Preliminary study of variation in leaf volatile oil of *Borrichia frutescens* (L.) DC. along the Texas Gulf Coast

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ABSTRACT

Borrichia frutescens from along the Texas Gulf Coast were grown in a common garden at College Station, TX. Analysis of the volatile leaf oils revealed a very unusual mosiac among individuals. The oils of individuals from South Padre Island grouped with individuals from near Galveston. No clear pattern of geographic variation was discerned. The volatile oil is often dominated by sabinene (16.2 - 30.35), but two individuals had 1.5 and 5.8%. β-phellandrene was the major component in several plants (17.6-21.0%). The major component for one plant was germacrene D (18.0%). Sixty-two terpenoids plus benzene aldehyde were identified in the oils.
Published on-line **www.phytologia.org** *Phytologia 97(3): 164-169 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Borrichia frutescens,* Asteraceae, terpenes, leaf essential oil, geographic variation.

Borrichia frutescens is a halophyte that grows on beaches, dunes, barrier islands in saline or brackish areas. It ranges along the Atlantic and Gulf Coast from Virginia to Florida and thence, westward to Texas and south to Yucatan as well as throughout the West Indies and Bermuda (Semple, 1978). Oddly, it ranges inland along the Rio Grande in south Texas and has a disjunct population in Coahuila (Semple, 1978).

Evaluation of plants from the Texas Gulf Coast for possible horticultural use in a common nursery at College Station, TX (30° 37' 24.24", -97° 22' 0.17") afforded an opportunity to examine the leaf volatile oils, free of environmental effects due to different habitats. Samples were obtained from plants grown in #3 black plastic containers filled with the commercial substrate Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, BC) amended with 6.53 kg·m-3 15N -3.9P-9.9K controlled release 3 to 4 month formulation fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio). Plants from which tissues were harvested were at a mature flowering growth stage, grown in full sun, manually weeded, and irrigated as needed to maintain leaf turgidity.

The phytochemistry of *B. frutescens* has been little studied. There is a report on triterpenoids with sitgmastanol, stigmasterol, oleanoic aicd and zoapatanolide A found in *B. frutescens* (Delgado et al., 1992). Cantrell et al. (1996) reported several new triterpenes in *B. frutescens.* There have, apparently, been no reports on the leaf volatile oil of *Borrichia*.

The purpose of the present work was to report on a comprehensive analysis of the steam volatile leaf oils of *B. frutescens* and give a preliminary analysis of variation among individuals.

MATERIALS AND METHODS

Specimens were collected (SC) and grown in a common nursery on the campus of Texas A & M, College Station, TX. Specimens used in this study, *Borrichia frutescens* :

S. Carver B3, *Adams lab 14235*, 26° 06.742' N, 97° 10.212' W, Laguna St. and Campeche, S. Padre Isl., TX; *S. Carver B4*, *Adams lab 14236*, 27° 17.363' N, 97° 39.710' W, End of Rd. 771, Rivera Beach, 9.4 mi. e of Rivera, TX; *Carver B6*, *Adams lab 14237,* 26° 08.435' N, 97° 10.492' W, Convention Center, South Padre Island, TX; *S. Carver B7*, *Adams lab 14238*, 26° 04.353' N, 97° 22.510' W, next to Whataburger, Port Isabel, TX; *S. Carver B8*, *Adams lab 14239*, 26° 04.715' N, 97° 12.712' W, Shore Dr. Port Isabel, TX; *S. Carver B10*, *Adams lab 14240*, 26° 07.175' N, 97° 09.945' W, Gulf and E. Mars streets, S. Padre Island, TX; *S. Carver B16*, *Adams lab 14241*, 28° 33.601' N, 96° 32.247' W, Public Beach, Magnolia Beach, TX; *S. Carver B21*, *Adams lab 14242*, 29° 22.040' N, 94° 45.607' W, Hwy 87, side of road, Port Bolivar, TX; *S. Carver B22*, *Adams lab 14243*, 29° 40.091' N, 94° 04.279' W, on beach, McFaddin NWR, 16 mi sw Port Arthur, TX; *S. Carver B23*, *Adams lab 14244*, 29° 42.612' N, 93° 51.539' W, on bank of Sabine River, 1st Ave., Sabine, TX; *S. Carver B25*, *Adams lab 14245*, 28° 56.415' N, 95° 17.888' W, on Bay Beach Park View, Surfside, TX; *S. Carver B26*, *Adams lab 14246,* 29° 33.079' N, 94° 22.336' W, in ditch, jct. TX 124 and 87, High Island, TX.

