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Artigo

VOLATILE COMPONENTS FROM GALLS INDUCED BY *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) ON LEAVES OF *Baccharis dracunculifolia* (Asteraceae)

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The volatile components of the galls induced by the insect *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) on leaves of *Baccharis dracunculifolia* (Asteraceae) were analyzed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame-ionisation detection (GC-FID), and then comparison with volatile oil samples from healthy leaves collected in the vicinity. The galls produced around 3.5% of the total organic volatiles whereas healthy leaves rendered an average yield of 0.6%. The observed higher proportions of germacrene D, bicyclogermacrene, limonene, and β-pinene in the galls suggest that all these compounds are important targets in the search for natural enemies of this Psyllid. Moreover, higher relative percentages of (*E*)-nerolidol and spathulenol were found in healthy leaves.

Keywords: *Baccharis dracunculifolia*; *Baccharopelma dracunculifoliae*; volatiles.

INTRODUCTION

Galls are specialized modifications of plant tissue caused by various agents, such as microorganisms, nematodes, mites or insects that seek protection against predators or adverse weather conditions, being common the formation of a nutritive tissue in the lining of the larval chamber.[1-3]

The occurrence of many different galls on *Baccharis dracunculifolia* (Asteraceae), which are mostly caused by insects classified in the family Cecidomyiidae (Diptera), is well known.[1,4] Five psyllids of the genus *Baccharopelma* and linked to specific *Baccharis* species were described.[5] However, the gall-inducing insect *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) induces the most frequently gall found in this plant species. A comprehensive study on this gall regarding the stages of its morphogenesis in leaves of *B. dracunculifolia* has been published and even though the formation of nutritive tissue has not been observed, salivary sheaths left by the inducer can be taken as food for the subsequent development of the nymphs.[6] The remarkable transformation that the leaf undergoes immediately after the deposition of one to several tiny eggs in its sheath reveals a strong metabolic interference. Most probably, the egg gripper does not reach the main leaf vein, preventing great changes in the metabolism of other nearby plant parts and causing major localized reaction only at the attacked leaf. However, Lara and Fernandes (1994) observed a significant reduction in the number of healthy leaves and in the length of the stems supporting a *Baccharopelma* gall in *B. dracunculifolia*.[7]

The observed variability in the chemical composition of volatile substances obtained from plants is due to environmental or other factors that are characteristic of the species. Several authors have already studied the factors affecting volatile metabolites from species belonging to the *Baccharis* genus, either cultivated or growing in nature, such as light, rainfall, mineral content in the soil, interaction with insects and predators, location and altitude.[8-11] A very important factor that interferes with the chemical composition of volatiles extracted from *Baccharis* species is their known intense environmental relation with a great number of insects.[12] The volatiles from galls induced by a Psyllid (Hemiptera) on leaves of *Baccharis spicata* have been studied by Damasceno and collaborators (2010). The observed differences between the volatile composition of larval chambers and healthy leaves from *B. spicata* suggest changes in the biosynthetic pathways to secondary metabolites and can be regarded as a chemical defense against predators.[13]

Several authors have studied volatiles from leaves of *B. dracunculifolia* collected in different locations. Geographical and environmental factors have a strong influence on the composition of essential oils from *B. dracunculifolia* as shown by analyzing volatiles from plants collected indifferent places and altitudes.[10,11,14-16] On the other hand, the compositions of the volatiles from leaves of male and female specimens of five *Baccharis* species collected in the same place and at the same time, including *B. dracunculifolia* were compared, allowing to conclude for high degrees of similarity.[17] Compositions of volatiles obtained from male and female specimens of *B. trimera*, as well as from *B. milleflora*, collected using the same care presented also great similarities between their compositions, but showed that during blooming period the stage of morphological development can be strongly related to the secondary metabolic processes.[18,19]

We aim to present here, for the first time in the literature, GC-MS and GC-FID analyses of the volatiles obtained from galls produced by the galling Psyllid *Baccharopelma dracunculifoliae* on leaves of *Baccharis dracunculifolia*. A comparison between the volatile content of galls and healthy leaves collected in the vicinity is presented and highlighted using unsupervised statistical techniques, namely principal component analysis and hierarchical cluster analysis.

