Technical Note

Influence of Plant Material Handling Protocols on Terpenoid Profiles of One-Seed Juniper Saplings

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Abstract

Accurate estimation of one-seed juniper (*Juniperus monosperma* [Engelm.] Sarg.) intake by herbivores often requires harvesting, transporting, and storing plant material that is later used in pen experiments. Such manipulation could alter terpenoid profiles and modify herbivory levels significantly. We used gas chromatography mass spectrometry (GC/MS) to analyze the terpenoid profile of leaves from 10 short (0.5 m ± 0.05, mean ± SE) and 10 tall (1.14 m ± 0.06) one-seed juniper saplings subjected to 3 handling protocols: a) placed on dry ice after clipping and stored after 5 hours at −80°C for 3 weeks (Control); b) kept at ambient temperature for the first 24 hours and then frozen at −80°C for 3 weeks; or c) kept at ambient temperature for the first 24 hours, and then stored at 8°C for 3 weeks. Juniper saplings contained 51 terpenoids, 3 of which were unknown compounds. Fourteen terpenoids accounted for 95% of the total amount of volatiles. The most abundant compound was α-pinene, which accounted for 65% of total terpenoids present. Handling protocols were not associated with detectable differences in total terpenoid content (Means ± SE, Control: 21.68 ± 1.42 mg·g^{-1} dry matter [DM]; Frozen after 24 hours: 19.55 ± 1.08 mg·g^{-1} DM; Refrigerated after 24 hours: 18.80 ± 1.13 mg·g^{-1} DM). However, total terpenoid amount and concentration of a few major compounds tended to decrease with increasing storage temperature. Handling protocols induced detectable variations in a small number of minor terpenoids. We observed large among-plant variation in terpenoid profiles that was not fully explained on the basis of sapling size. This study suggests that the length of storage period of one-seed juniper branches should not exceed 3 weeks and that storage refrigeration temperatures should be kept below 8°C to prevent significant alterations in terpenoid profiles.

Resumen

Estimación precisa del consumo de *Juniperus monosperma* por herbívoros a menudo requiere cosecha, transporte y almacenaje de material de planta que luego es usado en experimentos a corral. Tal manipulación podría alterar el perfil de terpenos y modificar significativamente los niveles de herbivoría. Usamos cromatografía gaseosa junto a espectrometría de masa para analizar el perfil de terpenos de hojas de 20 renuevos de *Juniperus monosperma* pequeños (0.5 m ± 0.05; n = 10) y grandes (1.14 m ± 0.06; n = 10), sujetas a 3 protocolos de manipulación: a) puestas en hielo seco después de cosecha y almacenadas luego de 5 horas a −80°C por 3 semanas (Control); b) mantenidas a temperatura ambiente durante las primeras 24 horas, y luego congeladas a −80°C por 3 semanas; o c) mantenidas a temperatura ambiente durante las primeras 24 horas, y luego conservadas a 8°C por 3 semanas. Los renuevos de *Juniperus* presentaron 51 terpenos, 3 de los cuales fueron desconocidos. Catorce terpenos representaron el 95% de la cantidad total de volátiles. El compuesto más abundante fue α-pinene, representando el 65% del total de terpenos presentes. El contenido total de terpenos no difirió entre los tratamientos de manipulación (Control: 21.68 ± 1.42 mg·g^{-1} DM; Congelado luego de 24 horas: 19.55 ± 1.08 mg·g^{-1} DM; Refrigerado luego de 24 horas: 18.80 ± 1.13 mg·g^{-1} DM). A pesar de que la cantidad de terpenos totales y algunos terpenos mayores tendieron a disminuir al aumentar la temperatura de almacenaje, los protocolos de manipulación solo indujeron variaciones detectables en algunos terpenos menores. Detectamos gran variación en el perfil de terpenos entre plantas que no fue completamente explicado por el tamaño de los renuevos. Este estudio sugiere que el almacenaje de ramas de renuevo de *Juniperus* no debe exceder las 3 semanas y que la temperatura de refrigeración durante almacenaje debe mantenerse por debajo de los 8°C para prevenir alteraciones significativas en el perfil de terpenos.

