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(Organizador)

CARACTERÍSTICAS DOS SOLOS E SUA INTERAÇÃO COM AS PLANTAS

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APRESENTAÇÃO

A obra “Características dos solos e sua interação com as plantas” aborda uma apresentação de 18 capítulos, no qual os autores tratam as mais recentes e inovadoras pesquisas voltadas para a área da Ciência do Solo.

O envolvimento das plantas com o solo requer conhecimento técnico de alto nível, pois a interação Solo – Planta – Ambiente é sem dúvida um universo complexo de informações e resultados que são influenciados por vários agentes externos e internos e que respondem no potencial produtivo de uma cultura. Entretanto, essa interação exige modelagem de dados que muitas vezes são inacabáveis, fazendo assim estimativas conforme os parâmetros estudados.

Porém, com a pesquisa voltada cada vez mais para o estudo do ambiente como um complexo sistema de produção, torna-se favorável para conhecer mais sobre os processos químicos, físicos e biológicos envolvidos no solo e na planta.

Assim, o conhecimento da relação Solo - Planta é fundamental para o entendimento desse sistema de produção, no qual a sua interação com as diversas características define seu potencial.

Por fim, espero que esta obra atenda a demanda por conhecimento técnico de qualidade e que novas pesquisas surjam neste contexto.

Leonardo Tullio

SUMÁRIO

CAPÍTULO 9

NUTRITIONAL AND PHENOLOGICAL INFLUENCE IN ESSENTIAL OILS OF *Eugenia dysenterica* ("CAGAITEIRA")

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RESUMO: O padrão de variabilidade química dos óleos essenciais das folhas de *E. dysenterica* cultivadas foi obtido por análises quimiométricas. Trocas na fenologia das amostras, previamente definidas a partir de três Unidades Químicas Operacionais (UCOs), combinadas com a precipitação e conteúdo de Mn^{2+} foliar, como variáveis ambientais, indicaram que 49,9% da variação total dos óleos podem ser explicados por esses preditores. O particionamento da variação mostrou que 46,5% da variação total foram explicadas conjuntamente pela origem das amostras (UCO) e variáveis ambientais. A maior contribuição pura foi atribuída aos preditores ambientais (20,9%), seguida da UCO (15,8%). Embora as trocas na fenologia das amostras contribuíram com um pequeno percentual (3,4%), ela foi significativa. A maior contribuição total para a variação nos óleos foi observada para o preditor ambiental (27,9%), seguida da UCO (18,2%), enquanto que o preditor fenológico total explicou 10,8% da variabilidade dos óleos essenciais. Os resultados forneceram uma plataforma para o qual os órgãos governamentais possam compreender a variabilidade química em espécies vegetais em relação aos estádios de desenvolvimento da planta e às influências ambientais.

PALAVRAS-CHAVE: cagaiteira, óleos essenciais, variabilidade química, fenologia, influência ambiental

ABSTRACT: Variability patterns of leaf essential oils from cultivated *E. dysenterica* were performed using chemometric analyses. Phenological changes in samples from three previously defined Operational Chemical Units

(OCUs), combined with rainfall and foliar Mn^{2+} contents as environmental variables, indicated that 49.9% of total variation in oil data should be explained by these predictors. Variation partitioning showed that 46.5% of oil variability can be jointly explained by OCU origin and environmental variables. The largest pure contribution was due to environmental predictors (20.9%), followed by OCU origin (15.8%). Even though samples' phenological stage offered a small pure contribution (3.4%), it was significant. The highest total contribution to the oil variations was observed for environmental predictor (27.9%), followed by UCO origin (18.2%), while total phenological predictor explained 10.8% of the essential oil variability. Results provide a platform from which to further pursue the understanding of chemical variability in plant species along to phenological changes and in relation to environmental influences.
KEYWORDS: cagaiteira, essential oil, chemical variability, phenology, environmental influence.

1 I INTRODUCTION

The biosynthesis of essential oils involves genetic control, even though the influence of environmental factors has been demonstrated for a variety of species (BASER; BUCHBAUER, 2010). This chemical plasticity often occurs under biotic or abiotic stress and plays an important role in an individual's adaptation to the environment, allowing the formation of new communities (BRUNETTI et al., 2013). The adaptive characteristics of essential oils may thus be useful as indicators of changes that affect the structure of the plant population under chemical, genetic, and ecological aspects. The factors responsible for the chemical structure of plant populations, in addition to the way in which the plant adapts to a local level, may lead to new strategies to government programs for the conservation of native species.

Cagaiteira (*E. dysenterica* DC.; Myrtaceae) is a deciduous perennial plant found in the various phytophysiognomies of central Brazilian Cerrado. Its populations have been severely depleted and fragmented due to overcollecting and habitat destruction caused by the expansion of the agricultural frontier. Habitat fragmentation and spatial isolation increase populations' genetic drift and differentiation among them, as well as reduce their future adaptation to environmental changes (SANO et al., 2010). Therefore, knowledge of genetic diversity and structure among *E. dysenterica* populations is required for the development of appropriate conservation and breeding programs.

