



# Distribution of Antiherbivory Compounds in *Flourensia cernua*

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## Introduction

*Flourensia cernua* is being used as a shrub model to study the role of terpenes in intake by browsing ruminants at the Jornada Experimental Range

Tarbrush consumption by small ruminants is related to leaf surface concentration of individual terpenes (Estell et al., 1998), but plant to plant variability in concentration is high (Estell et al., 1994)

Secondary chemical concentrations and profiles of many plant species differ among and within plants

Leaf age and location on the plant are 2 factors that influence within-plant chemical profiles of some species (Barnola et al., 1997; Laitinen, 2003)

Young leaves are often more heavily defended in woody plant species (e.g., terpenes, Meyer and Karasov, 1991; gallotannins, Ossipov et al., 1997; total phenolics, Massei et al., 2000)

Our objective was to identify sources of within-plant sources of variation in leaf chemistry in an effort to minimize sample variation and optimize sampling protocols

## Materials and Methods

40 tarbrush plants (n = 20 different plants per study) were selected randomly in a heavily infested area on the Jornada Experimental Range during active summer growth (late August)

Each plant was divided into 4 quadrants (northeast, southeast, northwest, and southwest)

Experiment 1: all leaves were removed from 2 leaders (current year's growth) from 3 positions (outer canopy, subcanopy, and basal) in all 4 quadrants and placed on dry ice and stored at -10°C

Experiment 2: 10 leaders (current year's growth) from each quadrant (outer canopy only) were removed and 3 leaf age categories were formed (immature, moderate, and mature) by separating leaders into thirds and removing leaves

5 leaves from each sample were extracted in duplicate for 5 min at room temperature in 5 ml of ethanol containing 5 ng/ul of 2-carene (internal standard)

Leaf surface terpenes were analyzed by gas chromatography-mass spectroscopy to determine identity and concentration (Tellez et al., 1997) with Kovats indices and mass spectral libraries (Adams, 1995)

Total volatile concentration was estimated from cumulative concentrations of all compounds within a leaf category

Data were transformed to natural logarithms and subjected to univariate analysis of variance (SAS Inst. Inc., Cary, NC) with a completely randomized design to compare mean concentrations among quadrant and leaf position (Exp. 1) and leaf age (Exp. 2) for each compound; means were separated using Tukey's honestly significant difference

Because quadrant effects were minimal, data were pooled over quadrant and reanalyzed without quadrant in the model