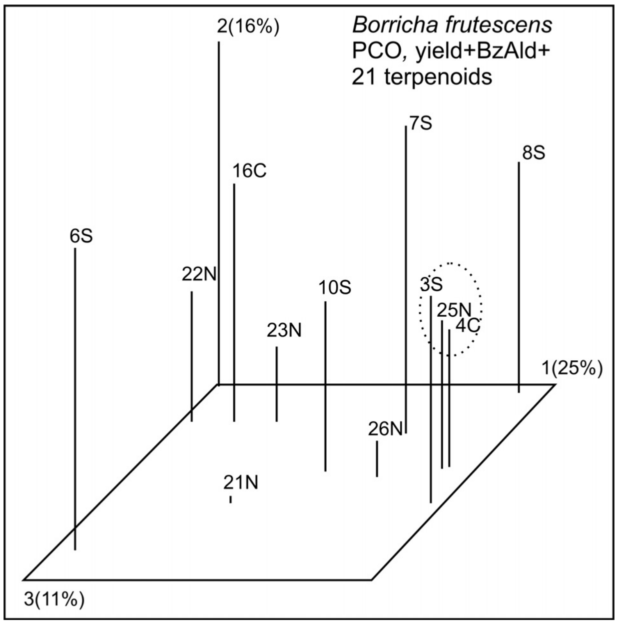
Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields. Oils were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf oils of *Borrichia frutescens* were clear except B10, which was faint yellow (Table 1). The yields ranged from 0.29 to 0.70%, except for plant B6 that had a yield of 1.86%. Thus, there appears to be a polymorphism in oil production.

The composition of the volatile leaf oils revealed a very unusual mosiac among individuals (Table 2). The volatile leaf oil of *Borrichia frutescens* is often dominated by sabinene (16.2 - 30.35, plants B21, B23, B10, B6, Table 2), but two individuals had 1.5 and 5.8% (plants B4, B7, Table 2). β-phellandrene was the major component for B4 (21.0%) and B26 (20.3%). The major component for B7 was germacrene D (18.0%). Plants B6, 7 and 10 are from a small area on south Padre Island, yet their oils vary widely (Table 2). Note also the considerable variation among B21, 23, 26 from the northern region (Table 2). It might be noted that benzene aldehyde is one of the few non-terpenoids in the volatile leaf oil and it varies continuously from 2.3 to 5.4% (Table 2). Sixty-two terpenoids plus benzene aldehyde were identified in the oils.

To visualize the overall similarities among the volatile leaf oils, similarities were computed among individuals using 22 components plus % oil yield. Principal Coordinates Ordination (PCO) of the matrix of similarities resulted in five eigenroots before they began to asymptote. These eigenroots accounted for 25.2, 16.5, 11.4, 9.7 and 7.9% of the variance among individuals (70.7% of total). The lack of loading on the first three eigenroots indicates that there are multiple modes of variation in oils among these individuals. PCO ordination shows (Fig. 1) this is indeed the case. Notice the lack of tight groups in the ordination (Fig. 1) with only plants 3S, 4C, 25N from south, central and north in a group. Plants 6S, 7S, 8S, all from the south Padre Island area, are quite distinct in their volatile oils.



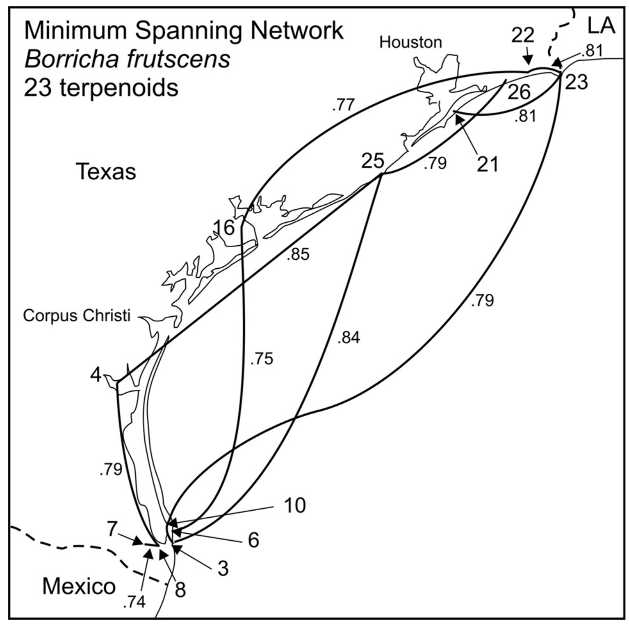


Figure 1. PCO of *Borrichia frutescens* individuals. Figure 2. Minimum spanning network.