According to a recent finding (VILELA et al., 2013), the structure of *E. dysenterica* populations based on oil chemical variability agrees with the pattern of genetic variability among populations, based on morphological descriptors (TELLES et al., 2003), isozymes (TRINDADE; CHAVES, 2005), and genetic markers (BARBOSA et al., 2015). On the other hand, there is a clear environmental influence on phenotypic differentiation, given the morphological and chemical characteristics of regions where these populations are found (DUARTE et al., 2012; TRINDADE; CHAVES, 2005).

Results from these studies have shown that populations located closer than 120 km from each other have a high similarity based on these genetic and chemical descriptors. Populations located below this range can be considered chemically and genetically homogeneous and therefore be defined as an operational chemical unit (OCU) for purposes of conservation and management (TRINDADE; CHAVES, 2005; VILELA et al., 2013).

Regarding the chemovariability in leaf oils, southeast wild populations in Goiás State, have been classified into three OCUs (Fig. 1): C1, populations from Catalão (**1**), Três Ranchos (**2**), and Luziânia (**3**); C2, Campo Alegre de Goiás (**4**) and Cristalina (**5**); and C3, Goiânia (**6**) and Senador Canedo (**7**) (VILELA et al.,2013). However, so far the influence of phenological changes in the plant as well as of environmental factors on the chemical variability of essential oils in *E. dysenterica* OCUs has not been described.

Chemovariations in essential oils can be used as an additional tool in establishing conservation areas and meeting the representation of concepts and minimum viable population for the species' management and conservation strategies.

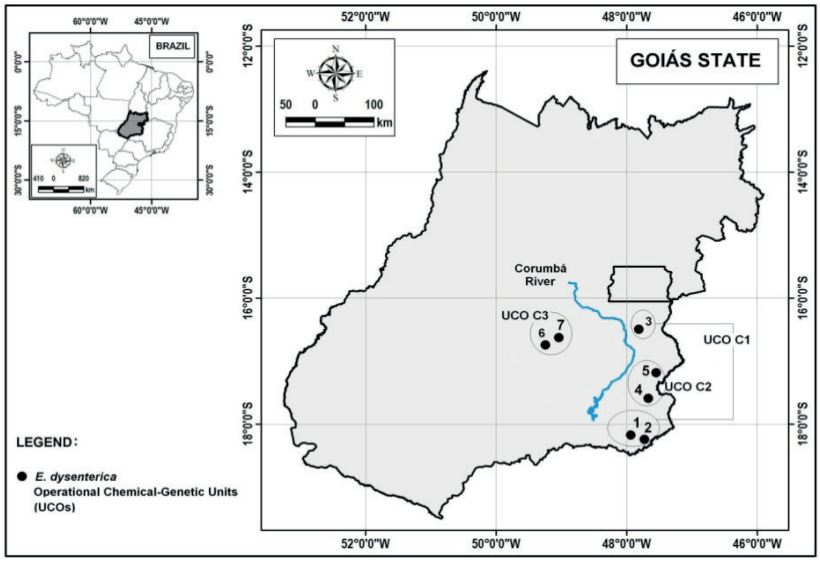

Figure 1. *E. dysenterica* operational chemical units (UCOs) of Goiás State, Brazil: C1: wild populations from **1** = Catalão, **2** = Três Ranchos and **3** = Luziânia; C2: **4** = Campo Alegre de Goiás and **5** = Cristalina; C3: **6** = Goiânia and **7** = Senador Canedo.

We now report on the chemical composition in leaf essential oils and their variability patterns from cultivated *E. dysenterica* germplasm collection representing the three OCUs over different phenological changes, to provide additional resources for conservation, management, domestication, and breeding programs concerning this native Cerrado species.

2 I MATERIAL AND METHODS

2.1 Study area

This study was carried out in a single experimental field (S 16°35¢39², W

49°17¢23², 716 m) belonging to the School of Agronomy of Universidade Federal de Goiás (SA/UFG), Goiânia, Goiás State, Brazil. The area consists exclusively of 19-year-old trees in the spacing 6.0 m ´ 6.0 m. The germplasm collection contains 440 plants from 110 progenies originated from ten *E. dysenterica* wild populations from southeast Goiás and incorporating the three OCUs. Plants were grown in the form of a randomized block design with four replications, and their local environmental effect was fully randomized.

In the area where the research was conducted, the average annual rainfall is considered to be very low, averaging 114 mm, ranging from 0.0 mm in the driest months (May to August) to 393 mm in the wet months (November to January). Climatic data were obtained from the meteorological station distant 2 km from the study area. The soil of the region is a dystrophic red-yellow latosol; are deep soils, well drained for most of the year, Al^{3+} toxicity and are poor in essential nutrients, such as Ca^{2+}, Mg^{2+} and K^{+} (2.1, 0.8 and 0.09 $cmol_c\ dm^{-3}$, respectively), and some micronutrients. The study area has never been fertilized or has received limestone.