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EXPERIMENTAL

Collection of plant material

The galls induced by *Baccharopelma dracunculifoliae* (HEMIPTERAE, Psyllidae) were collected in triplicate on March 31st 2012 in a private land localized in the Road PR 513, Ponta Grossa, Paraná, Brazil, at the geographical position 25°08'30" S and 49°58'51" W and altitude of 1,061 meters, indistinctly from male and female specimens of *Baccharis dracunculifolia*. The vouchers (conserved in ethanol) were deposited in the herbarium of the Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil, under the number 22814 HUPC after the identification conducted by Dr. Geraldo Wilson Fernandes at the Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. By opening fifteen galls, three of them had no nymph, three galls contained one nymph, six presented two nymphs, and three galls contained three nymphs per chamber ($x \pm s = 1.60 \pm 2.12$), and the occurrence of abundant white wax was observed in galls containing one to three nymphs.

The healthy leaves from *Baccharis dracunculifolia* DC. plants were collected on private lands along the PR513 road, Ponta Grossa, Paraná, Brazil (980 to 1,061 m of altitude), in places up to 2 km surrounding the galls collection point. The leaves were collected in January 20th (female) and 23th (male), March 20th (male and female), May 24th (female), May 23th (male) and out of the flowering period in August 20th and October 23th. Only in March the healthy leaves samples were collected at the same place and time. Representative vouchers of plant specimens have been deposited at Museu Botânico Municipal de Curitiba, Paraná, Brazil, under the numbers 476334 and 476335.

Extraction of the volatiles

Each replicate of approximately 13 grams of fresh galls was ground in a blender with 500 mL of water and hydrodistilled for 2.5 hours in a 1 liter flask attached to a glass apparatus constructed according to the design and measures recommended by Stahl & Schild (1981).[20] The volatiles were taken in ethyl ether, decanting the remains of water and evaporating the solvent at room temperature. The yielding of each essential oil was calculated with respect to the mass of fresh galls used.

Healthy leaves dried at room temperature for 2 days after collection were kept in a closed amber container, protected from light, at -18 ºC till the day of the extraction. The volatiles were obtained by hydrodistillation in the glass apparatus described above, using between 80 and 100 g of each plant material and between 0.8 and 1.0 L of distilled water, for 2.5 hours and the samples were separated as described above.

Volatiles analyses

The volatiles from healthy leaves were analyzed by a Varian® CP-3800 Gas Chromatograph using the software Saturn® GC-MS Work station 5.51, operating in EI mode at 70 eV, with a mass scan range of 40-650 m/z at a sample rate of 1.0 scans^{-1}. The analyses were carried out using a capillary column CP-Sil-8 CB LowBleed/MS 30 m long with a diameter of 0.25 mm and a film of 0.25 µm. The temperature of the injector was kept at 250 °C and the temperature of the interface was 240 ºC. A flow rate of 1 mL min^{-1} was adopted and helium was the carrier gas. The injection volume was 1.0 µL of sample solution (diluted in ethyl ether). The temperature was programmed as follows: 50 °C in the first 1 minute, going up 3 °C min^{-1} up to 240 °C; split ratio of 1/50. In order to quantify the chemical compounds in each oil sample, a Shimadzu Gas Chromatograph 14B coupled with a flame ionization detector (GC-FID) and an OV-5 column (30 m x 0.25 mm d.i. x 0.25 µm) was used. Nitrogen was the gas carrier, with a constant pressure of 80 kPa, a split ratio of 1/150 and injection volume of 1 µL of oil (diluted in ethyl ether). The temperature of the detector and the injector was kept at 300 and 250 °C, respectively. The initial temperature in the column was 50 °C (3 min), with a heating rate of 5 °C min^{-1} until the temperature reached 270 °C, with an isotherm of 8 min. Results are presented (Table 1) in terms of the relative composition of each sample from healthy leaves.