Key Words: *Juniperus monosperma*, plant volatiles, gas chromatography, storage conditions, plant variation

INTRODUCTION

Terpenoids have been shown to deter herbivory in one-seed (*Juniperus monosperma* [Engelm.] Sarg.) and other juniper species (Riddle et al. 1996; Pritz et al. 1997; Dearing et al. 2000) as well as in a number of native woody invasive plants of western North America (Pfister 1999). This herbivore deterrence is a result of terpenoid toxicity, bitter flavor, and aversive odors, all of which are the result of distinct terpenoid mixtures that occur in specific plant tissues (Langenheim 1994). Terpenoid

This study was funded by the Joe Skeen Institute for Rangeland Restoration, the Corona Range and Livestock Research Center, the New Mexico Agricultural Experiment Station, and the USDA-ARS Jornada Experimental Range.

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Manuscript received 23 January 2006; manuscript accepted 24 August 2006.

chemical diversity arises from the sequential combination of a basic five-carbon polymer that can suffer chemical structure rearrangement by oxygenation, conjugation, and cyclization reactions giving distinct mono- and sesquiterpenoids. By synthesizing specific mono- and sesquiterpenoids, plants achieve different degrees of defense against specific herbivores and pathogens (Langenheim 1994). Riddle et al. (1996) found a close association between goats' intake and mono- and sesquiterpenoid profiles in Ashe's (*Juniperus ashei* Buchh.) and redberry (*Juniperus pinchotii* Sudw.) juniper plants. Estell et al. (1998) reported that the intensity of tarbush (*Flourensia cernua* DC) defoliation by cattle, sheep, and goats was explained by differences in mono- and sesquiterpenoid profiles of plants. Accurate characterization of terpenoid profiles in one-seed juniper is therefore essential to predict levels of juniper intake by browsers.

Controlled feeding trials using juniper often require harvesting, transporting, and storing sampled plant material from distant sites. Such manipulation procedures could alter terpenoid profiles significantly. For instance, plant tissue damage in conifers can result in induced relocation, rearrangement, and volatilization of mono- and sesquiterpenes from wounded sites (Langenheim 1994; Litvak et al. 2002). Furthermore, changes in the terpenoid profile of harvested plant material could also occur during storage, depending on the chemical properties of each compound, storage temperature, and length of storage time (Animut et al. 2004). No detailed work has been conducted, to our knowledge, examining variation in one-seed juniper terpenoid profiles that occur as a result of harvest, handling, and storage procedures commonly used in pen experiments in which harvested plant material is fed to test subjects.

The primary objective of this study was to determine the influence of handling protocols on terpenoid profiles of one-seed juniper saplings. In addition, given that resistance to herbivores in woody plants is predicted to increase rapidly during the sapling growth stage (Boege and Marquis 2005), we also determined the influence of sapling size on one-seed juniper terpenoid profiles. We predicted that plant materials that were not frozen immediately after harvesting would suffer terpene profile alterations, but that refrigeration would be sufficient to suppress significant terpene losses from harvested material. We also predicted that larger saplings would exhibit higher terpene levels than their smaller (younger) counterparts.

METHODS

Plant material was harvested at Corona Range and Livestock Research Center (CDRRC) during spring 2005. The CDRRC straddles the border between Torrance and Lincoln counties in New Mexico (34°15′36″N, 105°24′36″W; elevation = 1 900 m), has an average annual rainfall of 400 mm, and is approximately 298 km from the laboratory where analyses were performed.

Juniper saplings were randomly sampled at a heavily infested loamy ecological site dominated by the Tapia-Dean soil association (USDA-SCS 1970). Topography at this site is moderate to strongly sloping with soils that absorb water at moderate to rapid rates and exhibit good moisture-storage capacity and consolidated caliche layers at varying depths. In excellent condition, this site has good cover of perennial grasses. Pinyon pine (*Pinus edulis* Engelm.) and one-seed juniper are the most common woody invaders. Woodlands at our harvest site had been mechanically cleared in the 1980s.