2.2 Plant material

Leaves essential oils were obtained from cultivated plants belonging to three OCUs. Each OCU was considered as a homogeneous composite sample collected in three periods: March 2013, during wet season with plant's vegetative phenophase; July 2013, dry season and with plant's senescence; and October 2013, at the onset of the rainy season and the plant's fruiting stage. There were, hence, 36 composite samples (3 OCUs ´ 3 times ´ 4 replicates). *E. dysenterica* is a deciduous tree, so it loses its leaves in the flowering phase. Thus, samples in this stage were not included in data collection. A voucher specimen is deposited at the Herbarium (UFG40611).

2.3 Oil analyses

To assess essential oils, leaves were dried for 7 days at 30°C until constant weight. After being powdered, each dried phytomass (150 g) was submitted to hydrodistillation (3 h) using a Clevenger-type apparatus. At the end of each distillation oils were collected, dried with anhydrous Na_2SO_4, transferred to glass flasks, and kept at a temperature of -18°C until analysis. Oil yields (%) were based on the dried weight of plant samples.

A Varian CP3900 gas chromatograph (GC) with flame ionization detector (FID) was used for the compositional analysis of oils. Samples (0.4 ml in hexane 20% v/v) were injected in the split mode in a DB-5 (J&W Scientific) fused silica capillary column (30 m × 0.25 mm, 0.25 mm film thickness). Chromatographic conditions: injector and detector temperature were 220°C and 240°C, respectively; column temperature was programmed from 60°C to 240°C at 3°C min^{-1}; carrier gas: N_2 at a flow of 1 ml min^{-1}. Constituents' relative percentages were determined from GC peak areas without correction factors. Gas chromatography-mass spectrometry (GC/MS) were performed

with a Shimadzu QP505A with a flow rate of 1 ml min^{-1} (helium); column, injector, interface, and programmed heating temperatures were the same as above. Samples' injection with a 1:20 ratio, in EI mode at 70 eV, mass of 40-400 *m/z*, and speed of 1 scan s^{-1}. Oil constituents were identified by comparing their arithmetic indices (AI) and mass spectra with those of the literature (ADAMS, 2007). AI were calculated by linear hydrocarbon (C_8-C_{32}) co-injection (DOOL & KRATZ, 1963).

2.4 Leaf nutrients

Macro and micronutrients from each sample were determined by usual methods (MALAVOLTA; VITTI; OLIVEIRA, 1997). Concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+},Fe^{3+}, Mn^{2+}, Co^{2+} and Mo^{2+} were measured by flame atomic absorption spectrometry (AAS, Perkin Elmer), phosphorous was determined by spectrophotometry (DU-70 Spectrophotometer, Beckman), total nitrogen by micro-Kjeldahl digestion, and sulfur by gravimetric analysis.

2.5 Statistical analyses

Multivariate analysis was conducted by CANOCO software (TER BRAAK; ŠMILAUER, 2012). Oil constituents were ordered in a response matrix (36´30), with lines representing samples and oil constituents in columns as variables. Six climatic variables and 13 parameters of leaf nutrients were arranged in an explanatory data (environmental matrix; 36´21). Multichotomic variables representing factors on different levels were added to a better representation of the experimental design. These factors were the plant's phenological stages (senescence, fruiting, and vegetation), seasonal change (dry, transitional, and rainy season) and samples' original OCUs (C1-C3).

Redundancy analysis (RDA) was used to measure the association between response and explanatory matrices. Monte Carlo permutation test (999 permutations) was used to test the eigenvalue significance of the first canonical axis as well as the sum (trace) of all canonical axes. Variance inflation factor of variables (VIF) was used to the selection of explanatory variables, avoiding multicollinearity in multivariate regressions (TER BRAAK; ŠMILAUER, 2012).

Total variation partitioning of response data was obtained through partial RDAs (pRDAs) using the explanatory data reordered into three sets: OCU origin, phenological stages and environmental data (foliar nutrients and climate variables). Significant terms in each set were selected by forward selection procedure with VIF acting to decrease error type I. Variation partitioning yielded fractions of response data variation, which represent variation within and across sets of explanatory variables, as well as an unexplained variation by predictor data sets. Prior to the multivariate analysis, the response matrix was transformed to log(x+1), mean-centered, and standardized. Multiple comparisons were established by ANOVA with Tukey's *post-hoc* test using SAS GLM procedure. *p*-Values below 0.05 were regarded as significant.

