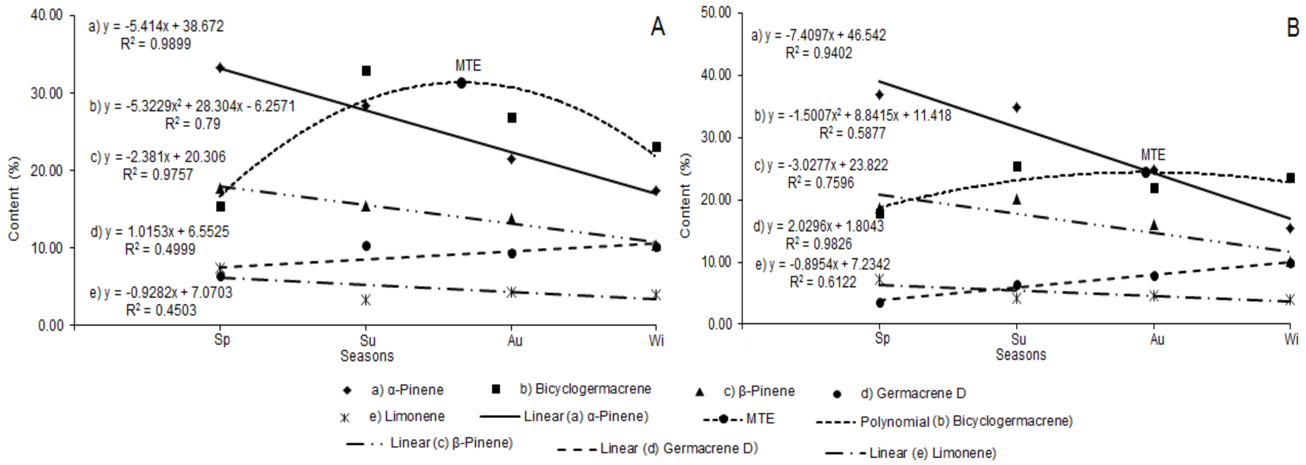

Figure 1 - Seasonal variability of the major constituents of the essential oils of *Nectandra megapotamica* in young leaves-YL (1.A) and old leaves-OL (1.B) collected in Santa Maria, RS, Brazil. Legend: Sp – spring; Su – summer; Au – autumn; Wi – winter; MET – maximum technical efficiency.

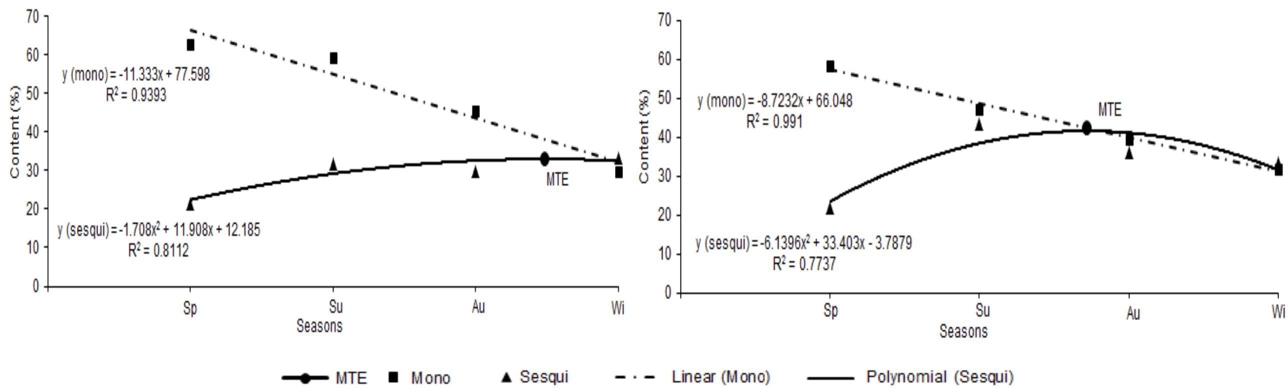

Figure 2 - Seasonal variability of the major constituents of the essential oils of *Nectandra megapotamica* grouped into classes: monoterpenes and sesquiterpenes in young leaves- YL (2.A) and old leaves- OL (2.B) collected in Santa Maria, RS, Brazil. Legend: Sp – spring; Su – summer; Au – autumn; Wi – winter; MTE – maximum technical efficiency; Mono – monoterpenes (except β-mircene and eucalyptol); Sesqui – sesquiterpenes (except Caryophyllene).

