One seed juniper mono- and sesqui- terpenoids : analytical and ecological aspects

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Introduction

One-seed juniper (*Juniperus monosperma* (Englem. Sarg) covers large areas of New Mexico. Domestic livestock could be used as a tool to slow down encroachment and to prevent re-invasion following tree clearing practices. However, richness in mono- and sesqui- terpenoids in this species may serve as defense against herbivory and may affect the success of this practice. Development of simple and accurate analytical standards to identify terpenoids in one-seed juniper is key to investigating practical and ecological aspects of one-seed juniper manipulation by domestic browsers.

1 Terpenoid identification and quantification

Procedure:

1. Current year's growth was ground in liquid nitrogen to < 0.5 mm.
2. Ethanolic extraction of terpenes by shaking 0.5 g of ground material in 5 ml of ethanol for 5 min. at 150 RPM in a 20 ml scintillation vial containing 5 mg/ml of Longifolene as internal standard (IS).

Sample collection from juniper saplings

1. Analysis by gas chromatography (GC) coupled to a mass spectometer (MS) using a Finnigan MAT Magnum GC//MS (Thermoelectron Co., USA) with a CTC-A200s auto-sampler and equipped with a DB-5 column that uses helium as carrier.
2. GC/MS settings: split injection flow (ratio 20:1); injection volume 1 µl.; MS detector temperature: 220ºC; injector temperature: 220ºC; transfer line temperature: 240ºC; initial column temperature 60ºC; final column temperature: 240ºC; and column temperature rate of increase: 3ºC/ min.
3. Terpenoid identification by comparing mass spectra and retention indices with known libraries.
4. Terpenoid quantification based on the percent peak area relative to total peak area and known concentration of IS, expressed on a dry matter basis (mg g^{-1} DM).

Detail of the GCMS (Left.) and filters (Right)

2 Mono- & Sesqui-terpenoids in one-seed juniper

Compound	Concentration (mg g^{-1} DM)	SEM [a]	CV (%)	Relative Concentration (%)	Cumulative Concentration (%)
α-pinene	12.94	0.70	24.05	64.67	64.67
β-phellandrene	1.48	0.13	38.72	7.39	72.06
3-carene	1.19	0.25	92.42	5.93	77.99
unknown 1	0.96	0.06	26.54	4.78	82.77
Myrcene	0.50	0.03	23.65	2.51	85.29
unknown 3	0.31	0.03	37.49	1.53	86.81
β-eudesmol	0.29	0.02	29.05	1.46	88.28
α-eudesmol	0.25	0.02	26.98	1.27	89.55
Terpinolene	0.23	0.02	42.85	1.15	90.69
germacrene_B	0.22	0.02	40.97	1.08	91.78
α-phellandrene	0.19	0.03	60.48	0.95	92.72
8-α-acetoxyelemol	0.18	0.01	35.41	0.90	93.62
β-pinene	0.17	0.01	29.49	0.87	94.49
e-caryophyllene	0.11	0.01	29.34	0.54	95.03
γ-eudesmol	0.10	0.01	34.15	0.51	95.54
unknown 2	0.09	0.01	28.07	0.43	95.97
bornyl_acetate	0.08	0.01	34.84	0.42	96.39
Camphene	0.08	0.01	42.86	0.39	96.78
8-α-11-elemodiol	0.07	0.01	78.81	0.37	97.15
Elemol	0.06	0.00	29.54	0.30	97.45
Sabinene	0.05	0.01	52.74	0.24	97.69
γ-terpinene	0.05	0.01	55.96	0.23	97.92
Tricyclene	0.04	0.00	27.81	0.18	98.10
(e)-β-ocimene	0.03	0.01	126.07	0.17	98.27
Verbenene	0.03	0.01	105.09	0.14	98.41
Α-humulene	0.03	0.00	52.83	0.14	98.55
2-carene	0.03	0.00	51.17	0.13	98.68
Camphor	0.02	0.00	88.95	0.12	98.80
germacrene_D	0.02	0.00	42.35	0.12	98.92
e-nerolidol	0.02	0.00	26.10	0.09	99.01
p-cymene	0.02	0.00	53.40	0.09	99.10
Verbenone	0.02	0.00	52.17	0.08	99.18
cis-sabinene_hydrate	0.02	0.00	43.14	0.08	99.26
Α-bulnesene	0.01	0.00	39.03	0.07	99.33
terpin-4-ol	0.01	0.00	42.26	0.06	99.40
Α-thujene	0.01	0.00	31.47	0.06	99.46
trans-sabinene_hydrate	0.01	0.00	51.17	0.06	99.52
cis-pinene_hydrate	0.01	0.00	55.53	0.06	99.57
α-campholenal	0.01	0.00	105.17	0.05	99.63
para-cymen-8-ol	0.01	0.00	111.39	0.04	99.67
(z)-β-ocimene	0.01	0.00	57.93	0.04	99.71
para-mentha-2,4-(8)-diene	0.01	0.00	75.52	0.04	99.75
meta-cymen-8-ol	0.01	0.00	131.54	0.03	99.78
α-terpineol	0.01	0.00	55.79	0.03	99.81
Sylvestrene	0.01	0.00	98.04	0.03	99.84
β-selinene	0.01	0.00	25.68	0.03	99.87
α-terpinene	0.01	0.00	25.08	0.03	99.90
α-selinene	0.01	0.00	30.57	0.03	99.93
Pinocarvone	0.01	0.00	45.38	0.03	99.96
trans-carveol	<0.01	0.00	39.80	0.02	99.98
o-cymene	<0.01	0.00	63.77	0.02	100.00
Total amount [b]	20.01	0.86	19.32	100.00	-
Maximum value	12.94	-	131.54	64.67	-
Minimum value	<0.01	-	23.65	0.02	-