3 I RESULTS AND DISCUSSION

The largest variations for minimum and maximum temperatures occurred in July 2012 (11.8°C, senescence) and October 2012 (32.0°C, fruiting), respectively. In turn, the highest average relative humidity and total rainfall were observed during the vegetative phenophase (62.0% and 228.4 mm). During senescence there was no rainfall, which is typical of July in central Brazilian Cerrado. For species with big-bang flowering strategies such as *E. dysenterica*, whose synchronization is precise and the flowering period is short, the onset of flowering is environmentally cued by changes in humidity over the dry/rainy season transition (PROENÇA; GIBBS, 1994).

As regards foliar data collection, foliar nutrients did not reveal any interaction effects between OCU origin and phenological changes. Nutrients such as Ca^{2+}, Mg^{2+}, S, Fe^{3+}, Co^{2+}, Mo^{2+}, and Na^{+} failed to share significant differences according to OCU origin or phenological changes, respectively (results did not show). The main foliar nutrient features with the highest contents were N and K^{+} during the fruiting stage and P, K^{+}, Zn^{2+} and Mn^{2+} in samples from OCU C3. Senescence samples showed the highest values of Cu^{2+} and Mn^{2+}, whereas the vegetative phenophase was characterized by the highest Co^{2+} contents. The fruiting phase also showed the lowest values of Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+} and Mo^{2+}.

There has been little research on the effect of foliar nutrients on timing of phenological events (JOCHNER et al., 2013), in comparison to the effects of soil fertilization on phenology, with most predominantly related to agriculture and horticulture (YANG; ZHANG; LI, 2011), and less frequently to forest science (COLGAN et al., 2015). In Cerrado, seasonal variation of nutrients is related to uptake and retranslocation, which is important for the conservation of elements in low nutrient availability (VILELA; LACERDA, 1992). It has been reported that *E. dysenterica* accumulates a small amount of nutrients in its leaf and that variation in soil fertilization does not change mineral ratio in the leaves (BRITO et al., 2003). However, the influence of nutrient content such as P, K^{+}, Mg^{2+} and Cu^{2+} in this species' leaf oil has been reported (VILELA et al., 2013). Despite a small contribution (8%), it was significant in explaining leaf oil variability.

Similarly to other studies (MOGHADDAM et al., 2014), oil yields show differences in relation to seasonal progress and sample origin (OCU). The highest oil yields (0.43%) were obtained from Goiânia and Senador Canedo (C3), during the dried (senescence) to rainy (vegetative) transition. In total, 30 constituents were identified, accounting for 85-98% of volatiles (Table 2).

Constituent	RI[b]	Phenological stage	Operational chemical unit[c]		
			C1	C2	C3
a-Pinene[d]	930	Senescence	3.05 ^{Aa}	2.83 ^{Aa}	4.16 ^{Aa}
		Fruiting	1.00 ^{Ba}	1.02 ^{Ba}	0.73 ^{Ba}
		Vegetative	1.81 ^{ABa}	1.86 ^{ABa}	2.36 ^{ABa}
β-Pinene[d]	973	Senescence	1.29 ^{Aab}	0.95 ^{Ab}	3.27 ^{Aa}
		Fruiting	0.65 ^{Bab}	0.32 ^{Bb}	0.61 ^{Ba}