The volatile components from galls were qualitatively analyzed by a Shimadzu GC-17A chromatograph coupled to a mass spectrometer QP5050A, equipped with a DB5-MS capillary column (30 m x 0.25 mm; film thickness of 0.25 µm); injector temperature, 250 °C; interface temperature, 280 ºC; oven heating program: 60 °C (3 min), then 6 °C min^{-1} till 240 °C (5 min); helium as carrier gas, 1.2 mL min^{-1}; split mode injection, ratio 1:50; injections of 1.0 µL, samples dissolved in *n*-heptane:petrol ether 1:1; spectrometer operating in scan mode, 40-550 u.m.a., electron impact of 70 eV. For quantification of components, a Shimadzu GC-17A Gas Chromatograph attached to a flame ionization detector was used with a RTX-5MS capillary column (30 m x 0.25 mm; film thickness of 0.25 µm); injector temperature, 250 °C; detector temperature, 280 ºC; column heating program: 50 °C (3 min), then 5 °C min^{-1} till 250 °C (10 min); helium as carrier gas, 1.0 mL min^{-1}; injections of 1.0 µL, samples dissolved in *n*-heptane:petrol ether 1:1, split ratio 1:25. Results are presented (Table 2) in terms of the relative composition of each sample of galls volatiles.

In all the performed analyses, the identifications were made by using NIST107, NIST21 and WILEY8 libraries considering the relative retention indices (RRI) calculated by using the data obtained from a series of *n*-alkanes (C10-C30).[21,22] Sample standards of heptanal, α-pinene, limonene, linalool, caryophyllene, viridiflorol, guaiacol, camphor, *trans*-anetol, safrol, thymol, eugenol and *tert*-butyl-hydroxytoluene were used to validate the GC-systems and guarantee the reliability of the calculated indices.

Statistical analysis

Data are presented as means ± SD, when appropriate. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to the data set from healthy leaves samples (Table 2; 8 volatiles x 13 volatile compounds = 104 data points). For this purpose, the columns (response variables) were standardized to unit variance (auto-scaling) prior to the statistical analysis.[23] For PCA, a two-dimension scatter-plot was generated in order to study data structure, while a dendrogram for samples was constructed using the Ward's agglomerative method aiming at grouping samples according to similarities in the volatile composition. Analyses were performed using the Statistica v. 7 (Statsoft, USA) and Chemoface (UFLA, Brazil) software.

RESULTS AND DISCUSSION

Table 1 shows the results of the analyses of volatile components from the healthy leaves of *B. dracunculifolia*, whereas Table 2 presents data regarding the galls samples. The relative compositions are presented in a crescent order of component RRI, which were measured in apolar columns and compared with the data published by Adams using a DB5 column or similar.[22,24]

As presented in Table 1, although the proportions of (*E*)-nerolidol and spathulenol obtained from healthy leaves are relevant, great variability in the proportions of both components can be observed. These

Table 1. Relative compositions of the volatiles obtained from healthy leaves of *Baccharis dracunculifolia* collected from areas located near to the galls collection point (all data in %)

