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Is Differential Use of *Juniperus monosperma* by Small Ruminants Driven by Terpenoid Concentration?

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Abstract Differential plant use by herbivores has been observed for several woody plant species and has frequently been attributed to plant secondary metabolites. We examined the relationship between terpenoid concentration and *Juniperus monosperma* herbivory by small ruminants. Two groups of animals (10 goats or 5 goats plus 4 sheep) browsed 16 paddocks (20×30 m) containing one-seed juniper for six days during two seasons. Juniper leaves were sampled from 311 saplings immediately after browsing. Saplings were categorized by size (short [<0.5 m], medium [0.5–1.0 m], or tall [>1.0 m]), and by browsing intensity (light [<33 %], moderate [33–66 %], or heavy [>66 %]). Juniper bark was collected from 12 saplings during spring. Total estimated terpenoid concentrations in leaves and bark were 18.3±0.3 and 8.9±0.8 mg/g, respectively, and the dominant terpene in both tissues was α-pinene (11.1±0.2 and 7.6±0.7 mg/g, respectively). Total terpenoid concentration of juniper leaves was greater in spring than summer (20.6±0.5 vs. 16.7±0.3 mg/g, respectively) and was lower in short saplings than medium or tall saplings (16.5±0.6 vs. 19.8±0.4 and 19.5±0.4 mg/g, respectively). Total terpenoid concentration of leaves also differed among the three defoliation categories (21.2±0.6, 18.7±0.5, and 16.1±0.4 mg/g for light, moderate, and heavy, respectively). The smallest subset of terpenoids able to discriminate between light and heavy browsing intensity categories included eight compounds ([*E*]-β-farnesene, bornyl acetate, γ-eudesmol, *endo*-fenchyl acetate, γ-cadinene, α-pinene, *cis*-piperitol, and *cis*-*p*-menth-2-en-1-ol). Our results suggest terpenoid concentrations in one-seed juniper are related to season, sapling size, and browsing by small ruminants.

Keywords Goats · Sheep · One-seed juniper · Terpenes · Herbivory · Resource availability hypothesis · Oxygenated terpenes · Targeted grazing

Introduction

Shrub encroachment into rangelands is a global concern with serious ecological implications for soil erosion, water quality, and biodiversity. One-seed juniper (*Juniperus monosperma* [Engelm.] Sarg.) is one such woody species dominating millions of hectares of rangelands in the western United States (Romme et al. 2009). Woody plants generally are chemically defended by plant secondary metabolites (PSM) and are typically regarded as low quality forage for domesticated livestock due to palatability and toxicity issues (Estell 2010). However, both positive constituents (nutrients) and negative defense mechanisms (physical and chemical) interact in a complex manner to affect consumption of woody plants by ruminants (Estell 2010).

Differential use of individual plants within a species by herbivores is common for many woody plants; this variable intake has been related to the presence and/or concentration of terpenoids in some species (Adams et al. 2013a; Estell et al. 1998a; Markó et al. 2008). Terpenoids have been found to be aversive to intake of several woody species by ruminants (Bray et al. 1991; Riddle et al. 1996; Schwartz et al. 1980a,

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b). Markó et al. (2008) and Adams et al. (2013a) observed lower total essential oil concentrations in browsed than unbrowsed *J. communis* and ashe juniper (*J. ashei)*, respectively. α-Pinene content was negatively related to redberry *(J. pinchotti)* and ashe juniper consumption by goats and deer (Adams et al. 2013a, b; Riddle et al. 1996). Utsumi et al. (2009) identified 11 terpenoids related to one-seed juniper intake by protein-supplemented goats or sheep; however, α-pinene was unrelated to intake in that study.

Because loss of grasslands due to shrub encroachment, conversion of rangeland to cropland, and a variety of other reasons is occuring at a time when global demand for red meat is rising, methods are needed to increase woody plant use by ruminants (Estell et al. 2012). A better understanding of factors controlling intake may lead to mechanisms to increase consumption of less palatable plant species. In addition to supplying an alternative source of nutrients, prescription grazing with small ruminants may provide a biocontrol tool for one-seed juniper (Utsumi et al. 2010). Targeted grazing (e.g., with goats) could allow land managers to extend mechanical removal treatments through suppression of seedling recruitment and sapling density of woody species (Archer 1995; Launchbaugh and Walker 2006). A study was conducted by Utsumi et al. (2010) to examine targeted grazing with sheep and goats to control one-seed juniper encroachment. In that study, browsing intensity was greater during summer than spring, and for short vs. medium or tall saplings. Our objective in this companion study was to determine the relationship between one-seed juniper terpenoid content and herbivory by small ruminants under field conditions and the effect of season and sapling size on this relationship. We hypothesized that terpenoid concentration and extent of herbivory would be inversely related, and that terpenoids would be lower during summer and in short juvenile saplings.