		Vegetative	1.46 ABab	1.18 ABb	2.61 ABa
Myrcene[d]	986	Senescence	0.49 Ab	0.25 Ab	2.09 Aa
		Fruiting	0.25 Bb	0.08 Bb	0.41 Ba
		Vegetative	0.52 ABb	0.26 ABb	1.62 ABa
Limonene[d]	1025	Senescence	1.33 Aa	1.69 Aa	4.07 Aa
		Fruiting	1.36 Aa	0.71 Aa	0.90 Aa
		Vegetative	2.59 Aa	1.53 Aa	2.72 Aa
(*Z*)-β-Ocimene[d]	1033	Senescence	1.94 Aa	1.27 Aa	0.49 Aa
		Fruiting	0.92 Aa	0.75 Aa	0.45 Aa
		Vegetative	1.52 Aa	1.65 Aa	0.83 Aa
(*E*)-β-Ocimene[d]	1043	Senescence	0.61 Aa	0.30 Aa	0.25 Aa
		Fruiting	0.27 Aa	0.10 Aa	0.31 Aa
		Vegetative	0.67 Aa	0.27 Aa	0.64 Aa
Linalool	1097	Senescence	0.84 Ba	0.76 Ba	0.53 Ba
		Fruiting	0.69 Ba	0.80 Ba	0.55 Ba
		Vegetative	1.10 Aa	1.01 Aa	0.94 Aa
a-Terpineol	1188	Senescence	1.55 Ab	1.43 Aa	0.80 Ab
		Fruiting	0.18 Ba	1.00 Ba	0.10 Ba
		Vegetative	0.85 Ab	1.51 Aa	0.87 Ab
a-Copaene	1375	Senescence	3.49 Bb	4.82 Ba	1.63 Bc
		Fruiting	5.93 Ab	8.41 Aa	3.35 Ac
		Vegetative	6.33 Ab	9.75 Aa	2.66 Ac
(*E*)-Caryophyllene	1421	Senescence	12.25 Bab	14.65 Ba	13.67 Bb
		Fruiting	24.16 Aab	30.58 Aa	19.53 Ab
		Vegetative	29.12 Aab	34.10 Aa	22.36 Ab
a-Guaiene[d]	1437	Senescence	0.23 Aa	1.41 Aa	0.90 Aa
		Fruiting	0.40 Aa	1.47 Aa	0.46 Aa
		Vegetative	0.59 Aa	0.98 Aa	0.43 Aa
a-Humulene	1455	Senescence	13.57 Ba	7.31 Bb	13.83 Ba
		Fruiting	24.19 Aa	13.59 Ab	22.43 Aa
		Vegetative	21.57 ABa	11.12ABb	18.87 ABa
g-Muurolene	1475	Senescence	1.05 Aa	1.15 Aa	0.40 Ab
		Fruiting	0.90 Aa	1.18 Aa	0.54 Ab
		Vegetative	0.10 Aa	1.08 Aa	0.45 Ab
β-Selinene[d]	1485	Senescence	0.65 Ab	0.51 Ab	3.65 Aa
		Fruiting	0.90 Ab	0.45 Ab	2.37 Aa
		Vegetative	0.17 Ab	0.74 Ab	2.45 Aa
d-Selinene[d]	1490	Senescence	0.75 Aa	3.47 Aa	1.25 Aa
		Fruiting	2.76 Aa	3.55 Aa	1.99 Aa
		Vegetative	1.59 Aa	1.95 Aa	1.86 Aa
cis-β-Guaiene[d]	1494	Senescence	0.23 Ab	0.66 Ab	2.30 Aa
		Fruiting	1.19 Ab	0.78 Ab	2.33 Aa
		Vegetative	0.33 Ab	0.45 Ab	1.73 Aa
a-Muuroleno[d]	1498	Senescence	1.98 Aab	0.95 Ab	3.04 Aa
		Fruiting	3.70 Aab	1.57 Ab	4.68 Aa
		Vegetative	1.68 Aab	0.86 Ab	3.82 Aa
a-Bulnesene[d]	1505	Senescence	0.10 Aa	2.11 Aa	0.26 Aa
		Fruiting	3.63 Aa	3.22 Aa	1.19 Aa
		Vegetative	0.91 Aa	1.66 Aa	0.78 Aa

g-Cadinene	1515	Senescence	3.95 Ab	5.01 Ab	20.37 Aa
		Fruiting	2.74 ABb	2.11 ABb	15.58 ABa
		Vegetative	1.96 Bb	1.45 Bb	15.11 Ba
7-*epi*-a-Selinene[d]	1518	Senescence	0.97 Aa	1.79 Aa	0.71 Aa
		Fruiting	3.96 Aa	0.84 Aa	0.65 Aa
		Vegetative	0.41 Aa	0.63 Aa	0.75 Aa
d-Cadinene	1524	Senescence	14.03 Ab	18.93 Aa	4.40 Ac
		Fruiting	1070 Ab	19.99 Aa	6.86 Ac
		Vegetative	13.58 Ab	18.84 Aa	4.39 Ac
a-Calacorene[d]	1541	Senescence	1.28 Aa	1.06 Aa	0.41 Aa
		Fruiting	0.27 Aa	0.34 Aa	0.56 Aa
		Vegetative	0.21 Aa	0.20 Aa	0.56 Aa
Caryophyllene oxide[d]	1584	Senescence	11.05 Aa	10.05 Aa	4.98 Aa
		Fruiting	0.78 Ba	0.32 Ba	0.51 Ba
		Vegetative	1.12 Ba	1.02 Ba	0.79 Ba
Ledol[d]	1603	Senescence	1.17 Aa	0.46 Aab	0.10 Ab
		Fruiting	0.28 Ba	0.10 Bab	0.10 Bb
		Vegetative	0.10 Ba	0.10 Bab	0.10 Bb
Humulene epoxide II[d]	1610	Senescence	6.81 Aa	3.41 Ab	3.67 Aab
		Fruiting	0.91 Ba	0.23 Bb	0.32 Bab
		Vegetative	1.04 Ba	0.32 Bb	0.52 Bab
1,10-di-*epi*-Cubenol	1618	Senescence	1.43 Aa	1.81 Aa	0.35 Aa
		Fruiting	0.10 Ba	0.10 Ba	0.10 Ba
		Vegetative	0.23 Aa	0.19 Aa	0.10 Aa
Muurola-4,10(14)-dien-1β-ol[d]	1629	Senescence	5.14 Aa	4.08 Aa	0.83 Ab
		Fruiting	0.10 Ba	0.10 Ba	0.10 Ba
		Vegetative	0.40 Ba	0.38 Ba	0.10 Ba
allo-aromadendrene epoxide[d]	1634	Senescence	2.31 Aa	0.95 Aa	1.06 Aa
		Fruiting	0.10 Ba	0.10 Ba	0.10 Ba
		Vegetative	0.10 Ba	0.10 Ba	0.10 Ba
Selina-3,11-dien-6a-ol[d]	1637	Senescence	1.95 Aa	1.93 Aa	0.69 Aa
		Fruiting	0.10 Ba	0.10 Ba	0.10 Ba
		Vegetative	0.10 Ba	0.10 Ba	0.10 Ba
Selin-11-en-4a-ol[d]	1655	Senescence	0.86 Aa	0.66 Aa	0.40 Aa
		Fruiting	0.10 Ba	0.10 Ba	0.10 Ba
		Vegetative	0.10 Ba	0.10 Ba	0.10 Ba
Monoterpene hydrocarbons[d]		Senescence	8.40 Aa	7.28 Aa	14.31 Aa
		Fruiting	4.44 Aa	2.97 Aa	3.40 Aa
		Vegetative	8.57 Aa	6.75 Aa	10.78 Aa
Oxygenated monoterpenes[d]		Senescence	2.39 Aab	2.19 Aa	1.33 Ab
		Fruiting	0.87 Bab	1.80 Ba	0.65 Bb
		Vegetative	1.95 Aab	2.52 Aa	1.80 Ab
Sesquiterpene hydrocarbons[d]		Senescence	54.49 Ba	63.80 Ba	66.82 Ba
		Fruiting	85.43 Aa	88.05 Aa	82.51 Aa