n=20

[a] SEM: Standard error of means.

[b] Total amount was calculated as the sum of the concentration of all individual compounds.

3 Can juniper be stored for pen feeding studies?

Terpene	Handling Protocol [a]			P value
	Control (mg g^{-1} DM)	Frozen after 24 hours (mg g^{-1} DM)	Refrigerated after 24 hours (mg g^{-1} DM)	
Total amount [b]	21.68 ± 1.42	19.55 ± 1.08	18.79 ± 1.13	0.23
α-pinene	14.10 ± 1.99	12.71 ± 0.84	12.02 ± 0.78	0.23
β-phellandrene	1.57 ± 0.17	1.43 ± 0.13	1.43 ± 0.14	0.73
3-carene	1.21 ± 0.29	1.12 ± 0.25	1.23 ± 0.25	0.95
unknown 1	1.04 ± 0.09	0.94 ± 0.07	0.89 ± 0.07	0.37
Myrcene	0.55 ± 0.04	0.49 ± 0.03	0.47 ± 0.03	0.27
unknown 3	0.34 ± 0.03	0.29 ± 0.03	0.29 ± 0.03	0.47
Β-eudesmol	0.28 ± 0.02	0.31 ± 0.02	0.28 ± 0.02	0.54
Α-eudesmol	0.25 ± 0.02	0.28 ± 0.02	0.24 ± 0.02	0.22
Terpinolene	0.25 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.62
germacrene-B	0.24 ± 0.03	0.22 ± 0.02	0.20 ± 0.02	0.46
α-phellandrene	0.21 ± 0.03	0.18 ± 0.02	0.18 ± 0.03	0.79
8-α-acetoxyelemol	0.18 ± 0.03	0.17 ± 0.02	0.19 ± 0.02	0.77
β-pinene	0.19 ± 0.02	0.17 ± 0.01	0.16 ± 0.01	0.45
E-caryophyllene	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.42

a Values are means ± SEM (standard error of the means).

b Total amount was calculated as the sum of the concentration of all individual compounds.

The refrigeration of juniper branches allowed us to conduct pen feeding trials with harvested plant materials to explore different aspects of juniper-herbivore interactions with sheep (Left) and goats (Right) at NMSU.

4 Do terpenes vary from plant to plant and with plant size?

1. Terpenes vary significantly from plant to plant as denoted by CV% in Table 2.
2. Saplings between 0.5 m to 1 m had subtle differences in α-campholenal, germacrene_D, (e)-β-ocimene, and unknown 3 (Table 2), compounds that have not been shown to deter herbivory

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