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37. ESSENTIAL OILS OF SEVEN BRAZILIAN *BACCHARIS*: A PROSPECTIVE APPROACH ON THEIR ECOLOGICAL RÔLE

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Abstract
A description of the habitat and areas where seven *Baccharis* species (*B.caprariifolia* male and female, *B. dracunculifolia* male and female, *B. erioclada* male and female, *B. myriocephala*, *B. platypoda*, *B. tridentata*, *B. vincifolia*) occur, is presented. The essential oils of the aerial parts were obtained by hydrodistillation and examined by gas chromatography and gas chromatography/mass spectroscopy. The major constituents were identified by mass spectra comparison with those in the Wiley/NBS library and with those of standard commercial, synthetic and isolated compounds. A seasonal and daytime variation in oil composition between female and male plants was observed. The essential oil variation was first correlated to pollination but preliminary field observations showed this to be a complex problem and the floral scent was not easily related to a pollination advertisement or vector.

Introduction

The genus *Baccharis* L. from the Compositae occurs in South, Central and North America. The Brazilian Cerrado is one of the richest areas in *Baccharis* species. Cerrado is the Brazilian name for a characteristic vegetation of the Central part of Brazil which is intermediate between forest and pasture presenting a great plant diversity. The plants are dioecious with male and female capitula appearing on separate plants (Boldt, 1989). This genus has been much investigated from the phytochemical point of view but no systematic work on its essential oil has been reported. It should be mentioned though, that vassoura oil (*B. dracunculifolia* DC. (Boldt, 1989)) and carqueja oil (*B. genistelloides* Pers.) which were produced and used in perfumery, attracted the attention of several research groups including ours (Queiroga *et al.*, 1990; Motl & Trka, 1983; Weyerstahl *et al.*, 1990, 1992; Bauer *et al.*, 1978).

This study aims to survey the essential oil composition of several *Baccharis* naturally occurring in our part of Brazil and to apply this knowledge to interspecific, interspecimen and ecological correlations. The species studied are in Table 1.

TABLE 1. Details of the material studied***

Entry	Plant	Collecting site	Voucher specimen	oil yield (%)	References
1	*B. caprariifolia* DC. female	Salesópolis - S.Paulo	UEC-46656 (2IV/87)	0.05	Bohlman *et al.*, 1973
2	*B. caprariifolia* DC. male	Salesópolis - S.Paulo	UEC-46656 (2IV/87)	0.07	Bohlman *et al.*, 1973
3	*B. dracunculifolia* DC. female	Campinas - S.Paulo	UEC-47072 (18/XII/85)	0.18	**Queiroga *et al.*, 1990; Weyerstahl *et al.*, 1992; Motl & Trka, 1983; Bauer *et al.*, 1978; Weyerstahl *et al.*, 1990; *Zdero *et al.*, 1989
4	*B. dracunculifolia* DC. male	Campinas - S.Paulo	UEC-47072 (18/XII/85)	0.19	*Jarvis *et al.*, 1991
5	*B. erioclada* DC. female	Campos de Jordão- S.Paulo	UEC-1499 (23/V/78)	0.30	*Jarvis *et al.*, 1991
6	*B. erioclada* DC. male	Campos de Jordão- S.Paulo	UEC-1499 (23/V/78)	0.21	
7	*B. myriocephala* DC.	Jundiaí - S.Paulo	UEC-50276 (9/IX/88)	0.60	*Jarvis *et al.*, 1987
8	*B. platypoda* DC.	Santana do Riacho- Minas Gerais	UEC-51399 (2/VII/89)	0.49	*Jarvis *et al.*, 1991
9	*B. tridentata* Vahl	Santana do Riacho- Minas Gerais	UEC- 51400 (2/VII/89)	0.10	*Jarvis *et al.*, 1991
10	*B. vincifolia* Baker	Campinas- S.Paulo	UEC	0.38	

* References concerning phytochemical studies with no emphasis on the essential oil.
** Publications concerning essential oil data.
*** Some of the above *Baccharis* species have recently been reclassified, for more details see Hellwig (1993).