Methods and Materials

Study Site The study was conducted at the New Mexico State University Corona Range and Livestock Research Center in central New Mexico (34° 15'36"N, 105° 24'36"W; elevation: 1,900 m; average annual precipitation: 327±96 mm; 65 % of rainfall between May and September). The site has gentle to moderate topography dominated by the Tapia-Dean soil association (USDA-Soil Conservation Service 1970). One-seed juniper and pinyon pine (*Pinus edulis* Engelm.) are the dominant woody species. The study site was located in a 341 ha pasture that is heavily infested with one-seed juniper, and is lightly winter-grazed by cattle each year. Woodlands were mechanically removed from most of the pasture in the late 1980's but heavy reinvasion of one-seed juniper saplings has occurred in most cleared areas.

Study Description The study was part of a larger effort to examine prescription grazing with small ruminants as a biocontrol tool for one-seed juniper (Utsumi et al. 2010). Experiments were conducted in July and August 2006 (summer) and May and June 2007 (spring) in a 1.3 ha area with a high density of one-seed juniper saplings (527±136 saplings/ha; 0.82±0.41 m height). Targeted grazing treatments applied in the companion study consisted of goats alone or with sheep at two stocking densities during two seasons. Mature Western White Face ewes (N=4; mean BW=69.2± 0.9 kg) and Boer-Spanish does (N=15; mean BW=47.9± 1.1 kg) experienced with New Mexico rangelands containing juniper were used to apply treatments. Briefly, treatments consisted of goats alone (N=10) or goats plus sheep (N=5 goats and 4 sheep), each at two stocking densities (high= 10 m^2/animal unit/day; low=60 m^2/animal unit/day). Groups grazed in 20×30 m cells during the day (0700 to 1800 hr) for 6 d, either continuously (low density) or rotated daily through 10×10 m subcells (high density). The two animal groups grazed low and high density paddocks in consecutive 6 d periods each season, with groups switched between density levels for the two blocks (sequence determined randomly). Each treatment was replicated twice and two adjoining control plots were excluded from grazing, resulting in two complete blocks per season (four species/density combinations plus a control cell in each block). Previous data from the same animals were used to identify goats as high or low juniper consumers and to stratify them among treatment groups, which remained intact throughout the study. All protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee (Protocol No. 2006–038).

Sampling Density and height of all juniper saplings in the study plots were recorded prior to grazing. Saplings were classified as tall (>1 m), medium (1–0.5 m), or short (<0.5 m). Frequency of defoliation of all saplings was measured immediately after completion of each treatment application. Extent of sapling defoliation was estimated as described by Bonham (1989). Degree of defoliation (0–33 %, 34–66 %, and 67–100 % for light, moderate, and heavy, respectively) was categorized based on percentage of total branches browsed. The number of saplings in each defoliation category was determined for each height category in each grazing cell.

Leaf samples were collected from 311 juniper plants immediately after defoliation measurements were recorded (N= 139 and 172 samples in spring and summer, respectively). This sample size represented nearly half of the total number of junipers (632 individual saplings) in all grazing cells (excluding control plots). Efforts were made to sample three saplings in each of the three defoliation categories for all three height categories in each grazing cell. However, this was not always

possible because combinations of sapling size and defoliation categories did not occur evenly in each cell. Approximately 10 to 20 g of leaves were removed from 10 randomly selected leaders of current year's growth on each plant. Leaders (including leaves and small terminal twigs) were placed in plastic bags immediately after clipping and placed on dry ice, transported to the laboratory, and frozen at −20 °C.

During both sampling periods, heavy bark use was noted on many of the saplings in the study plots. Consequently, bark samples were collected during the spring sampling period from juniper saplings outside the treatment paddocks. Twelve juniper saplings ranging from 0.5 to 1.5 m in height and within 50 m of the perimeter of the grazed plots were randomly selected, and approximately 15 g of bark were stripped from the main stem with scissors and handled as described for leaves.

Analytical Procedures Approximately 10 g of both leaves and bark were ground in liquid nitrogen in a Micro-mill Grinder (Bel-art products, Pequannock, NJ, USA) to approximately a 0.5 mm particle size, and were mixed thoroughly, placed in a plastic bag, and stored at −80 °C. After thawing, dry matter was determined (0.5 g in duplicate at 105 °C for 24 hr). Terpenoids were extracted in ethanol (Tellez et al. 1997; Utsumi et al. 2006). Samples (0.5 g) were extracted in a 30-ml widemouth bottle (capped) on an orbital shaker (Henry Troemner, LLC, West Deptford, NJ, USA) for 5 min at 150 RPM with 5 ml of 100 % ethanol fortified with 5 mg/ml of longifolene (99 %, Aldrich Chemical Co., Milwaukee, WI, USA) as an internal standard. Extracts were filtered through a fiberglass (Fisherbrand G8) filter (2.5 cm o.d.) with a disposable plastic syringe into 20-ml vials and stored at −20 °C until analysis. Blanks were prepared as described above without juniper.