Components	Healthy leaves samples								
	♀1	♂2	♀3	♂4	♀5	♂6	♀♂7	♀♂8	$\bar{x}$ ± s
limonene	*	0.21	0.06	0.05	**	0.07	0.07	0.12	0.10 ±0.06
α-terpineol	*	0.42	0.63	0.47	0.18	0.92	0.26	0.14	0.43 ±0.28
caryophylene	*	0.36	4.45	2.88	0.56	5.72	5.91	8.50	4.67 ±2.98
germacrene D	1.04	1.12	4.52	3.90	1.76	7.98	6.40	13.10	6.28 ±4.13
bicyclogermacrene	1.80	0.66	6.48	3.95	2.20	9.07	6.70	13.72	7.02 ±4.36
δ-cadinene	1.29	1.10	4.77	3.26	2.05	3.98	2.98	5.66	3.78 ±1.63
(*E*)-nerolidol	44.36	4.51	15.25	18.55	8.80	19.66	17.78	19.63	16.61 ±11.79
palustrol	1.32	1.67	1.00	0.63	0.88	**	0.91	0.68	0.96 ±0.37
spathulenol	17.80	31.23	18.57	21.73	29.87	16.15	16.23	9.55	18.68 ±7.28
caryophylene oxide	5.33	18.06	7.41	6.08	11.17	5.33	5.72	3.38	6.52 ±4.72
globulol	1.47	4.26	2.10	2.63	3.02	1.87	0.86	0.46	1.82 ±1.22
viridiflorol	2.46	3.32	3.16	2.66	4.20	2.73	2.94	1.93	2.94 ±0.67
α-cadinol	6.53	5.43	4.81	4.44	7.25	3.85	4.98	3.90	4.87 ±1.21
Monoterpene hydrocarbons	0.0	0.63	0.69	0.52	0.18	0.99	0.33	0.26	0.45 ±0.32
Sesquiterpenes hydrocarbons	4.13	3.24	20.22	13.99	6.57	26.75	21.99	40.98	17.23 ±12.97
Oxygenated sesquiterpenes	79.27	68.48	52.30	56.72	65.19	49.59	49.42	39.53	57.56 ±12.72
Yielding (w/w, dried)	0.65	0.62	0.63	0.55	0.71	0.67	0.69	0.55	
Total identified	83.40	72.35	73.21	71.23	71.94	77.33	71.74	80.77	

* = detected only by GC-MS. ** = not detected. ♀ = feminine specimen; ♂ = male specimen; ♀♂ = out of the flowering period. 1 = January 20th; 2 = January 23th; 3 = March 20th; 4 = March 20th; 5 = May 24th; 6 = May 23th; 7 = August 20th; 8 = October 23th.

two sesquiterpene alcohols appeared as principal components of the volatiles from *B. dracunculifolia* leaves in several earlier published researches but also in varied relative concentrations.[11,25] When female and male specimens are collected at the same time and place, as in the case of samples ♂3 and ♂4, greater similarity between the relative volatile compositions could be expected once the plants grow under very similar conditions.[18,19] Moreover, the comparison between the data related to the leaf samples collected on different days and locations, namely the samples ♀1 and ♂2, and ♀5 and ♂6, demonstrates that these factors have a great influence on the composition of volatile metabolites. Interestingly, as shown in Table 1, higher concentrations of the sesquiterpenes caryophyllene (8.5%), germacrene D (13.1%) and bicyclogermacrene (13.7%) were found in the volatiles from healthy leaves collected in October (♀♂8), far away from the blooming period that occurs between February and April, when the *Baccharopelma* attack occurs.

The first interesting difference between the analyzed volatile samples are the yields: fresh galls produced over 3.5% whereas dried healthy leaves an average of 0.63%, showing the remarkable effect on the secondary metabolism of leaf volatiles in its transformation into gall. Comparing the compositions of the volatiles extracted from healthy leaves (Table 1) with those from galls (Table 2) higher proportions of monoterpene compounds are observed in the volatiles from galls (~14% to ~22%) as compared to the observed from leaves (below 1%).

In the volatiles from galls (Table 2), the principal components are β-pinene (5.72 ± 1.98%), limonene (10.12 ± 4.15%), caryophyllene (6.50 ± 3.12%), germacrene D (15.65 ± 5.39%), and bicyclogermacrene (27.78 ± 13.68%), which appear in lower proportions in the healthy leaves (Table 1): limonene (0.10 ± 0.06%), caryophyllene (4.67 ± 2.98%), germacrene D (6.28 ± 4.13%), bicyclogermacrene (7.02 ± 4.36%). The proportions of (*E*)-nerolidol (16.61 ± 11.79%) and spathulenol (18.68 ± 7.28%) in the healthy leaves volatile samples are high as compared with concentrations found for these compounds in the galls, (*E*)-nerolidol (7.81 ± 2.84%) and spathulenol (1.67 ± 0.56%).