We randomly selected and clipped nine leaders (current year's growth) on each of 20 saplings classified into two size (height) classes, small (0.5 m ± 0.05; n = 10, mean ± SE) and large (1.14 m ± 0.06; n = 10). Three leaders from each plant were placed together in a plastic bag and assigned to one of the following three treatments: 1) *Control:* placed in a container with dry ice (−78.5°C) immediately after clipping and kept there for the 5 hours of sample collection and transportation to the laboratory, then at the laboratory frozen at −80°C for 3 weeks; 2) *Frozen after 24 hours:* placed in a container at ambient temperature (approximately 20°C–25°C) for the first 24 hours (no dry ice) after clipping, then frozen at −80°C for 3 weeks; and 3) *Refrigerated after 24 hours:* placed in a container at ambient temperature for the first 24 hours (no dry ice) after clipping, then refrigerated at 8°C for 3 weeks.

After 3 weeks of storage, all sampled plant material was ground to particle diameters of less than 0.5 mm in liquid nitrogen, and approximately 10 g of each sample was kept for ethanolic extraction, terpenoid analysis, and dry mass determination. Ethanol extraction method was selected based on data from previous terpenoid extractions conducted by Tellez et al. (1997) and Estell et al. (1994) that showed that this method does not cause alterations in the chemical structure of terpenes observed with other high-temperature extracting methods such as steam distillation. Ethanol was the solvent selected based on data from sequential extractions with increasingly polar solvents that indicated that ethanol can extract a wider array of molecular weight terpenes than less polar solvents such as hexanes and ether (Tellez et al. 2001). Ethanol extractions were conducted in duplicate by shaking in 5 ml of ethanol 0.5 g (± 0.04) of ground material for 5 minutes at 150 RPM in a 20-ml scintillation vial containing 5 $mg \cdot ml^{-1}$ of Longifolene as an internal standard (IS). The 5-minute extraction time was used based on data reported by Tellez et al. (1997) that showed that 5-minute and 5-day solvent extractions of tarbush terpenes produced similar results.

The extracted solution was filtered into a 20-ml scintillation vial using a G8 glass wool filter and a 10-ml plastic syringe. The filtered solution was stored at −20°C until analysis. Blank samples without juniper were also prepared as described above. Analysis of the extracts was conducted by gas chromatography (GC) coupled to a mass spectrometer (MS) as described by Tellez et al. (1997). We used a Finnigan MAT Magnum GC/MS (ion trap mass spectometer, Thermoelectron Corporation, Waltham, MA) with CTC-A200s auto-sampler and equipped with a DB-5 column (30 mm long, 0.25 mm ID, 0.25 μm film; J&W Scientific, Santa Clara, CA) that used helium as carrier gas (1 $ml \cdot min^{-1}$) and worked with a split injection flow of 20 $ml \cdot min^{-1}$ (ratio 20:1), and an injection volume of 1 μl. The GC/MS programmed temperature run was set as follows: MS detector temperature = 220°C; injector temperature = 220°C; transfer line temperature = 240°C; initial column temperature = 60°C; and final column temperature = 240°C. The rate of column temperature increase was 3°C/min. Samples were injected in duplicate. Compounds were identified by comparing mass spectra and retention indices with those reported by Adams (1995). The relative amount of each compound was determined based on the percent peak area

relative to total peak area and known concentration of IS, expressed on a dry matter basis ($mg \cdot g^{-1}$ dry matter [DM]). Total terpenoid amount was calculated as the sum of all individual compounds, expressed on a dry matter basis. Dry matter was also estimated in duplicate by drying 0.5g of fresh plant material at 105°C for 24 hours.

One-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used for data analysis in a completely randomized design. ANOVA analyses were conducted using Proc GLM (SAS 1999) to assess differences in dry matter among plants and plant-to-plant variation in both total and individual terpenoid concentration in relation to handling protocol and sapling size. Multivariate discriminant analysis (DA) was conducted using Proc DISCRIM (SAS 1999) to determine whether individual samples could be discriminated into significantly different groups on the basis of handling protocol or sapling size using the entire suite of terpenoids in a single analysis. When significant discrimination was achieved ($P \leq 0.1$), stepwise discriminant analysis was conducted using Proc STEPDISC (SAS 1999) to identify the least set of terpenoids likely to classify samples into known handling protocol groups or sapling size classes. Significance level to enter a variable into the discriminant function in the stepwise iterations was fixed at $P = 0.10$. Prior to conducting all statistical analyses, we tested departures from normality and homogeneity of variance assumptions using Shapiro-Witt's and Levene's tests, respectively. Natural logarithm transformations were used to correct departures when observed (Steel and Torrie 1980). All diagnostic analyses were performed using SAS statistical software (SAS 1999).