Extracts were analyzed by gas chromatography/mass spectrometry using a Finnigan ion trap mass spectrometer (EI, 70 eV, Thermoelectron Corporation, Waltham, MA, USA) coupled to a Varian gas chromatograph (model 3400) equipped with a CTC-A200s autosampler and a DB-5 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film, 5 mol/100 mol phenyl-methylpolysiloxane coating; J&W Scientific, Santa Clara, CA, USA). Helium served as the carrier gas (1 ml/min), the split flow was 20 ml/min (ratio 20:1), and injection volume was 1 μl (duplicate injections). Column conditions were as follows: detector temperature 260 °C; injector temperature 220 °C; transfer line temperature 240 °C; initial column temperature 60 °C; final column temperature 240 °C; rate of temperature increase 3 °C/min (Adams 1995; Tellez et al. 1997). Compounds were identified by comparing mass spectrum and retention time with authentic compounds when available. Otherwise, compounds were tentatively identified by comparison with spectral libraries (Adams 1995) and Kovats retention indices. Concentrations of individual compounds were estimated with the internal standard and averaged across duplicate injections prior to statistical analysis. The total concentration was estimated from the sum of individual concentrations.

Statistical Analyses Data were collected in conjunction with a study to examine the use of small ruminants to suppress one-seed juniper sapling encroachment (Utsumi et al. 2010). Three saplings for each defoliation/height combination were not always present, but samples were fairly evenly distributed across the four treatments. Thus, data were pooled across treatments prior to analysis (N=139 and 172 saplings for summer and spring, respectively; N=73, 125, and 113 for short, medium, and tall saplings, respectively; N=132, 100, and 79 for heavy, moderate, and lightly browsed saplings, respectively). Within sapling size/browsing intensity combinations, N=35, 52, 45, 19, 41, 40, 19, 32, and 28 for heavy browsing/short saplings, heavy browsing/medium saplings, heavy browsing/tall saplings, moderate browsing/short saplings, moderate browsing/medium saplings, moderate browsing/tall saplings, light browsing/short saplings, light browsing/medium saplings, and light browsing/tall saplings, respectively. Relationships between terpenoid content and amount of herbivory, sapling size, and season were examined with analysis of variance for a randomized complete block design (two blocks per season) using Proc Mixed with season as a fixed effect (SAS 2004). Means were separated with *LSD* (α=0.05). Multivariate analysis using stepwise discriminant analysis (SAS 2004) was conducted on the subset of 211 saplings that received light or heavy herbivory to identify the smallest set of terpenoids capable of discriminating between saplings in the two groups. Bark samples were collected for descriptive purposes only and were not subjected to statistical analysis.

Results

The volatile profile of one-seed juniper leaves consisted of 65 terpenoids, including one unknown compound (Table 1). Total estimated terpenoid concentration was 18.3 mg/g of DM. The dominant compound in the profile was α-pinene (60.8 % of total volatiles). Only four other compounds comprised more than 4 % of the total concentration (limonene + β-phellandrene, 7.6 %; 3-carene, 4.3 %; β-eudesmol, 4.2 %; α-eudesmol, 4.0 %). Total estimated terpenoid concentration in juniper bark was 8.9 mg/g of DM (less than half the concentration in leaves). Bark contained 55 total compounds (Table 1), 40 of which were also present in leaves. Only α-pinene (>86 % of total concentration) and two other compounds (myrcene and limonene) were above 1 % of the total concentration in bark.