However, there were no significant differences in the chemical composition of EO in relation to seasonality. Yet, for the other genera of Lauraceae, several studies have analyzed the infuence of seasonality and/or phenological stage on income and/or chemical composition of the EO. According to Marchetti et al. (2006), the best yield of EO in the leaves of *Cryptocarya mandioccana* Meisner occurred in the reproductive phase compared to other phases of development. Li et al. (2013) observed large variations in the yield of EO of *Cinnamomum cassia* Presl., from 0.54 to 2.12%, in the leaves under different stages of development.

Leaves of rosewood, *Aniba roseodora* Duckei Kosterm, collected monthly in the Amazon Forest, Forest Reserve Ducke - INPA, Manaus-AM showed strong dependence on the yield of EO to the season, dry or wet, with lower yields in rainy season (Araujo et al. 2013). According to the authors, the water solubilized linalool, oxygenated monoterpenoid and major component, eliminating it from the EO producing cells due to rapid internal circulation of water in the tree. The other factor considered was the age of the leaves; the older the leaves, the lower the content of linalool presented. For leaves of *Aniba duckei*, also collected in the Reserve Ducke, Cunha (2011) observed higher content of linalool (76.69/62.40%) and yield of EO (1.84/1.59%) for two samples obtained in the dry season (April and September) when the change of leaves and fruiting of the species occur, in comparison to the rainy season (November to February) when flowering takes place (60.38/56.26% linalool and 1.40/1.21% of EO). Leite et al. (2001) also observed the occurrence of high linalool content in YL of this species, 73-78%, higher values than the OL. Chantraine et al. (2009) studied the EO of *A. roseodora* in French Guiana and correlated the variation in the yield of EO to the organ of the tree, age, season, phenological stage, and geographic location. Castellani et al. (2006) found seasonal variation in the production of EO of *Ocotea odorifera*, recommending the collection of leaves and twigs in the Sp because they had income of 0.86 and 0.83%, respectively.

The highest yield (1.13%) of EO of *Laurus nobilis* L. obtained from its dry above the ground collected in northwestern Iran was observed during the flowering, in September (Verdian-Rizi 2009). There was no significant variation in the chemical composition at different phenological stages (vegetative period, anthesis, peak flowering period and with the presence of seeds in plants), but the monoterpenes predominated at all stages evaluated, in the order of 70%. Therefore, the results of this work along with the data described in the literature indicated that the species of Lauraceae could show seasonal variation in yield and chemical composition of EO as well as suffer influence of the age of the tree or plant organ, affecting the content of different volatile constituents synthesized.

However, literature also described the occurrence of physiological varieties in the species of Laureaceae, such as in canela-sassafrás (*Ocotea* *odorifera* (Vell.) Rowher), which in the cold weather of the Itajaí Valley in Santa Catarina, produced safrole as the major constituent and in the tropical climate in Rio de Janeiro produced methyleugenol and nitrophenylethane (Gotllieb 1999).

Seasonal and phenological influence

The phenological events for *N. megapotamica* are foliation, flowering, fruiting, rustification, and rest. Foliation, flowering and fruiting occur in the Sp. The buds emerge between late Au and early Wi, while in early Sp, the opening of flowers and leaf senescence take place with the formation of fruits between the end of this season and early Su (Souza and Moscheta 2000; Alberti and Morellato 2010; Vogel et al. 2012). The fruits ripen in Su (Krügel et al. 2006), while the YL grow. In the Au, the rustification of these leaves occurs to withstand the rigors of Wi, where there is vegetative rest with differentiation of axillary buds in flower buds at the end of this season.

At the times of collection, it was observed that the events did not occur alone, and no control of the season on the phenology occurred, i.e., a given event might start at a season and extended over others, although the stimulus for the induction of the same was related to the changes in climate. There is a demand for energy obtained from the metabolism, which is stimulated by the light that overcomes the forest canopy, favored by the deciduousness of some species in the Sp. Senescence of the leaves of *N. megapotamica* in Seasonal Deciduous Forest takes place between August and December (Vogel et al. 2012). This could explain the difference in the yields of EO between YL and OL at this season, where there was probably a reallocation of resources from OL to other organs of the tree. In the Au, another factor that could have contributed to the lower yield observed was the change in the characteristics of light and solar radiation, compared to Sp and Su. According to Li et al. (1996), luminosity lower rates are associated with the reduction in the content of EO and may also influence their chemical composition. Zanon et al. (2013) analyzed the photoperiod (number of hours with natural light throughout the day) for Santa Maria-RS from August 25th, 2010 to June 16th, 2011, period that includes Sp, Su, Au and Wi. According to the authors, the photoperiod was longer in Sp (12.1-14.9 h light per day, mean 13.2 h day^{-1}) when compared to Wi (13.7-11.1 h day^{-1}, mean 12.5 h day^{-1}), which is the season where the highest and lowest mean incomes for the OE of *N. megapotamica* were observed.