**Table 1:** Effect of leaf type on leaf terpenes in tarbrush<sup>1</sup>

Chemical	RT <sup>2</sup>	µg/g DM ± SEM			P value
		Canopy	Subcanopy	Basal	
Santolina triene	274	1.7 ± 0.3 <sup>a</sup>	2.0 ± 0.6 <sup>a</sup>	7.7 ± 4.7 <sup>b</sup>	0.012
Tricyclene	299	4.9 ± 0.7	4.4 ± 0.5	3.9 ± 0.8	0.166
α-Thujene	304	3.7 ± 0.9	2.2 ± 0.3	2.4 ± 0.4	0.117
α-Pinene	314	24.3 ± 4.3 <sup>a</sup>	24.2 ± 4.6 <sup>a</sup>	12.4 ± 1.9 <sup>b</sup>	0.015
Camphene	337	107.3 ± 10 <sup>a</sup>	98.0 ± 9.3 <sup>ab</sup>	52.1 ± 8.1 <sup>b</sup>	0.044
Sabinene	379	4.1 ± 0.5	3.0 ± 0.4	7.1 ± 3.1	0.121
β-Pinene	384	8.1 ± 1.2	8.6 ± 1.1	6.1 ± 1.4	0.109
Myrcene	405	20.4 ± 2.0	16.8 ± 2.1	26.4 ± 5.8	0.559
Mesitylene	412	1.0 ± 0.1	1.3 ± 0.3	1.8 ± 0.3	0.089
Yomogi alcohol <sup>3</sup>	419	63.3 ± 9.5	43.1 ± 7.7	36.2 ± 7.3	0.093
3-Carene	443	16.3 ± 1.0	17.6 ± 1.0	19.7 ± 1.5	0.146
α-Terpinene	455	1.7 ± 0.3 <sup>ab</sup>	1.3 ± 0.1 <sup>b</sup>	3.6 ± 1 <sup>a</sup>	0.025
p-Cymene	469	5.5 ± 0.9 <sup>a</sup>	3.8 ± 0.6 <sup>ab</sup>	2.0 ± 0.3 <sup>b</sup>	0.008
Limonene	480	19.2 ± 1.5 <sup>a</sup>	17.8 ± 1.5 <sup>a</sup>	12.2 ± 1.3 <sup>b</sup>	0.004
1,8-Cineole	484	31.4 ± 7.7	20.9 ± 4.7	9.6 ± 2.6	0.609
(Z)-beta-Ocimene	493	1.4 ± 0.2	1.6 ± 0.1	2.1 ± 0.4	0.382
(E)-beta-Ocimene	519	3.7 ± 2.3 <sup>b</sup>	1.4 ± 0.2 <sup>b</sup>	3.8 ± 0.9 <sup>a</sup>	0.005
trans-Decahydronaphthalene	533	1.4 ± 0.2 <sup>b</sup>	3.0 ± 1.4 <sup>b</sup>	3.2 ± 0.4 <sup>a</sup>	0.003
γ-Terpinene + Artemisia ketone	543	1.8 ± 0.3	1.4 ± 0.1	1.5 ± 0.2	0.708
cis-Sabinene hydrate	560	11.6 ± 3.3	9.0 ± 2.4	4.1 ± 1.2	0.175
Artemisia alcohol	597	219.3 ± 30.5	192.1 ± 27.6	102.0 ± 24.5	0.075
Terpinolene	609	1.1 ± 0.1 <sup>b</sup>	1.2 ± 0.1 <sup>b</sup>	2.8 ± 0.3 <sup>a</sup>	0.000
trans-Sabinene hydrate	633	5.8 ± 1.5	3.9 ± 0.9	2.7 ± 0.5	0.220
cis-p-Menth-2-en-1-ol	682	7.1 ± 1.2	5.2 ± 0.6	4.9 ± 0.9	0.417
α-Campholenol	693	2.3 ± 0.3	2.2 ± 0.2	3.2 ± 0.8	0.866
trans-Pinocarveol	727	8.1 ± 1.9	8.5 ± 2.1	6.0 ± 1.1	0.483
Camphor + trans-Verbenol	738	15.5 ± 1.6 <sup>a</sup>	10.4 ± 1.1 <sup>ab</sup>	8.4 ± 1.4 <sup>b</sup>	0.003
Isoborneol	771	1.4 ± 0.2 <sup>b</sup>	1.4 ± 0.2 <sup>b</sup>	2.7 ± 0.6 <sup>a</sup>	0.007
cis-Chrysanthenol + Pinocarvone	780	51.3 ± 18.3	62.7 ± 30.2	29.6 ± 9.2	0.963
Borneol	793	162.4 ± 16.5	149.6 ± 18.2	97.7 ± 10.7	0.270