Table 1 Mean concentrations of terpenoids in one-seed juniper leaves and bark

Chemical[a,b]	Leaves		Bark	
	Mean	*SEM*[c]	Mean	*SEM*[d]
Total volatiles[e]	18267.86	307.21	8845.72	825.32
Tricyclene	21.84	0.50	9.72	0.92
α-Thujene[b]	9.47	0.44	13.40	2.32
α-Pinene	11107.34	218.81	7631.90	710.17
Camphene	60.16	1.79	25.22	3.09
Verbenene[b]	20.43	1.89	14.68	5.31
Sabinene	22.34	1.79	67.52	5.93
β-Pinene	134.68	2.87	85.28	8.90
Myrcene	310.33	6.85	195.64	17.63
2-Carene	13.79	0.74	–	–
α-Phellandrene	145.19	8.12	–	–
3-Carene	800.12	59.06	19.19	8.57
α-Terpinene	5.13	0.24	2.16	0.31
p-Cymene	31.86	0.94	6.15	2.93
Limonene + β-Phellandrene[b]	1378.49	29.62	100.51[f]	8.68
(*Z*)-β-Ocimene	1.75	0.10	1.52	0.38
(*E*)-β-Ocimene[b]	22.54	1.56	25.49	3.89
γ-Terpinene	61.18	1.97	2.98	0.39
cis-Sabinene hydrate	6.70	0.27	1.35	0.27
Terpinolene	161.08	5.06	51.91	3.23
α-Pinene oxide[b]	–	–	6.40	1.22
trans-Sabinene hydrate + Linalool	9.37	0.58	1.88[g]	0.52
cis-*p*-Menth-2-en-1-ol[b]	29.03	0.86	–	–
α-Campholenal[b]	1.46	0.18	19.44	2.70
trans-Pinocarveol[b]	–	–	28.43	4.18
trans-*p*-Menth-2-en-1-ol[b] + *trans*-Pinene hydrate[b]	13.34	0.42	–	–
Camphor	18.00	0.82	5.51	0.90
trans-Verbenol[b]	–	–	33.39	4.90
Isopulegol[b]	18.63	0.75	–	–
Camphene hydrate[b]	8.79	0.65	–	–
trans-Pinocamphone[b]	–	–	4.15	0.68
Pinocarvone[b]	2.55	0.16	7.77	1.31
Borneol	4.43	0.22	2.75	0.45
Terpin-4-ol	14.48	0.50	2.99	0.50
p-Cymen-8-ol[b]	2.14	0.12	–	–
α-Terpineol	6.69	0.42	–	–
Myrtenal[b]	–	–	1.78	0.46
cis-Piperitol[b]	4.84	0.35	–	-
Myrtenol[b]	–	–	5.73	0.88
Verbenone[b] + *trans*-Piperitol[b]	9.00	0.38	24.59[h]	3.75
trans-Carveol[b]	–	–	5.36	0.79
endo-Fenchyl acetate[b]	2.77	0.17	–	–
Myrtenyl acetate[b]	–	–	11.33	3.91
Carvone[b]	7.45	0.72	–	–
trans-Sabinene hydrate acetate[b]	14.43	0.49	2.65	0.80
Methyl citronellate[b]	2.32	0.11	–	–
Bornyl acetate[b]	64.32	1.80	53.16	9.25
trans-Verbenyl acetate[b]	5.22	0.24	–	–
trans-Pinocarvyl acetate[b] + Carvacrol[b]	3.61	0.19	–	–
cis-Piperityl acetate[b]	16.46	0.67	–	–
trans-Carvyl acetate[b] + Δ-Elemene[b]	2.63	0.11	3.61[i]	0.69
trans-Piperityl acetate[b] + Terpinen-4-yl acetate[b]	8.27	0.38	–	–
α-Terpinyl acetate[b]	8.53	0.50	–	–
α-Cubebene[b]	–	–	8.37	3.70
trans-Myrtanyl acetate[b]	–	–	5.90	0.84
β-Elemene[b]	3.18	0.23	2.03	0.44
β-Caryophyllene	116.84	2.91	42.90	9.44
cis-Thujopsene[b]	–	–	19.08	6.08
Unknown 1 (KI=1452)	68.41	1.84	–	–
α-Humulene	45.00	2.16	1.28	0.32
(*E*)-β-Farnesene[b]	3.13	0.09	–	–
Thujopsadiene[b]	–	–	1.30	0.43
γ-Muurolene[b]	–	–	2.95	0.74
Germacrene D[b]	19.89	0.73	13.33	3.47
β-Selinene[b]	12.61	0.34	4.88	1.05
Viridiflorene[b] + α-Selinene[b]	11.24	0.43	4.46[j]	0.91
Germacrene A[b]	7.36	0.30	6.11	1.53
γ-Cadinene[b]	15.71	8.25	–	–
Δ-Cadinene[b]	18.07	1.19	–	–
Selina-3,7(11)-diene[b]	110.96	7.35	–	–
Unknown 2 (KI=1553)	–	–	19.48	2.77
Germacrene B[b]	186.41	5.63	82.29	21.42
(*E*)-Nerolidol	15.92	0.56	–	–
Caryophyllene oxide	6.99	0.24	8.43	1.71
Cedrol[b]	–	–	13.07	4.57
γ-Eudesmol[b]	112.65	6.16	11.89	1.57
β-Eudesmol[b]	762.52	23.04	41.27	6.33
α-Eudesmol[b]	737.97	18.36	32.39	4.81
8-α-11-Elemodiol[b]	567.38	25.52	–	–
8-α-Acetoxyelemol[b]	730.62	24.72	48.77	16.27
Cryptomeridiol[b]	121.79	3.77	–	–

[a] Compounds were identified with Kovats retention indices and mass spectral libraries; concentrations (μg/g DM) were estimated based on relative proportions of longifolene (internal standard)

[b] Tentatively identified (Adams 1995); authentic standards used to identify all other volatiles

[c] *SEM* = standard error of mean; *N*=311 leaf samples (pooled across season, sapling size, and browsing intensity)

[d] *SEM* = standard error of mean; *N*=12 plants sampled during spring

[e] Total volatiles = cumulative estimated concentrations of individual compounds