RESULTS

Percentage dry matter did not differ among individual plants, plant size groups, or handling protocols ($P > 0.05$) and averaged 50.2% ± 1.2 (mean ± SE). Ethanol-extracted volatiles accounted for 4.1% ± 0.3 of dry matter and included 51 terpenoids. Only three terpenoids could not be identified. The most abundant terpenoid (α-pinene) accounted for 65% of the volatiles. Fourteen of the 51 compounds accounted for 95% of total oil content (Table 1). Concentration of individual terpenoids varied greatly, ranging from 0.004 to 12.941 $mg \cdot g^{-1}$ of dry mass.

Total volatiles extracted and concentration of major terpenoids did not differ among handling protocol treatments ($P > 0.05$; Table 2). Differences among handling protocols were detected only when all terpenoids were considered simultaneously in the multivariate discriminant analysis ($P = 0.08$; Table 3). Ten minor terpenoids accounting for only 0.7% of the total terpenoid fraction were responsible for the discrimination.

Total volatiles and concentrations of major terpenoids (with the exception of unknown 3) did not differ between plant size groups ($P > 0.05$). Again, differences between small and large plant categories were detected only when all terpenoids were considered together in the multivariate discriminant analysis ($P < 0.05$). Four minor terpenoids, α-campholenal, (e)-β-ocimene, unknown 3, and germacrene_D, accounting for only 1.9% of the total volatile fraction were responsible for this discrimination. Smaller saplings had higher concentrations of α-campholenal and germacrene_D (0.015 vs. 0.006 $mg \cdot g^{-1}$ DM, SE: 0.002; and 0.028 vs. 0.023 $mg \cdot g^{-1}$ DM, SE 0.002, respectively) whereas taller plants had higher concentration of (e)-β-ocimene and unknown 3 (0.051 vs. 0.019 $mg \cdot g^{-1}$ DM; SE: 0.008, and 0.329 vs. 0.246 $mg \cdot g^{-1}$ DM; SE: 0.026).

Concentration of individual terpenoids as well as total terpenoid content varied among plants; variation was greatest, however, for minor terpenoids (Table 1). Plant-to-plant variation was detected in both total amount of volatile compounds and in 47 of the 51 individual compounds identified ($P < 0.05$). Concentration of α-terpinene, trans-carveol, bornyl-acetate, α-selinene, elemol, and 8-α-11-elemodiol did not differ among plants ($P > 0.05$).

DISCUSSION

Compared to our control (ideal condition), exposure of samples to ambient temperature for 24 hours after harvest followed by storage at −80°C or 8°C for 3 weeks did not induce statistically detectable differences in the concentration of major compounds and in the total terpenoid amount. However, total terpenoid amount and concentrations of a few major compounds tended to be lower in refrigerated samples compared to the frozen control. Animut et al. (2004) also found that a few terpenes of eastern red cedar (*Juniperus virginiana* L.) branches tended to decrease slightly after a 7-day storage period under refrigerated conditions. The effects of handling and storage protocols in this study were statistically detectable only when 10 minor terpenoids that exhibited high natural plant-to-plant variation were considered together in a multivariate analysis. Temperature and length of storage period investigated in our study were well below thresholds known to alter plant terpene composition significantly (Diaz-Maroto et al. 2003; Njoroge et al. 2003; Vanamala et al. 2005). However, differences in major terpenoid levels among handling protocols would have possibly reached statistical significance had we stored refrigerated samples for a longer period of time.