Terpin-4-ol	822	4.3 ± 0.7	3.3 ± 0.5	3.1 ± 0.5	0.604
m-Cymen-8-ol	831	1.0 ± 0.1 <sup>b</sup>	1.0 ± 0.1 <sup>b</sup>	3.3 ± 0.7 <sup>a</sup>	0.000
p-Cymen-8-ol	839	2.6 ± 1.4	1.3 ± 0.2	3.7 ± 1.3	0.054
α-Terpinol	856	2.6 ± 0.6	1.6 ± 0.2	3.1 ± 0.8	0.121
Myrtenal	868	1.5 ± 0.1 <sup>b</sup>	1.2 ± 0.1 <sup>b</sup>	2.5 ± 0.4 <sup>a</sup>	0.001
Myrtenol	870	1.5 ± 0.4 <sup>b</sup>	1.2 ± 0.2 <sup>b</sup>	3.4 ± 1 <sup>a</sup>	0.002
cis-Chrysanthenyl acetate <sup>3</sup>	1040	2.0 ± 0.4	2.3 ± 0.5	4.4 ± 1.1	0.146
Bornyl acetate	1103	3.2 ± 0.3	2.6 ± 0.3	3.3 ± 0.4	0.570
Carvacrol	1141	3.9 ± 1.5	2.1 ± 0.2	3.4 ± 1.2	0.654
α-Cubebene	1270	2.3 ± 0.7 <sup>ab</sup>	1.5 ± 0.2 <sup>b</sup>	2.9 ± 0.4 <sup>a</sup>	0.048
Eugenol	1283	9.4 ± 8.0	10.4 ± 8.9	2.6 ± 0.6	0.738
Cyclosativene	1315	4.1 ± 0.4	4.1 ± 0.4	4.3 ± 0.9	0.826
α-Copaene	1337	4.4 ± 0.8	4.1 ± 0.5	3.9 ± 0.5	0.993
β-Bourbonene	1360	9.7 ± 1.1 <sup>a</sup>	9.7 ± 1.1 <sup>a</sup>	5.7 ± 0.8 <sup>b</sup>	0.009
β-Cubebene	1374	5.4 ± 0.7 <sup>a</sup>	3.8 ± 0.6 <sup>ab</sup>	3.0 ± 0.5 <sup>b</sup>	0.022
(Z)-Jasmone	1388	22.0 ± 3.0 <sup>a</sup>	17.0 ± 5.0 <sup>a</sup>	5.5 ± 1.2 <sup>b</sup>	0.000
(E)-Caryophyllene	1447	30.7 ± 5.2 <sup>a</sup>	24.4 ± 2.8 <sup>a</sup>	16.0 ± 2.5 <sup>b</sup>	0.006
α-Humulene	1530	13.7 ± 1.6	10.5 ± 1.1	9.5 ± 1.4	0.067
allo-Aromadendrene	1551	5.6 ± 0.9	6.1 ± 0.8	6.2 ± 1.0	0.827
Drima-7,9(11)-diene	1573	5.8 ± 0.6	6.1 ± 0.6	4.2 ± 0.6	0.216
γ-Murolene	1589	8.3 ± 0.8 <sup>b</sup>	9.3 ± 0.8 <sup>b</sup>	14.5 ± 2.1 <sup>a</sup>	0.008
Germaacene D	1599	50.5 ± 6.0 <sup>a</sup>	35.6 ± 3.8 <sup>a</sup>	20.1 ± 2.7 <sup>b</sup>	0.000
β-Selinene	1615	17.4 ± 1.9	16.4 ± 1.8	10.8 ± 2.0	0.074
epi-Cubebol	1637	10.1 ± 2.4	9.1 ± 2.5	8.1 ± 1.9	0.767
Bicyclogermaacene	1641	7.2 ± 1.6	6.4 ± 1.7	7.4 ± 2.0	0.532
α-Murolene	1652	18.3 ± 3.6	15.2 ± 0.8	17.9 ± 2.0	0.728
γ-Cadinene	1687	7.9 ± 2.0	7.7 ± 2.1	7.2 ± 2.3	0.740
cis-Calamenene	1702	5.4 ± 1	5.0 ± 1.0	5.2 ± 1.3	0.822
Δ-Cadinene	1707	5.3 ± 0.8 <sup>ab</sup>	3.8 ± 0.7 <sup>b</sup>	9.0 ± 1.9 <sup>a</sup>	0.019
Cadina-1,4-diene <sup>3</sup>	1729	7.4 ± 0.6	6.8 ± 0.6	6.5 ± 0.8	0.429
Elemol	1766	23.5 ± 3.4 <sup>a</sup>	15.4 ± 2.3 <sup>a</sup>	7.6 ± 1.5 <sup>b</sup>	0.000
Ledol	1811	59.0 ± 8.3	57.5 ± 8.0	53.1 ± 9.0	0.971
Germaacene D-4-ol	1831	28.7 ± 2.6 <sup>a</sup>	22.2 ± 2.1 <sup>ab</sup>	18.7 ± 2.8 <sup>b</sup>	0.013