[f] Limonene only

[g] *trans*-Sabinene hydrate only

[h] Verbenone[b] only

[i] Δ-Elemene[b] only

[j] α-Selinene[b] only

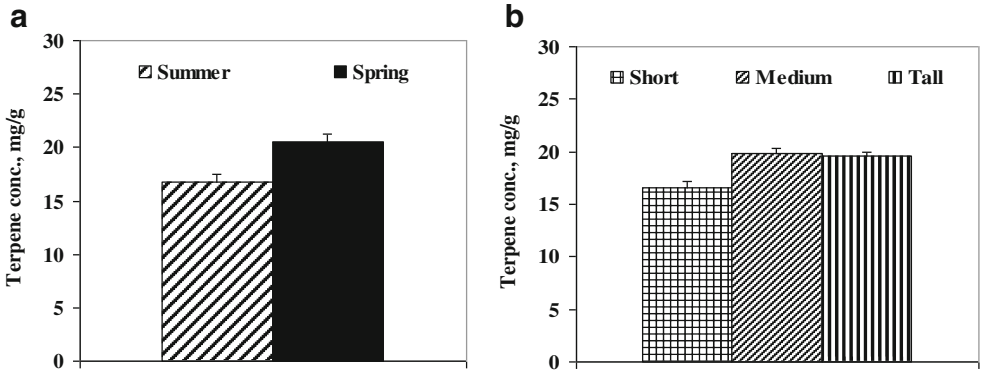

Fig. 1 Effect of season (**a**) and sapling size (**b**) on total terpene concentration (mg/g DM) in one-seed juniper; summer = July-August, 2006; spring = May-June, 2007; sapling size = short (<0.5 m), medium (0.5–1 m), and tall (>1 m); *N*=139 and 172 for summer and spring, respectively; *N*=73, 125, and 113 for short, medium, and tall saplings, respectively

Total terpenoid concentrations differed ($P<0.001$) between seasons (20.6±0.5 vs. 16.7±0.3 mg/g for spring and summer, respectively; Fig. 1a) and among sapling heights ($P<0.001$). Short saplings contained lower concentrations of total volatiles than medium or tall saplings (16.5±0.6 vs. 19.8±0.4 and 19.5±0.4 mg/g, respectively; Fig. 1b). Total concentrations also differed among the three browsing categories ($P<0.001$), with 21.2±0.6, 18.7±0.5, and 16.1±0.4 mg/g of DM for light, moderate, and heavy use categories, respectively (Fig. 2a). A sapling size x browsing intensity interaction was detected ($P<0.003$) that revealed an inverse relationship between herbivory and terpenoid concentration for short saplings (Fig. 2b). Eight compounds discriminated ($P<0.001$) saplings into lightly and heavily browsed groups ([*E*]-β-farnesene, bornyl acetate, γ-eudesmol, *endo*-fenchyl acetate, γ-cadinene, α-pinene, *cis*-piperitol, and *cis*-*p*-menth-2-en-1-ol; Table 2). The discriminant function correctly classified 79 % of saplings into heavy vs. light herbivory categories in the cross-validation procedure. Classification error rates were lower for heavily vs. lightly browsed saplings (11 vs. 39 %). [*E*]-β-farnesene weighed most heavily in discriminating between saplings in the heavy and light herbivory categories (Table 2). With the exception of α-pinene, all of these compounds were minor constituents of juniper leaf tissue (0.01–0.6 % of DM), and most were oxygenated compounds. Concentrations of most of these compounds were lower in heavily browsed saplings (Table 2).

Discussion

The study was part of a larger effort to examine the efficacy of prescription grazing with small ruminants as a bio-control method for one-seed juniper (Utsumi et al. 2010). The goal of this study was to evaluate the role of terpenoids in one-seed juniper herbivory during two seasons and for three different sapling sizes. Use of one-seed juniper was greater during summer than spring and for short than medium or tall saplings. These results support our hypotheses that variations in browsing patterns were due to variations in terpenoid content of saplings of different sizes and in different seasons.

Plant Chemistry Total terpenoid concentrations and dominant compounds are consistent with previous results using the same methodology (Utsumi et al. 2006; 20.0 mg/g of DM and 64.7 % α-pinene). Other studies have reported α-pinene in one-seed juniper ranging from 52 to 63 % of the total essential oil (Adams et al. 1981; Dearing et al. 2000). Though little information is available on terpenoid concentrations in one-seed juniper bark, Adams (1987) examined oil