Results

The GC (gas chromatography) analyses of seven *Baccharis* (Tables 2 and 3) revealed that the composition of the essential oils is complex with more than 100 constituents. The identification process relied heavily on some commercial, synthetic and isolated standards mentioned in our

Key to Tables 2 & 3.
RI = retention index obtained following Van den Dool & Kratz (1963) and arranged in order of elution of the components in a HP-ULTRA-1 capillary column. RIb = Retention index compared to that of Jennings & Shibamoto (1980) RIis = retention index compared to that of isolated standard which were further characterized by IR, proton and carbon-13 NMR; RIss = retention index compared to that of a synthetic standard reported in Queiroga *et al.* (1990). RIcs = retention index compared to that of a commercial standard. *Compounds 1, 2, 3, 4 and 5 were detected in the commercial *B. dracunculifolia* oil but never in the recently obtained volatiles from fresh plant hydrodstillation.

TABLE 2. Percentage oil composition of three female and male Baccharis plants.

	Method	*B. caprarifolia*		*B. dracunculus*		*B. erioclada*	
		♀	♂	♀	♂	♀	♂
		MONOTERPENES					
α-thujene	RIb,MS			0.07	0.23		0.18
α-pinene	RIcs,MS		0.17	1.18	0.13	8.45	0.26
sabinene	RIb,MS	1.2	0.08				
β-pinene	RIcs,MS	1.08	0.77	3.76	1.90	21.44	1.16
β-myrcene	RIb,MS						0.09
α-phellandrene	RIb,MS						
δ-3-carene	RIcs,MS						
α-terpinene	RIb,MS	2.05	0.74		0.14		0.16
limonene	RIcs,MS	1.15	0.85	4.65	0.93	15.16	2.68
γ-terpinene	RIb,MS		0.12			0.37	0.14
α-terpinolene	RIb,MS	1.87	0.18	0.55	0.40	1.89	0.52
α-terpineol	RIcs,MS		5.85	1.42	2.54	3.45	1.55
trans-geraniol	RIcs,MS						
		SESQUITERPENES					
α–cubebene	RIb,MS					0.55	0.23
α-ylangene	RIb,MS						
α-copaene	RIcs,MS			0.33	0.22	0.38	0.67
β-elemene	RIb,MS	2.06	0.65	1.23	1.14	0.39	0.99
α-gurjunene	RIcs,MS			0.45	0.28	0.24	0.62
β-caryophyllene	RIcs,MS	17.09	15.73	5.40	5.90	4.21	10.7
calarene	RIcs,MS						
aromadendrene	RIcs,MS		0.44	1.63	1.06		0.15
humulene	RIcs,MS	3.94	1.76	1.55	2.25	1.09	3.54
alloarimadendrene	RIcs,MS		0.37	0.92	0.83	0.31	0.42
cabreuva ox. 1	MS						
cabreuva ox. 2	MS						
β-selinene	RIb,MS			9.90	11.00		
β-cadinene	RIss,MS			1.20	0.98		
α-muurolene	RIb,MS		0.97	0.90	0.48	0.55	1.02
γ-cadinene	RIb,MS	5.35	2.96			1.92	4.55
δ-cadinene	RIb,MS			5.07	3.58		
isohumbertiol 3	MS						
isohumbertiol 4	MS						
epiglobulol	RIss,MS		1.33				
nerolidol	RIis,MS	8.85	0.43	20.80	12.02	1.92	4.55
spathulenol	RIis,MS	4.54	8.41	2.58	3.79	6.61	12.57
caryophyllene ox.5	RIcs,MS						
globulol	RIss,MS	4.65	7.02	2.46	2.26	1.02	1.46
viridiflorol	RIss,MS	2.55	3.76	1.34	1.12	2.27	3.38
guaiol	RIcs,MS	2.55	0.45	0.86	0.83	1.32	0.36
Unknown M+ 222							
α-cadinol	RIis,MS	1.81	4.73	2.55	3.01	1.30	2.15
Unknown M+240-15							
armodendrenodiol	RIss,MS	2.03	0.26	1.86	1.19	1.19	2.51
Unknown M+238							

TABLE 3. Percentage composition of the volatiles of four *Baccharis* species.