Spathulenol	1833	8.3 ± 1.8	10.4 ± 1.6	12.0 ± 2.7	0.570
Caryophyllene oxide	1846	35.6 ± 5.4	33.0 ± 4.3	30.2 ± 5	0.880
Unknown-01	1867	736.6 ± 222.3	668.6 ± 192.5	349.5 ± 100.3	0.671
Unknown-02	1893	47.5 ± 7.1	46.3 ± 6.7	39.0 ± 6.0	0.968
β-Oplophenone	1906	8.6 ± 1.2	9.1 ± 1.1	11.4 ± 3.4	0.851
1-epi-Cubebol <sup>3</sup>	1958	26 ± 3.5 <sup>a</sup>	22.8 ± 2.0 <sup>a</sup>	19.1 ± 4.8 <sup>b</sup>	0.018
epi-α-Murolol	1984	1.5 ± 0.2 <sup>b</sup>	1.8 ± 0.2 <sup>b</sup>	27.9 ± 24.0 <sup>a</sup>	0.001
(Z)-methyl jasmonate <sup>3</sup>	1996	233.9 ± 29.4 <sup>a</sup>	188.5 ± 24.8 <sup>a</sup>	121.9 ± 19.4 <sup>b</sup>	0.005
β-Eudesmol	2002	93.3 ± 14.6	82.8 ± 11.4	80.5 ± 13.1	0.722
Selin-11-en-4-α-ol	2014	123.2 ± 12.2	116.0 ± 10.4	103.7 ± 17.9	0.418
Unknown-03	2022	33.3 ± 8.7	30.9 ± 8.1	40.2 ± 13.6	0.968
Bulnesol	2045	14.6 ± 3.3 <sup>a</sup>	8.9 ± 1.7 <sup>a</sup>	5.0 ± 1.3 <sup>b</sup>	0.000
(Z)-Methyl epi-jasmonate	2067	12.5 ± 2.8	11.5 ± 2.3	7.9 ± 1.6	0.147
α-Bisabolol	2079	67.8 ± 6.8 <sup>a</sup>	54.6 ± 6.3 <sup>ab</sup>	48.4 ± 12.2 <sup>b</sup>	0.015
Oplopanone	2195	12.9 ± 1.6 <sup>a</sup>	11.1 ± 1.7 <sup>ab</sup>	7.2 ± 1.3 <sup>b</sup>	0.036
Unknown-04	2206	180.3 ± 61.7	162.5 ± 51.3	126.7 ± 44.4	0.971
β-Acoradienol	2247	20.5 ± 2.2	17.6 ± 2.1	14.8 ± 2.2	0.474
Nootkatone	2336	10.0 ± 1.4 <sup>a</sup>	8.9 ± 1.2 <sup>ab</sup>	6.2 ± 1.0 <sup>b</sup>	0.037
Cryptomeridiol	2362	87.6 ± 12.6	81.0 ± 13.2	74.3 ± 19.3	0.307
Flourensiadiol	2476	3860.1 ± 573.5	4093.5 ± 803.5	388.6 ± 620.4	0.973
Unknown-05	2592	38.6 ± 7.9	29.4 ± 3.8	27.7 ± 3.3	0.472
Unknown-06	2626	59.4 ± 19.9	51.6 ± 15.5	29.2 ± 10.1	0.209
Unknown-07 <sup>3</sup>	2754	132.4 ± 12.4	134.3 ± 12.5	153.8 ± 17.7	0.689
Unknown-08	2798	90.0 ± 21.8	61.8 ± 5.6	83.6 ± 10.2	0.243
Unknown-09	2876	402.7 ± 78.6	377.8 ± 71.3	383.9 ± 85.9	0.990
Unknown-10 <sup>3</sup>	3113	14.0 ± 3.2	8.7 ± 3.4	5.5 ± 1.3	0.249
Unknown-11 <sup>3</sup>	3234	119.2 ± 38.0	100.7 ± 32.8	154.9 ± 57.5	0.741
Unknown-12	3288	1276.1 ± 266.2	1180.4 ± 252.3	416.8 ± 313.3	0.703
Unknown-13	3332	98.1 ± 27.9	95.6 ± 27.6	90.7 ± 22.9	0.928
Unknown-14	3420	134.9 ± 30.3	143.3 ± 40.4	125.5 ± 39.6	0.970
Unknown-15	3458	100.9 ± 26.5	102.1 ± 28.3	189.1 ± 50.6	0.319
Cumulative		9272.0 ± 1114.0	8988.7 ± 1225.6	7933.0 ± 1037.3	0.844