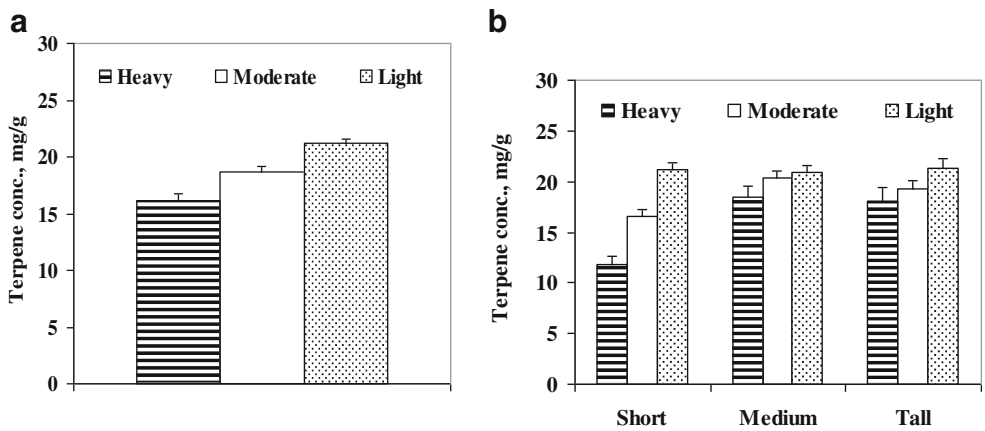

Fig. 2 Relationship between one-seed juniper use by small ruminants and total terpene concentration (mg/g DM) across (**a**) and within (**b**) plant size; sapling size = short (<0.5 m), medium (0.5–1 m), and tall (>1 m); browsing intensity = heavy (>66 %), moderate (33–66 %) , and light (<33 %); *N*= 132, 100, and 79 heavy, moderate, and lightly browsed saplings, respectively

Table 2 Smallest subset of terpenoids separating heavily and lightly browsed one-seed juniper using discriminant function analysis[a]

Step[c]	Compound	Discriminant Function Coefficient	Mean[b] ± *SEM*	
			Heavy	Light
1	(*E*)-β-Farnesene	0.253	2.5±0.13	3.9±0.21
2	Bornyl acetate[d]	0.010	53.1±2.61	76.6±3.51
3	γ-Eudesmol[d]	−0.006	124.6±9.68	93.5±11.07
4	*endo*-Fenchyl acetate[d]	−0.114	2.8±0.29	2.8±0.26
5	γ-Cadinene	0.029	5.6±0.68	11.3±1.78
6	α-Pinene	0.0001	9702.6±313.78	12654.1±416.41
7	*cis*-Piperitol[d]	−0.056	4.8±0.54	4.2±0.40
8	*cis*-*p*-Menth-2-en-1-ol[d]	0.020	26.0±1.16	31.4±1.87
	Wilks' Lambda	0.67		
	F	12.27		
	P	<0.001		

[a] Browsing intensity categories = heavy (>66 %) and light (<33 %)

[b] μ/g DM; *SEM* = standard error of mean; *N*=132 and 79 for heavy and light browsing categories, respectively

[c] Order of compound entry into discriminant function by stepwise selection procedure

[d] Oxygenated compounds

yields from hexane extracts of leaves and bark/sapwood of *J. monosperma*, and observed approximately 2 to 3-fold greater yields from leaves than bark/sapwood. Of the 80 compounds listed in Table 1, 15 were present only in bark, and 25 were present only in leaves. Although not statistically comparable, most of the compounds present in both tissues were numerically greater in leaves (32 of 40 compounds), many by an order of magnitude. However, it is not possible to make inferences about bark terpenoids with respect to browsing because only 12 samples were collected during spring season from outside the browsed area.

Season Terpenoid profiles and concentrations of *Juniperus* species have been reported to vary among individual plants and due to season (Animut et al. 2004; Riddle et al. 1996; Utsumi et al. 2006, 2009; von Rudloff 1975), so seasonal patterns of juniper intake were not unexpected. Utsumi et al. (2009) reported mean total terpenoid concentrations of 17.0, 23.6, 20.0, and 21.2 mg/g of DM for one-seed juniper harvested during summer, fall, winter, and spring, respectively. Oil concentration of *J. communis* also varied with season, being lower in summer than for the other three seasons (Markó et al. 2008). Adams (1987) reported oil yields from one-seed juniper leaves harvested between May and December were fairly stable through July and then increased in September and peaked in November. Adams (1987) also followed seasonal trends of a closely related species (*J. osteosperma*) through a full year and observed fairly stable oil yields during summer months which increased during fall, peaked in January, and declined during spring to the previous summer levels.

Use of short juvenile saplings was heaviest in summer (Utsumi et al. 2010), which coincides with lowest concentrations of terpenoids (Fig. 2a). This observation is consistent with our previous findings that intake of one-seed juniper harvested in the fall was approximately half that harvested during the other three seasons and contained more total terpenoids (Utsumi et al. 2009). Bark girdling/stripping was more prevalent in spring (Utsumi et al. 2010) when terpenoid concentrations in leaves were greater (Fig. 2a).