Compounds	**RI**[8]	***B. myri.***	***B. plat.***	***B. trid.***	***B. vinc.***	**Method**
			MONOTERPENES			
α-thujene	1117					RIb,MS
α-pinene	1123	0.82			0.60	RIcs,MS
sabinene	1147					RIb,MS
β-pinene	1152	0.11			0.40	RIcs,MS
β-mircene	1156					RIb,MS
α-phellandrene	1167					RIb,MS
δ-3-carene	1172					RIcs,MS
α-terpinene	1177					RIb,MS
limonene	1184	0.15			0.35	RIcs,MS
γ-terpinene	1203	1.38				RIb,MS
α-terpinolene	1219			0.09	3.41	RIb,MS
α-terpineol	1234					RIcs,MS
trans-geraniol	1325					RIcs,MS
			SESQUITERPENES			
α-cubebene	1409	1.01		0.03	4.98	RIb,MS
α-ylangene	1428					RIb,MS
α-copaene	1432	2.86		0.08	0.98	RIcs,MS
β-elemene	1445	1.57	0.15	0.04	1.31	RIb,MS
α-gurjunene	1463	0.24	0.23		0.56	RIcs,MS
β-caryophyllene	1475	4.52	0.82	0.10	8.66	RIcs,MS
calarene	1481			0.02	0.46	RIcs,MS
aromadendrene	1495	1.76	0.49	0.02	0.56	RIcs,MS
humulene	1505	1.03	0.66	0.05	2.18	RIcs,MS
alloaromdendrene	1508	0.22	0.28	0.02	1.11	RIcs,MS
cabreuva ox. 1	1512					MS
cabreuva ox. 2	1529					MS
β-selinene	1537		1.90	0.02	0.62	RIb,MS
β-cadinene	1541		0.43			RIss,MS
α-muurolene	1554	2.23	0.53	0.07	0.54	RIb,MS
γ-cadinene	1567				6.49	RIb,MS
δ-cadinene	1579	9.44	3.45	1.60	8.88	RIb,MS
isohumbertiol 3	1588					MS
isohumbertiol 4	1611					MS
epiglobulol	1619			0.06		RIss,MS
nerolidol	1627	9,01	0.36	4.65	3.19	RIis,MS
spathulenol	1638	9.44	8.76	8.60	4.78	RIis,MS
caryophyllene ox.5	1641					RIcs,MS
globulol	1645	2.45	2.70	0.22	1.78	RIss,MS
viridiflorol	1652	0.82	0.71	0.15	1.08	RIss,MS
guaiol	1663	0.91	2.36	0.047	1.29	RIcs,MS
Unknown M+ 222	1691	1.81	1.00	1.03		
α-cadionol	1707	2.28	3.09	0.05	1.29	RIis,MS
Unknown M+240-15	1735		0.06			
aromadendranediol	1744	1.58	1.60	0.04	0.68	RIss,MS
Unknown M+238	1760		1.44	0.65		