In order to examine geographic variation, a minimum spanning network was constructed to link the nearest neighbor for each plant based on the 23 oil characters. Figure 2 shows that 4 of the plants from the south (south Padre Island area) have their most similar oil to that of a plant from the northern or central area (cf. 3-25, 6-16, 10-23, 8-4, Fig. 2). Only plant 7 is most similar to a nearby plant 8 (Fig. 2). A similar pattern is found among the northern plants. The clearest geographical pattern is a north-south network of similar oil patterns. Of course, this is only a preliminary study based on limited samples. The lack of clustering among the five southern plants from a small area is remarkable. However, it is interesting to note that Carver et (2015) reported that analysis of morphological data did not cluster neatly into regional groups.

ACKNOWLEDGEMENTS

Thanks to Amy Tebeest for lab assistance. This research was supported in part with funds from Baylor University and Texas A&M AgriLife Research.

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Table 1. Yields (percent oil on foliage oven dry wt. basis) of volatile leaf oils for *Borrichia frutescens* individuals.

plant	% yield	oil color
B3, 14235	0.64	clear
B4, 14236	0.70	clear
B6, 14237	1.86	clear
B7, 14238	0.52	clear
B8, 14239	0.43	clear
B10, 14240	0.60	faint yellow
B16, 14241	0.58	clear
B21, 14242	0.43	clear
B22, 14243	0.29	clear
B23, 14244	0.48	clear
B25, 14245	0.52	clear
B26, 14246	0.38	clear

Table 2. Leaf essential oil compositions for *Borrichia frutescens* individuals based on FID gas chromatography. KI = Kovats Index (linear) on DB-5 column. Those compounds that appear to distinguish individuals are in boldface. Components with an * were used in similarity calculations. Southern sites: B6-S, B7-S, B10-S; Central: B4-C; Northern: B21-N, B23-N, B26-N.