From two-dimension PCA (Figure 1), it was possible to observe that leaf samples ♀3, ♂4, and ♂♀7 presented similar chemical compositions, but no clear separation was observed among all volatile samples. Cluster analysis was further applied to check for groups of similar samples and results are shown in Figure 2. Two distinct groups were formed when the Euclidean distance of 7 is taken into account. Using both HCA and PCA, it is possible to state that besides samples ♂♀8 and ♂6 were included in Cluster 2, they present very similar chemical composition.

As Table 2 shows, the replicate 2 and 3 present very similar chemical composition and those values were quite different from replicate 1, leading to larger deviations for some components, such as limonene, caryophyllene, germacrene-D and bicyclogermacrene. The latter two compounds are the major volatile components of galls and alternate in importance in replicates, which can have several causes. In this study, some collected galls were empty, while others contained one to three insects therein. The total absence of nymphs in the larval chamber or a greater or lesser number could lead to variations in the galls volatile composition.

CONCLUSIONS

This research work presented the volatile composition of the galls induced by the psyllid *Baccharopelma dracunculifoliae* in leaves of *Baccharis dracunculifolia*, for the first time. These results were compared with those obtained for volatiles from healthy leaves of the plant. The galls provide higher yield of volatiles compared to healthy leaves. The volatile substances present in greater relative proportions in the galls are β-pinene, limonene, germacrene D and bicyclogermacrene, whereas the healthy leaves contain more (*E*)-nerolidol and spathulenol.

Presented and discussed data as a whole suggest various new hypotheses: that both sesquiterpene alcohols (*E*)-nerolidol and spathulenol are unnecessary or prejudice the good development of the nymphs; that an increase in the content of non-oxygenated sesquiterpenes protect the nymphs by better isolating the chamber from

Table 2. Relative compositions of the volatiles from the galls induced by *Baccharopelma dracunculifoliae* on *Baccharis dracunculifolia* leaves (all data in %)

Components	Galls samples					
	RRI[a]	RRI[b]	1	2	3	$\bar{x}$ ± s
β-pinene	977	980	3.43	6.75	6.98	5.72 ±1.98
β-myrcene	991	991	4.12	2.09	2.53	2.92 ±1.07
limonene	1029	1031	5.36	12.95	12.07	10.12 ±4.15
trans-β-ocimene	1048	1050	0.47	0.66	0.71	0.61 ±0.13
linalool	1102	1098	0.25	0.09	nd	0.17 ±0.11
α-terpineol	1194	1189	0.32	nd	nd	0.32 ±*
bicycloelemene	1328	1330	0.47	0.21	0.28	0.32 ±0.13
δ-elemene	1338	1339	6.81	3.47	4.49	4.92 ±1.72
neryl acetate	1364	1365	2.43	0.63	1.60	1.56 ±0.90
α-copaene	1377	1376	0.37	0.34	nd	0.35 ±0.03
caryophyllene	1422	1418	8.59	8.00	2.92	6.50 ±3.12
β-gurjunene	1431	1432	0.28	0.20	0.14	0.21 ±0.07
aromadendrene	1441	1439	0.46	0.60	nd	0.53 ±0.10
α-caryophyllene	1456	1454	1.65	1.36	0.82	1.28 ±0.42
alloaromadendrene	1463	1461	0.91	0.47	0.40	0.59 ±0.27
γ-muulorene	1478	1477	0.77	0.83	nd	0.80 ±0.04
germacrene D	1484	1480	21.80	11.78	13.36	15.65 ±5.39
β-selinene	1489	1485	1.02	0.80	nd	0.91 ±0.16
bicyclogermacrene	1499	1494	12.42	32.29	38.63	27.78 ±13.68
δ-cadienene	1525	1524	1.18	1.66	0.46	1.10 ±0.60
(*E*)-nerolidol	1564	1564	10.90	5.30	7.24	7.81 ±2.84
palustrol	1573	1565	0.58	0.26	0.20	0.35 ±0.20
germacrene D-4-ol	1579	1574	1.02	0.50	0.62	0.71 ±0.28
spathulenol	1583	1576	2.32	1.40	1.29	1.67 ± .56
ledene oxide	1589	1890	3.88	2.13	2.03	2.68 ±1.04
viridiflorol	1598	1590	1.90	1.28	1.08	1.42 ±0.43
α-cadinol	1660	1653	0.90	0.53	0.26	0.56 ±0.32
aromadendrene oxide	1694	1702	1.03	0.17	0.23	0.48 ±0.48
Monoterpene hydrocarbons			13.38	22.45	22.29	19.37 ±5.19
Oxygenated monoterpenes			0.57	0.09	nd	0.33 ±0.34
Sesquiterpene hydrocarbons			59.16	62.64	63.11	61.64 ±2.16
Oxygenated sesquiterpenes			24.85	11.56	12.96	16.46 ±7.30
Yielding (w/w, fresh)			3.50	3.60	3.80	3.63 ±0.15