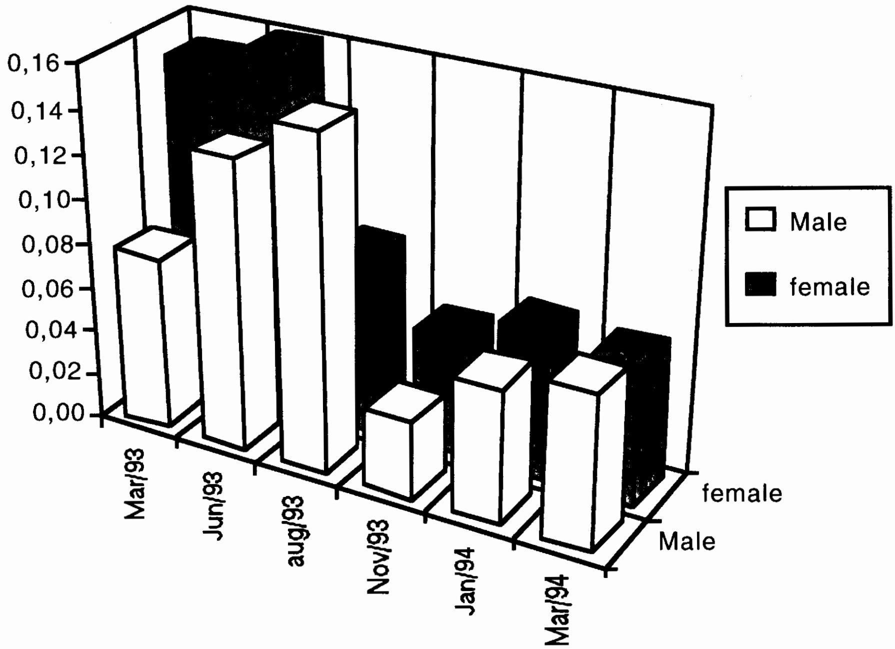

FIG. 1. Seasonal monoterpene/sesquiterpene variation in female and male *B. dracunculifolia* volatiles

first publication (Queiraga, *et al.*, 1990). Several constituents are present in trace amounts and about 15–20 components contribute to more than 1–2% of the total oil and are present in most species. There is a neat variation of oxygen containing mono and sesquiterpenes from one species to the other but more data are necessary to draw any taxonomic conclusions. Compounds 1,2,3,4 (Weyerstahl *et al.*, 1990; Weyerstahl *et al.*, 1992) and 5 (Schneider & Agrawal, 1986) were found in the commercial *B. dracunculifolia* oil but were not detected in fresh plant hydrodistilled oils. These compounds might be artifacts connected with the oil oxidation. A synthetic pathway was undertaken to obtain several standards possessing a cadinane skeleton in order to identify the three unknown constituents detected in *B. dracunculifolia*, *B. myriocephala* and *B. platypoda* and will be reported elsewhere.

The most striking fact was the variation observed among the female and male specimens of the same species (Table 2). The monoterpene/sesquiterpene ratio was higher in the female plants. It was first suggested that stress might be responsible for this variation. As one plant might be closer to a water source or growing in a different soil than the other. *B. dracunculifolia* was therefore submitted to water stress and 10^{-4} M aqueous magnesium chloride solution stress for 72 h before hydrodistillation. A monoterpene/sesquiterpene ratio decrease and a total oil yield increase of 100% was observed for the plant material submitted to water and aqueous magnesium chloride stress. Our second experiment proposed to analyse *B. dracunculifolia* essential oil of a female plant and a male plant during 12 months (Fig. 1).

The seasonal variation of the monoterpene/sesquiterpene ratio revealed that higher ratios were closely related to the low rainfall levels and to a low oil yield (samples collected in March, June and August 1993) and lower ratios were related to high rainfall levels (November 93, January and March 94) and to high oil yield. These results were analogous to our stress experiments but major differences were observed between the male and female plants during the flowering season. The obvious conclusion, at this point, was that the first observed female/male plant oil variation was probably related to the inflorescences and not to unaccounted stress conditions. From a daytime (8 am, 11 am, 2 pm and 5 pm) monitoring of female and male plants (Fig. 2) we could observe that the inflorescences are mainly responsible for monoterpene/sesquiterpene variation with a noticeable inversion occurring at about 10 am.