KI	Compound	B4-C	B26-N	B21-N	B23-N	B10-S	B6-S	B7-S
	% oil yields (w/DW)*	0.70	0.38	0.43	0.48	0.60	1.86	0.52
846	(2E)-hexenal	0.2	0.2	1.1	0.2	0.2	0.2	0.3
921	tricyclene	0.2	0.2	0.1	0.1	0.2	0.1	0.2
924	α-thujene	t	t	0.3	0.2	0.3	0.2	0.1
932	**α-pinene***	**8.3**	**8.3**	**4.4**	**3.8**	**6.2**	**4.4**	**6.7**
946	**camphene***	**10.4**	**9.1**	**4.2**	**3.5**	**7.3**	**5.2**	**8.6**
969	**sabinene***	**1.5**	**10.2**	**30.3**	**27.0**	**25.4**	**16.2**	**5.8**
974	**β-pinene***	**7.9**	**7.2**	**4.1**	**3.7**	**6.2**	**5.1**	**8.1**
988	**myrcene***	**0.6**	**7.8**	**3.4**	**2.6**	**1.2**	**3.7**	**1.1**
1003	**p-menthal-1(7),8-diene***	**t**	**3.2**	**2.9**	**1.9**	**t**	**t**	**t**
1014	α-terpinene	-	0.2	0.5	0.4	0.2	0.4	0.1
1020	p-cymene	-	t	0.3	t	t	t	t
1024	**limonene***	**10.7**	**10.2**	**9.0**	**6.5**	**6.9**	**5.1**	**4.7**
1025	**β-phellandrene***	**21.0**	**20.3**	**17.6**	**12.7**	**13.3**	**10.2**	**9.0**
1032	(Z)-β-ocimene	0.3	0.2	0.3	0.2	2.1	0.2	0.3
1036	**benzene aldehyde***	**4.3**	**2.3**	**4.8**	**3.1**	**3.3**	**3.2**	**5.4**
1054	γ-terpinene	t	0.4	1.6	0.8	0.5	0.6	0.3
1065	cis-sabinene hydrate	t	0.1	0.4	0.3	0.2	0.3	0.1
1085	p-mentha-2,4(8)-diene	t	0.1	0.2	0.2	0.1	0.2	0.1
1098	trans-sabinene hydrate	t	0.2	0.4	0.2	0.2	0.3	t
1100	n-nonanal	-	t	0.1	t	t	t	t
1118	cis-p-menth-2-en-1-ol	t	t	0.2	0.1	t	0.2	t
1136	trans-p-menth-2-en-1-ol	t	t	t	t	-	0.1	t
1141	**camphor***	**t**	**t**	**t**	-	**0.9**	**t**	**t**
1165	**borneol***	**0.7**	**1.7**	**0.2**	**0.3**	**0.3**	**t**	**2.3**
1174	**terpinen-4-ol***	**0.1**	**0.5**	**1.6**	**1.2**	**0.7**	**1.4**	**0.4**
1183	cryptone	t	0.2	0.2	t	-	t	t
1186	α-terpineol	t	t	t	t	-	t	t
1201	n-decanal	t	t	t	-	-	t	t
1215	2-methyl benzaldehyde	t	t	-	t	-	0.2	t
1284	**bornyl acetate***	**11.4**	**5.7**	**1.7**	**3.1**	**5.9**	**1.3**	**3.0**
1309	**p-vinyl guaiacol***	**0.3**	**0.1**	**0.6**	**0.1**	**t**	**0.7**	**t**
1335	δ-elemene	t	t	-	-	t	0.2	t
1374	α-copaene	t	t	-	t	t	t	t
1387	β-cubebene	0.6	0.1	t	0.6	0.4	t	0.4
1411	α-cis bergamotene	t	0.1	t	0.2	-	t	t
1417	(E)-caryophyllene	0.6	0.3	0.8	0.8	0.2	1.3	1.0
1430	β-copaene	t	t	-	t	-	-	t
1432	trans-α-bergamotene	0.6	0.4	0.5	0.5	t	t	0.3
1452	**α-humulene***	**0.3**	**0.1**	**0.2**	**0.3**	**0.1**	**5.6**	**1.7**
1452	neryl propionate	0.2	t	-	0.2	t	-	-
1484	**germacrene D***	**10.8**	**4.1**	**0.7**	**9.7**	**1.9**	**9.6**	**18.0**
1493	trans-muurola-4(14),5-diene	0.1	t	-	0.6	0.6	t	t
1500	**bicyclogermacrene***	**0.5**	**0.1**	-	**0.5**	**3.8**	**7.1**	**2.6**
1500	α-muurolene	0.3	t	-	0.2	-	t	0.3
1505	(E,E)-α-farnesene	1.0	0.6	2.2	0.3	0.3	0.5	1.0
1522	**δ-cadalene***	**0.3**	**t**	**t**	**3.5**	**1.3**	**0.4**	**t**
1529	kessane	t	-	-	-	-	-	t
1559	**germacrene B***	**-**	**0.6**	**t**	-	**0.2**	**0.8**	-
1559	43,109,65,238, sesquiterpene	0.5	t	-	0.5	3.1	2.2	1.0
1561	**(E)-nerolidol***	**0.1**	**-**	-	-	**0.3**	**0.4**	**1.7**
1573	isomer of 1159, sesquiterpene	0.3	-	t	0.2	1.2	1.0	0.4

KI	Compound	B4	B26	B21	B23	B10	B6	B7
1574	germacrene D-4-ol	-	0.1	t	t	0.1	0.3	0.4
1582	caryophyllene oxide	t	t	-	t	-	0.1	t
1594	caratol	-	-	0.1	0.9	-	t	-
1620	**germacrene D-4-ol isomer8**	**1.2**	**0.8**	**0.3**	**0.3**	**0.8**	**2.7**	**3.9**
1645	**cubenol***	**0.2**	**t**	-	**0.4**	**0.4**	**0.7**	**0.5**
1644	α-muurolol	0.3	t	-	0.2	0.3	t	0.2
1649	β-eudesmol	0.4	t	-	t	t	0.6	1.2
1652	α-cadinol	0.2	t	-	t	t	0.3	0.6
1678	41,159,177,220, sesquiterpene	-	t	-	2.1	-	-	t
1685	**germacra-4(15),5,10-triene-1-a**	**0.6**	**t**	-	**0.3**	**0.6**	-	**1.1**
1848	hexadecanal	t	t	t	-.1	t	t	t
1914	hexadecadienal	t	t	0.6	t	t	t	t
2111	phytol isomer	0.3	0.4	t	0.3	0.1	0.1	0.7
2135	55,83,159,320, diterpene	t	t	t	2.0	-	t	t

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.