Plant Size Plant age has been reported to affect terpenoid concentrations in some woody species. Though the relationship varies among species, younger plants often are more chemically defended than older (larger) plants (Bryant et al. 1991; Meyer and Karasov 1991; Swihart and Bryant 2001). Although plant height is not a direct measurement of plant age, it served as a surrogate in this study. We recognize that age and size may be confounded by differential growth among years due to environmental factors such as amount and timing of precipitation. Lower terpenoid concentrations in short juvenile juniper saplings (Fig. 1b) support the notion of lower chemical defense in younger leaf tissue (i.e., needle leaf stage), particularly in light of the inverse relationship between herbivory and terpenoid concentrations in short saplings (Fig. 2b). We were unable to detect a difference in terpenoids (total concentration or major individual compounds) between tall and short one-seed juniper saplings in a preliminary investigation (Utsumi et al. 2006). However, observations in that study were based on 10 saplings from each of two sizes (0.5 or 1.1 m height). Campbell and Taylor (2007) observed lower terpenoid levels (total concentration and all individual compounds except α-pinene) in juvenile redberry juniper plants (regrowth three months post fire) than at 11 months of age (which did not differ from unburned controls).

The results of our study are in agreement with Campbell and Taylor (2007) and in contrast with the general theory of greater investment of chemical defense for critical young tissue. However, our results may partially support the resource availability hypothesis (Coley et al. 1985) that predicts higher levels of plant defenses in slow vs. fast growing plants, a phenomenon which has been documented across a broad array

of plant taxa (Endara and Coley 2011). Terpenoid concentrations in our study were lower in saplings during stages (ontological or phenological) when growth rates were presumably highest (young individuals or peak growing season). During these periods, saplings may have allocated more carbon to synthesize primary metabolites to support active tissue growth, and diverted more photosynthate to synthesis of carbon-based defense compounds in periods of slower growth (older saplings or after peak growing season). We also observed ontological differences in leaf morphology, with seedlings and most juvenile sapling twigs containing spiny needle-like leaves and more rounded scale-like leaves in taller saplings. Though needles may serve as a general physical defense for young tissue prior to the synthesis of adequate terpenoid concentrations, goats generally prefer early needle-leaf stage (Lyons et al. 1998), suggesting this physical defense is less effective for protecting young saplings from herbivory by small ruminants.

Herbivory by Ruminants Terpenoids (individual compounds and/or total concentrations) have been reported to deter intake and/or preference in goats (Animut et al. 2004; Pritz et al. 1997; Riddle et al. 1996), deer (Bray et al. 1991; Schwartz et al. 1980a, b; Vourc'h et al. 2002), and sheep (Estell et al. 1998b; Villalba et al. 2006). Our data support the notion of a role of terpenoids in differential use of one-seed juniper by small ruminants. This relationship has been observed in other *Juniperus* species. Markó et al. (2008) observed differential use of juniper in Hungary. Essential oil concentration was highest in undamaged juniper and lowest in heavily browsed junipers in that study. Adams et al. (2013a) also reported more total oil in non-browsed ashe juniper than those browsed by deer and goats. In contrast, Adams et al. (2013b) observed no difference in oil yields between browsed and unbrowsed redberry juniper. Variable terpenoid concentrations and profiles observed for different juniper species (Adams 1987; Adams et al. 1981; von Rudloff 1975) may explain the conflicting results.

The companion study (Utsumi et al. 2010) revealed that pooled over season, approximately 63 % of the short saplings were in the heavy use category, compared to ~42 and 46 % of tall and medium sized plants, respectively. Slightly less than 20 % of saplings were in the light use category for all three plant sizes; thus, differences in herbivory levels were due primarily to relative proportions of heavy vs. moderately used plants in the three size categories. The fact that short saplings had a greater percentage of heavy use and a lower total volatile concentration supports our hypothesis of an inverse relationship between use and terpenoid concentrations. In the same study, approximately 55 % of tall saplings exhibited heavy bark stripping, while ~78 % of short plants were in the light category for bark use (Utsumi et al. 2010). Unfortunately, bark samples for chemical analysis were obtained from plants not exposed to browsing and therefore we are unable to make any inferences regarding relationships between bark use and terpenoid concentrations.

Of the eight compounds present in the smallest subset discriminating between low and high use junipers, five were greater in lightly browsed juniper ([*E*]-β-farnesene, bornyl acetate, γ-cadinene, α-pinene, *cis*-*p*-menth-2-en-1-ol), and three were greater for heavily used juniper (γ-eudesmol, *endo*-fenchyl acetate, *cis*-piperitol). Little information is available concerning the eight compounds that discriminated between the two defoliation categories with respect to mammalian herbivory, with the exception of α-pinene. α-Pinene has been reported to be negatively related to intake by ruminants (Adams et al. 2013a, b; Estell et al. 1998b; Riddle et al. 1996; Vourc'h et al. 2002). In contrast, α-pinene was positively related (Markó et al. 2008) or unrelated (Utsumi et al. 2009) to juniper consumption in other studies. γ-Eudesmol was a predictor of juniper intake for protein-supplemented goats and sheep (Utsumi et al. 2009). Five of the eight discriminatory compounds were not present in juniper bark.