Oxygenated sesquiterpenes[d]	Vegetative	79.43 Aa	83.79 Aa	76.21 Aa
	Senescence	30.72 Aa	23.34 Aab	12.09 Ab
	Fruiting	2.46 Ba	1.14 Bab	1.43 Bb
	Vegetative	3.18 Ba	2.04 Bab	1.91 Bb
Oil yield (%, wt/dry wt)	Senescence	0.19Bb	0.18 Bb	0.35 Ba
	Fruiting	0.24 Ab	0.25 Ab	0.47 Aa
	Vegetative	0.22 Ab	0.21 Ab	0.48 Aa

Table 1. Percentage[a] of essential oil constituents from cultivated *E. dysenterica* leaves according to operational chemical unit (OCU) and different phenological stages.

[a]Based on original data; [b]Retention Index; [c]C1: Catalão, Três Ranchos, and Luziânia; C2: Campo Alegre de Goiás and Cristalina; C3: Senador Canedo and Goiânia; [d] Rank-transformed in ANOVA. Averages followed by the same capital letter in the columns and by the same small letter in the rows did not share significant differences at 5% probability by Tukey's test.

Essential oils mainly reveal sesquiterpene hydrocarbon compositions (61.7-85.3%), results in accordance with those obtained by Duarte et al. (2012). ANOVA showed that interaction between OCU and sample phenophases only occurred in minor constituents, such as muurola-4,10(14)-dien-1β-ol, *allo*-aromadendrene epoxide and selina-3,11-dien-6a-ol. The highest amount occurred in senescence samples from OCU C1 (5.14%) and C2 (4.08%) for muurola-4,10(14)-dien-1β-ol. Differences were also found according to OCU origin and phenological stage but were devoid of any interaction. Thus, (*E*)-caryophyllene (24.75% and 28.52%),a-humulene (20.07% and 17.19%), and sesquiterpene hydrocarbons (85.33% and 79.81) revealed the highest amounts from samples in fruiting and vegetative phases, respectively, whereas g-cadinene (9.78%), and oxygenated sesquiterpenes (22.05%) predominate in senescence samples, all regardless of OCU origin.

On the other hand, quantitative differences were obtained solely from OCU origin regardless of the phenological stage, and with the highest percentage in samples from OCU C1 and C2, such as (*E*)-caryophyllene (C1: 21.84%; C2: 26.44%) and oxygenated sesquiterpenes (12.12%; 8.84%); OCU C1 and C3, as a-humulene (19.78%; 19.38%); only OCU C2, as d-cadinene (19.25%) or g-cadinene (17.03%) in OCU C3. Despite low to moderate percentages (0.23-10.1%), constituents as limonene, (*Z*) and (*E*)-β-ocimene, a-guaiene, d-selinene, a-bulnesene, 7-*epi*-a-selinene, a-calacorene, and monoterpene hydrocarbons did not reveal any significant differences between samples from different OCU and/or plant phenophases.

At the end of multivariate data modelling, five explanatory parameters were selected for the RDA, containing OCU (C2 and C3), phenological stage (fruiting), climate (average monthly rainfall), and leaf nutrient (Mn^{2+}) (Fig. 2).