<sup>1</sup>Data are untransformed means ± SEM for each chemical and leaf type; significance was determined using log-transformed values.

<sup>2</sup>Retention time.

<sup>3</sup>A quadrant effect (P < 0.05) was observed for 7 compounds.

a,b,c Means with different superscripts differ (P < 0.05).

**Table 2:** Effect of leaf age on leaf terpenes in tarbrush<sup>1</sup>

Chemical	RT <sup>2</sup>	µg/g DM ± SEM			P value
		Immature	Moderate	Mature	
Santolina triene	274	2.3 ± 0.3 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	1.3 ± 0.4 <sup>b</sup>	0.001
Tricyclene	299	7.3 ± 0.9	6.0 ± 0.7	6.8 ± 0.9	0.697
α-Thujene	304	7.0 ± 1.0	5.4 ± 1.1	4.7 ± 0.7	0.171
α-Pinene	314	45.8 ± 4.8	56.4 ± 27.6	38.7 ± 12.2	0.176
Camphene	337	130.1 ± 18.5	118.8 ± 17.8	110.1 ± 17.7	0.871
Sabinene	379	11.6 ± 2.1	10.0 ± 2.8	9.2 ± 2.2	0.364
β-Pinene	384	13.2 ± 2.6	7.8 ± 1.8	7.9 ± 1.8	0.148
Myrcene	405	9.4 ± 2.3	7.5 ± 1.7	9.3 ± 1.7	0.433
Mesitylene	412	4.0 ± 1.5	1.5 ± 0.4	1.1 ± 0.4	0.232
Yomogi alcohol	419	53.3 ± 7.1	50.8 ± 7.0	59.7 ± 9.5	0.978
3-Carene	443	9.6 ± 1.1	9.5 ± 0.6	8.8 ± 0.6	0.829
α-Terpinene	455	2.3 ± 0.3	1.9 ± 0.4	1.6 ± 0.3	0.164
p-Cymene	469	9.0 ± 1.1	11.1 ± 1.7	11.4 ± 2.0	0.820
Limonene <sup>3</sup>	480	18.9 ± 2.2 <sup>a</sup>	9.5 ± 1.3 <sup>b</sup>	8.3 ± 0.7 <sup>b</sup>	0.000
1,8-Cineole	484	67.0 ± 9.0	65.3 ± 9.4	63.9 ± 10.5	0.903
(Z)-beta-Ocimene	493	6.7 ± 4.8	12.1 ± 11.1	11.7 ± 10.8	0.317
(E)-beta-Ocimene	519	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.089
trans-Decahydronaphthalene	533	1.0 ± 0.2	1.3 ± 0.6	1.1 ± 0.3	0.915
γ-Terpinene + Artemisia ketone	543	5.1 ± 0.7	4.7 ± 1.0	3.9 ± 0.7	0.277
cis-Sabinene hydrate	560	36.8 ± 5.9	28.2 ± 6.1	25.2 ± 6.2	0.191
Artemisia alcohol	597	451.7 ± 65.3	271.6 ± 40.2	257.4 ± 42.8	0.192
Terpinolene	609	4.4 ± 0.4 <sup>a</sup>	3.0 ± 0.2 <sup>ab</sup>	2.5 ± 0.4 <sup>b</sup>	0.001
trans-Sabinene hydrate	633	17.9 ± 2.2	15.2 ± 2.5	14.5 ± 2.8	0.314
cis-p-Menth-2-en-1-ol	682	9.4 ± 1.6	14.4 ± 8.4	10.9 ± 4.7	0.597
α-Campholenol	693	2.0 ± 0.6	3.5 ± 2.0	3.0 ± 1.5	0.822
trans-Pinocarveol	727	6.6 ± 0.8	7.6 ± 0.8	7.5 ± 0.7	0.673
Camphor + trans-Verbenol <sup>3</sup>	738	9.5 ± 1.2	7.3 ± 1.2	6.9 ± 0.9	0.423
Isoborneol <sup>3</sup>	771	1.6 ± 1.0	2.3 ± 1.7	2.0 ± 1.5	0.773
cis-Chrysanthenol + Pinocarvone	780	123.4 ± 53.1	86.3 ± 42.2	61.7 ± 33.0	0.990
Borneol	793	349.1 ± 48.3	276.2 ± 40.3	248.0 ± 38.4	0.805
Terpin-4-ol	822	7.8 ± 1.1	7.2 ± 1.2	7.0 ± 1.2	0.730
m-Cymen-8-ol	831	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.4	0.615
p-Cymen-8-ol	839	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.611
α-Terpinol	856	1.4 ± 0.2	1.3 ± 0.3	1.5 ± 0.4	0.897
Myrtenal <sup>3</sup>	8				