RRI[a] = means of calculated relative retention indices using the apolar columns DB5 and RTX-5MS, and the *n*-alkane series C10-C30; RRI[b] = published relative retention indices (DB-5); nd = not detected or below 0.10%; = mean of the triplicates; ± s = standard deviation; * identified in only one sample.

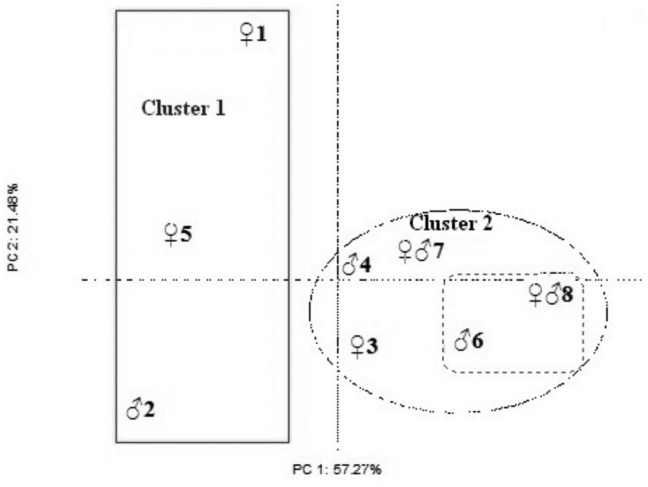

Figure 1. *2-Dimensional projection of healthy leaf samples according to the volatile composition*

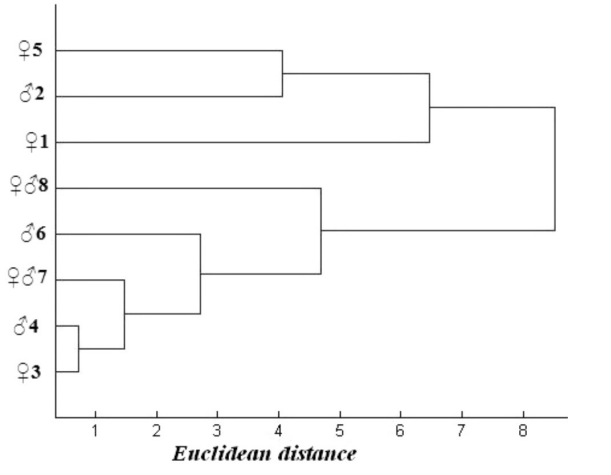

Figure 2. *Clustering of healthy leaf samples based on volatile composition. Note:* ***1****, January 20th;* ***2****, January 23th;* ***3****, March 20th;* ***4****, March 20th;* ***5****, May 24th;* ***6****, May 23th;* ***7****, August 20th;* ***8****, October 23th*

humidity; and that the insect can easily recognize the characteristic gall aroma using this to localize the galls all the time. The observed higher proportions of germacrene-D and bicyclogermacrene and the remarkable high monoterpene content in the galls due to the presence of limonene and β-pinene suggest that all these compounds should be tested as possible active targets in the search for natural enemies of *Baccharopelma*.

SUPLEMENTARY MATERIAL

Photos of *Baccharopelma dracunculifoliae* are available at http://quimicanova.sbq.org.br, in form of PDF archive with free access.

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