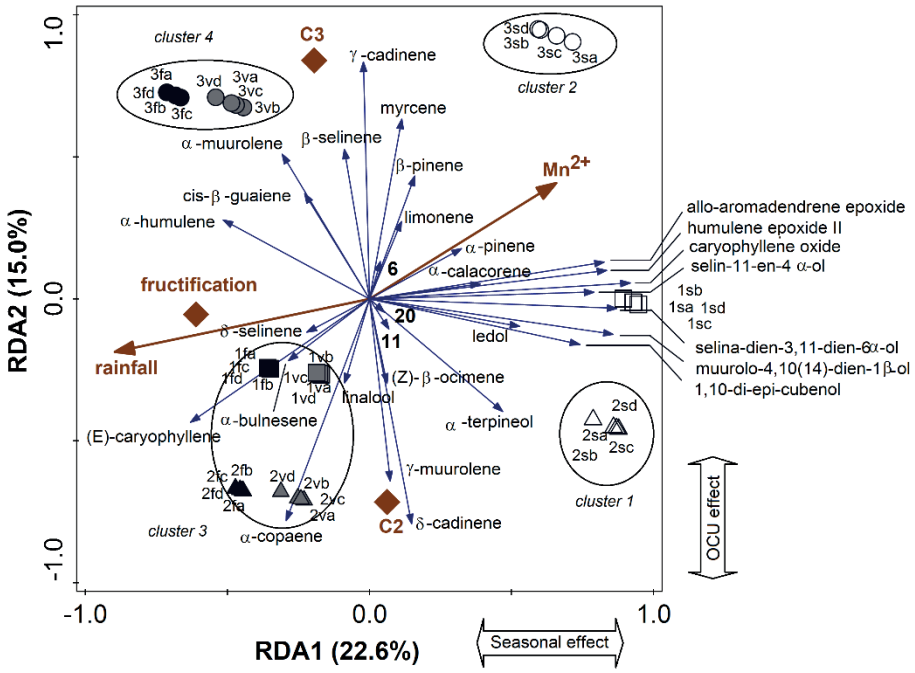

Figure 2. RDA triplot showing the distribution of *E. dysenterica* samples according to leaf essential oils explained by OCU origin (C2, C3), phenological stage (fruiting), foliar nutrients (Mn^{2+}), and climate (average monthly rainfall). The OCUs are represented by squares (C1), triangles (C2), and circles (C3), while samples in senescence, fruiting, and vegetative phenophases are represented by empty, gray-filled and black-filled symbols.

The RDA results indicated that correlations between the two data sets were higher in the first two canonical axes (0.918 and 0.898) with VIF-values low (< 5.9), suggesting no multicollinearity in models. Monte Carlo permutation test showed highly significant results for the axes (RDA1: 22.6% of explained variance, *F*-Fischer = 8.8, $p = 0.001$; RDA2: 15.0%, $F = 7.2$, $p = 0.001$), indicating that variation patterns in the original data do not arise by chance. The sum of the canonical axes was also highly significant (*trace* = 0.499; $F = 5.9$, $p = 0.001$), so that 49.9% of total variance in the essential oils was retained by selected explanatory variables. According to Fig. 2, an increase in the RDA1 axis is mainly associated with an increase in sesquiterpene oxidation in oils and foliar Mn^{2+} accumulation during senescence, whereas the average monthly rainfall decreases regardless of OCU origin. An increase in RDA1 showed that oxygenated sesquiterpenes, as caryophyllene oxide, strongly correlate with foliar Mn^{2+}. These conditions are related to samples from cold and dry winter coinciding with senescence, which suggests a seasonal influence on RDA1.

The positive and negative correlations of caryophyllene oxide and (*E*)-caryophyllene with foliar Mn^{2+}, respectively, are consistent with the effects of different dosages of Zn^{2+} and Mn^{2+} (EL-SAWI; MOHAMED, 2002) applied to cumin (*Cuminum cyminum* L). The effects of micronutrients in the biosynthesis of these terpenes exhibit the importance and need for a divalent metal as cofactor for enzyme synthases (PICAUD et al., 2005). It has been shown that Mn^{2+} promotes g-humulene formation, while other terpenes are reduced.

In *Mentha x piperita* L. the only by-product (d-cadinene) produced by (*E*)-b-

farnesene synthase in the presence of Mg^{2+} was completely absent in the presence of Mn^{2+} (CROCK; WILDUNG; CROTEAU, 1997), a result similar to the effect shown in Fig. 2, in which a decrease in d-cadinene is correlated with an increase in foliar Mn^{2+}. Also in relation to Fig. 2, an increase in RDA2 relates to samples of OCU C3, which showed the highest levels of monoterpenes and monoterpene hydrocarbons, regardless of sample phenology. Thus, RDA2 shows changes in the biosynthetic class of oils according to samples' OCUs.