Adams et al. (2013a) identified 12 terpenoids with greater concentrations in unbrowsed ashe juniper, while Markó et al. (2008) reported lower concentrations of 3-carene and myrcene in browsed juniper. Utsumi et al. (2009) observed 11 terpenoids that were negatively related to one-seed juniper consumption by sheep or goats, five of which were oxygenated compounds. Two of the five compounds in the present study that were browsing deterrents are oxygenated. Oxygenated monoterpenoids and/or sesquiterpenoids have been reported to have negative effects on preference and digestion of various juniper species by deer (Schwartz et al. 1980a, b). Furthermore, Malecky et al. (2012) reported that oxygenated terpenoids were less extensively degraded than hydrocarbon terpenoids in an *in vitro* fermentation system.

Given the inconsistencies among the aforementioned studies and others in the literature with respect to individual compounds and their relationships with within-species differential herbivory, it appears that individual compounds are less important than total concentration of essential oils. However, differences among herbivore species, concentrations and relative proportions of other compounds (and potential synergysm/antagonism), variations due to season and a host of other biotic and abiotic factors, as well as differing concentrations of nutrients and other co-occurring classes of compounds (e.g., tannins, phenolics, etc.) likely all contribute to the inconsistent results among studies regarding individual compounds. Other classes of PSM are present in one-seed juniper (e.g., phenolics and tannins; Nunez-Hernandez et al. 1989; Utsumi et al. 2009) that also could contribute to low palatability and differential use of juniper. Utsumi et al. (2009) reported a positive correlation between total terpenoids and total phenolics in one-seed juniper, while Adams et al. (2013a) noted a negative relationship between total oil concentration

and condensed tannins in ashe juniper. In this context, differences in enantiomeric composition of chiral constituents, especially terpenoids, also may play a rôle.

Practical Considerations Shrubs are expected to become increasingly important components of range livestock diets, given the increasing global demand for red meat and the concomitant decline in grasslands (Estell et al. 2012). Although we recognize the potential negative impacts of terpenoids on rumen microbes (Broudiscou et al. 2007; Nagy and Tengerdy 1968; Schwartz et al. 1980a) and animal physiology (Bisson et al. 2001; Pritz et al. 1997), some terpenes have been observed to increase rumen microbial activity. For example, α-pinene (the dominant terpene in one-seed juniper) stimulated bacterial activity and volatile fatty acid production (Broudiscou et al. 2007). However, nutrient requirements of animals consuming terpenoid-rich diets may increase to accommodate metabolic costs of detoxification (Illius and Jessop 1995; Utsumi et al. 2013).

Several strategies to expand the use of shrubs have been advanced that minimize the deleterious effects of PSM on herbivores, including targeting specific nutrients and additives for detoxification (e.g., protein, polyethylene glycol), using adapted individuals/breeds/species, implementing specific management strategies (e.g., stocking density that encourages learning and temporal associations that capitalize on complementarity), and targeted timing of use (Campbell et al. 2007; Estell et al. 2012; Mote et al. 2008; Provenza et al. 2007; Shaw et al. 2006; Utsumi et al. 2009, 2010, 2013; Villalba and Provenza 2005). Feeding strategies that allow animals to dilute toxins or increase length of time for clearance may improve consumption of juniper. Reducing length of eating bouts, increasing inter-bout length, and diet switching between forages may allow animals time to detoxify terpenoids and maintain blood concentrations below toxic levels and avoid feeding cessation (Dziba et al. 2006; Foley et al. 1999; Wiggins et al. 2006). Timing of use may be an important consideration for implementing some of these strategies. For example, Utsumi et al. (2009) found that during the fall when terpenoid levels were highest, dietary protein had little impact on one-seed juniper intake by sheep and goats, but during the other three seasons when concentrations were lower, protein improved intake. In a follow up study, Utsumi et al. (2013) found that several amino acids were depleted when animals consumed juniper, and supplemental protein counteracted this depletion to some extent and may improve the ability of ruminants to cope with terpenoid ingestion and increase juniper consumption. Strategies that help ruminants cope with PSM can be integrated with knowledge about factors that control PSM variation to assist producers with development of targeted grazing approaches that optimize browsing timing and location and maximize susceptibility to herbivory.

In summary, sapling size and season affect terpenoid concentrations in one-seed juniper. Terpenoid concentration and composition of one-seed juniper both affect herbivory by small ruminants. Given the lower terpenoid concentrations during summer and the heavy use of short saplings with low terpenoid concentrations, targeted use of short saplings in the summer should be an effective method for simultaneously using one-seed juniper as a dietary component and small ruminants as a biocontrol tool. Understanding the role of chemistry in differential use will lead to mechanisms to enhance utilization by livestock and improved methods for targeted biocontrol of shrub encroachment.

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