In plants, terpenes act as phytoalexins, insect repellents, pheromones, plant hormones, signaling molecules, allelochemicals, agents of pollinic attraction, and defense against herbivores (Harbone 1993; Burt 2004). Between the two major classes of constituents detected in the EO analyzed, monoterpenes were smaller molecules of lower density and higher volatility, which facilitated their diffusion (Bandoni and Czepak 2008). These properties explain their role in attracting the pollinators (Oliveira et al. 2003). However, along with the sesquiterpenoids, they also help to protect the plant in direct defense against the herbivores and indirectly in attracting the predators or parasitoids (Keeling and Bohlmann 2006; Mumm and Hilker 2006; Xiao et al. 2012). Hoskovec et al. (2005) demonstrated the high volatility of α-pinene compared to other compounds, which resulted in a high diffusion capacity in the environment. However, sesquiterpenes are larger, denser and less volatile molecules than the monoterpenes, which often have protective functions, such as the antimicrobial action of bicyclogermacrene (Cysne et al. 2005), and the fungitoxicity of germacrene D (Fach et al. 2002), among others. However, these classes of terpenic compounds may have structural and functional similarities because they have the same biosynthetic origin (mevalonate pathway in chloroplasts and via the deoxy-xylulose-5-phosphate in the cytoplasm) and are formed through the action of enzymes called terpene synthases (Taiz and Zeiger 2004; Degenhardt et al. 2009). The synthesis of monoterpenes requires fewer precursors as well as less enzymatic activity and energy in relation to sesquiterpenes. Thus, plants regulate the synthesis of terpenes in terms of their development and environment, where the space occupation during the growth may be related to greater metabolic efficiency and the need for defense and protection. These needs are initially performed by monoterpenes of higher volatility, which are widespread in the airspace surrounding the plant (Baby et al. 2010).

The relocation of resources from other plant organs for the structures on growing (YL and fruits) and/or with reproductive functions (inflorescences, flowers and seeds) is a common event during vegetative development of the plants, which occurs in Sp and Su for *N. megapotamica*.

Thus, it would be reasonable to use the energy available to synthesize, for example in the leaves, monoterpenes, which require lower energy for their synthesis, and can also perform similar functions to the sesquiterpenes, such as protection and defense that would require greater energetic cost. This might have occurred in OL, which produced less sesquiterpenes and more monoterpenes compared to YL.

The switch between α-pinene and bicyclogermacrene as major compounds in the sampled trees seems not to be of genetic nature because the three individuals recollected in different seasons showed similar variation to that observed in the EO for the other individuals sampled, indicating a physiological variability. However, the analysis of genetic variability of the sampled trees was not performed. Thus *N. megapotamica* could be classified in the group of aromatic species that presented large genetic variability and physiological activities. These individulas may be responding differently to the prevailing environmental conditions, with qualitative and quantitative alterations in secondary metabolites, which hinders to obtain a standardized composition (Passinho-Soares et al. 2006). A similar event occurs in the population of *C. mandiocanna* in the Atlantic Forest, which in the southern coastal region of São Paulo shows predominance of germacrene D and bicyclogermacrene in its EO. However, there is a predominance of the synthesis of germacrene D also in the northern growing population. According to Telascrea et al. (2007), this is due to the pressure of environmental and/or ecological selection, characterizing a chemical adjustment to the prevailing environmental conditions.

CONCLUSION

Young leaves produced more essential oil (0.40%) compared to OL (0.27%). Both were more productive in Sp (0.59 and 0.30%), recommended season to collect plant material. Monoterpenes were most abundant in Sp and Su (56.87%) during the growth and reproduction, whereas sesquiterpenes showed higher concentrations in Su, Au and Wi (34.66%), i.e., during the growth of YL, rustification and rest. The largest discrepancies between mono and sesquiterpenes were observed in OL. The observed variability could possibly be related to the adaptation of individuals to environmental conditions as well as to the needs of the plant.

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