In RDA, the pooled samples from clusters 1 and 3 originated from *E. dysenterica* sampling sites in Catalão, Três Ranchos, Luziânia, Campo Alegre de Goiás, and Cristalina (OCUs C2 and C3), whereas clusters 2 and 4 were only formed by samples from Goiânia and Senador Canedo (OCU C3). Such separation is geographically justified by the east (clusters 1 and 3) and west (clusters 2 and 4) of the Corumbá River basin (see Fig. 1), a finding which may foster consideration of different conservation units and permanence of at least one population from each region, based only on leaf oils. This geographical barrier could contribute at least partially to ecological isolation, a prerequisite for speciation between the two sites. These results are consistent with those based on morphological descriptors, isozymes (TELLES et al., 2003; TRINIDAD; CHAVES, 2005), and genetic markers (BARBOSA et al., 2015), in addition to the variability of leaf essential oils (VILELA et al., 2013).

Although RDA was able to quantify the total variance accounted by explanatory variables, she shed little light on the kind of relationship that exists between OCU origin, plant phenophases, and leaf nutrients in the sampled data. For this aim, variation partitioning was performed on response chemical data (PERES-NETO et al., 2006). In this work, the response matrix were conditioned to three sets of predictor variables: OCU, phenological stage of samples, as well as climate variables and foliar macro- and micronutrients grouped as environmental data set (Table 2).

Effects and variables	Covariables	Fraction of variation	Explained variation (%)	*F*	*P*[a]
Total effect					
OCU, phenological, environmental		[a-g]	49.9	6.0	0.001
Partial effects					
OCU	Phenological, environmental	[a]	15.8	4.7	0.001
OCU		[a+d+f+g]	18.2	3.7	0.001
Phenological	OCU, environmental	[b]	3.4	2.0	0.031
Phenological		[b+d+e+g]	10.8	4.1	0.002
Environmental	OCU, phenological	[c]	20.9	6.3	0.001
Environmental		[c+e+f+g]	27.9	6.4	0.001
Joint effects					
OCU, phenological		[d]	2.8		

Environmental, phenological	[e]	7.4
OCU, environmental	[f]	2.4
OCU, phenological, environmental	[g]	-2.8
Residuals	[h]	50.1

Table 2. Variance partitioning summary of leaf oil of *E. dysenterica* through partial RDAs.

[a]Based on the Monte Carlo permutation test (999 permutations). Predictors: OCU: C2 andC3; Phenological: fruiting phenophase; Environmental: average monthly rainfall and foliar Mn^{2+}.

Total variation (49.9%) in the oil data set ([a-g]) may be explained by predictor matrices, resulting in a model whose residue was 50.1% ([h]). The largest pure contribution was due to environmental set ([c] = 20.9%), followed by OCU ([a] = 15.8%). Despite offering a small pure contribution ([b] = 3.4%), samples' phenological stage was significant ($p < 0.031$). These results agree with the RDA analysis, whose explained variance in the first three canonical axes (RDA1: 22.6%; RDA2: 15.0%; RDA3: 7.3%) was attributed to environmental effect, OCU origin, and phenological influence in the samples. Moreover, the total contribution of OCU origin corresponds to 18.2% ([a+d+f+g]), whereas the phenology ([b+d+e+g]) explains 10.8% of total variability. The major contribution, in turn, was provided by the environmental predictor (27.9%; [c+e+f+g]). On the other hand, 46.5% of oil variability can be explained by OCU origin and environmental variables ([a+c+d+e+f+g]). The highest joint effect occurred between phenological and environmental sets, which explained 7.4% ([e]) of total variance in samples' oils. A negative contribution was obtained for the three sets of variables ([g] = -2.8%). This is due to the fact that joint effects are yielded by difference, not by parameters estimated by regression models.

Both the chemical composition of oils and the plant development, and thus phenological phases show great interannual variability. Individual (genes, age) and environmental factors (weather, diseases, competition etc.) can simultaneously contribute to these variations (BASER; BUCHBAUER, 2010). In contrast to many other studies (MOGHADDAM et al., 2014; PORRES-MARTÍNEZ et al., 2014), changes in the environmental variables during phenological stages were measured and a variation partitioning approach resulted in statistically significant pure and shared effects on the oil variations.

Variation partitioning has been used to assess the contribution of environmental factors and spatial variability associated with essential oils and phenolic content of wild and cultivated *Myrciaria cauliflora* (Mart.) O. Berg., Myrtaceae, jabuticabeira (DUARTE et al., 2012). Similarly to the present work, environmental factors also showed greater influence on phenolic contents in *M. cauliflora* leaves. Thus, chemovariations in the leaf oils of *E. dysenterica* could reflect specific phenological and environmental factors, its chemical composition being influenced by climatic parameters and foliar contents.

4 I CONCLUSIONS

The chemical variability of leaf essential oils from cultivated *E. dysenterica* is correlated with environmental factors such as rainfall and foliar nutrients, as well as phenological changes in the samples and the OCUs of original populations. Samples showed a higher chemical variability as defined by the Corumbá River basin, suggesting that at least two areas should be prioritized in government conservation and management programs of *E. dysenterica* in southeast Goiás. These results provide a platform from which we can further pursue our understanding of chemical variability in Cerrado plant species as regards ecological influences.

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