Further support was obtained studying a population of ten female and ten male plants of *B. dracunculifolia* growing in a very restricted area of the University campus. These results were reproduced. It is easy to assume and expect a clear association of floral scents and advertisement for pollinators, and dioecy obviously reinforces this assumption, if the needs of transporting pollen from male to female flower is regarded. Contrasting profiles of

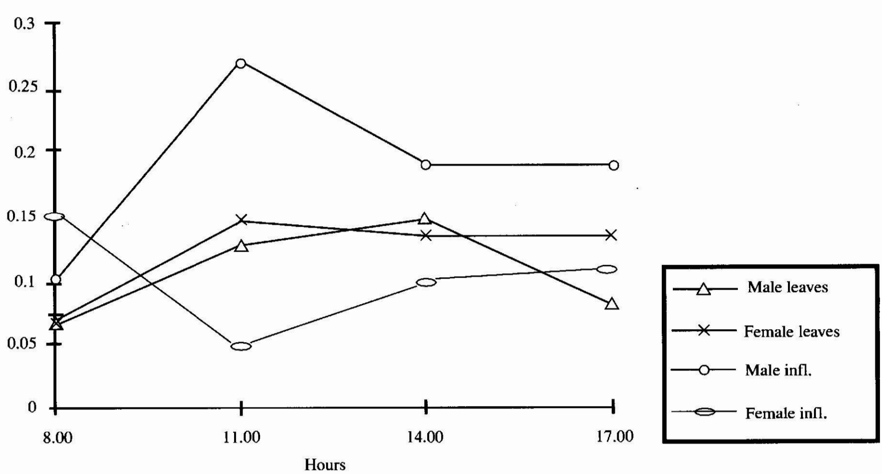

FIG. 2. Daytime monoterpene/sesquiterpene variation in female and male *B. dracunculifolia*.

monoterpene/sesquiterpene ratio between male and female plants of *B. dracunculifolia* (Fig. 2) points to a critical question about the signs that lead pollinators to visit both plants. Another question is about the biological meaning of the difference in the volatile profiles in such a dense population of male and female plants in such close proximity.

Concerning the two previous questions, we should not forget that visitors of capitula of *B. dracunculifolia* may be characterized as a guild of generalist-feeding insects and such a generalist guild is usually correlated to pollination of some others dioecious species (Givinish, 1980). Considering the daytime activity of wasps, flies and bees, species that truly feed on floral resources, it was the highest after 10.30 am, when these populations are surrounded by a sweet honey-like smell. However, volatile analysis show that male and female plants have a very different scent profile at this time.

This situation could be related to the floral scents of *Salix* L., a dioecious genus of Salicaceae (Tollsten & Krudsen, 1992) in which significant differences were observed in the volatile constitution of male and female fragrances of the predominantly anemophilous *S. repens* L.. The other two species, *S. caprea* and *S. repens*, were predominantly zoophilic and male and female flowers shared a common volatile in the infragrances, leading pollinators to both plants. In the anemophilous species, with no selective advertisement pressure for pollinators, the essential oil profile varied freely.

It is not easy to correlate the contrasting profiles of male and female *B. dracunculifolia* with pollination, because of the lack of a common sign for pollinators at the time capitula visitors are active in foraging on flowers.

A comparative analysis with other dioecious species has shown, that in spite of the male and female flower separation in different plants, dioecy has evolved both in anemophilic and zoophilic species (Bawa, 1980) meaning that biotic vectors for pollen do not necessarily follow this feature. However, pollen-kitt agglomerates pollen grains in Asteraceae and therefore do not allow wind pollination in *B. dracunculifolia*. If floral scents of this species were not able to advertise insects for both male and female flowers the answer to pollination might be the most frequent and constant visitors – the ants.

Ants were present in both male and female plants and did not vary according to weather or to air temperature changes during the day. Their behaviour could not be connected to a floral variable factor, such as variation in volatiles or rewards, mainly because they do not feed on capitula, but merely patrolled them along with the rest of the plant. It is also difficult to assume that ants are the pollinators due to the low probability of them visiting both male and female plants when the plant twigs are not overlapping.

Further resampling of volatiles of male and female inflorescences should be done as well as an exhaustive collection of visitors to both capitula, looking for pollen on their body, in order to detect insect species that could truly transport pollen grains among florets. A last point to be clarified in order to reveal the real need of pollination for seed-set, considering the high frequency of agamospermy in the Asteraceae, concerns the breeding system of *B. dracunculifolia*.

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