One-seed juniper volatiles composed approximately 4% of the DM of our samples and consisted of a complex mixture of 51 compounds, with α-pinene (a monoterpene) representing 65% of the total terpenoid content. Only 14 of these compounds accounted for over 95% of the total terpenoid fraction in our samples. Adams et al. (1981) reported that the steam-distillated oil fraction of one-seed juniper represented 3%–5% of the DM and contained at least 35 terpenoids, with α-pinene accounting for 52% of the total oil content. Dearing et al. (2000) also used steam distillation and reported that one-seed juniper oils represented 4% of the dry matter fraction, 63% of which was α-pinene.

The most abundant terpenoids in our samples exhibited the least plant-to-plant variation. Most of the terpenoids that have been shown to deter herbivory by sheep, goats, deer, and rodents were among the 14 most abundant compounds in the samples we analyzed. These include α-pinene (Riddle et al. 1996; Estell et al. 1998; Dearing et al. 2000; Vourc'h et al. 2002), myrcene (Vourc'h et al. 2002), and β-pinene (Riddle et al. 1996). Other major terpenoids present in one-seed juniper (terpinolene and 3-carene) individually tested by Estell et al. (2000, 2005) had no effect on forage intake by sheep. Most of the terpenoids that have been shown to deter herbivory

Table 1. Terpenoid constituents of ethanolic extracts of one-seed juniper saplings ($n = 20$) obtained by GC/MS analysis. This analysis included the processing of blank baseline samples that did not contain juniper material. Compounds were identified by comparing mass spectra and retention indices with those reported by Adams (1995).

Compound	Concentration ($mg \cdot g^{-1}$ DM)	SEM[1]	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
A-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
A-bulnesene	0.01	0.00	39.03	0.07	99.33
terpin-4-ol	0.01	0.00	42.26	0.06	99.40
A-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[2]	20.01	0.86	19.32	100.00	—
Maximum value	12.94	—	131.54	64.67	—
Minimum value	< 0.01	—	23.65	0.02	—

[1]SEM indicates standard error of means.
[2]Total amount was calculated as the sum of the concentration of all individual compounds.

Table 2. Concentration of 14 major terpenoids determined by GC/MS analysis of ethanolic extracts in one-seed juniper saplings exposed to one of three handling protocols. Protocols were: a) *Control:* placed on dry ice after clipping and after 5 h frozen at −80°C for 3 wk; b) *Frozen after 24 h:* kept at ambient temperature for the first 24 h and then frozen as control samples; and c) *Refrigerated after 24 h:* kept at ambient temperature for the first 24 h, and then stored at 8°C for 3 wk.

	Handling protocol[1]			
Terpene	Control (mg·g^{-1} DM)	Frozen after 24 h (mg·g^{-1} DM)	Refrigerated after 24 h (mg·g^{-1} DM)	*P* value
Total amount[2]	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
B-eudesmol	0.28 ± 0.02	0.31 ± 0.02	0.28 ± 0.02	0.54
A-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

[1]Values are means ± SEM (standard error of the means).
[2]Total amount was calculated as the sum of the concentration of all individual compounds.

exhibited high concentrations and lower among-individual variability in the one-seed juniper samples we analyzed. However, more research is needed to determine whether major and minor terpenoids operate jointly to increase levels of defense against herbivores (Langenheim 1994; Dearing et al. 2000).

Total volatile amount and concentration of at least 47 individual terpenoids varied significantly among one-seed juniper saplings. These results are not surprising given the vast information regarding intraspecific variation of terpenoids in junipers (Adams and Hagerman 1977; Adams 1979; Riddle et al. 1996; Adams 2004) and other species rich in terpenoids such as big sagebrush (*Artemisia tridentata* Nutt.) (Welch and McArthur 1981) and tarbush (Estell et al. 1998). Interestingly, strong plant-to-plant variation observed in this study occurred among saplings collected from a fairly uniform area within a range of approximately 100 m. Within-species genetic variation (Welch and McArthur 1981) associated with differential expression of genes that control terpenoid synthesis may explain the pattern we observed (Dudareva et al. 2004). Terpenoid variation among plants could also occur as a result of plant age (Boege and Marquis 2005), although we were unable to clearly discriminate small and large plants on the basis of concentration of major terpenoids. Major compounds, many of which have been shown to deter herbivory, did not vary significantly among the current year's growth of sampled plant sizes. Further research on the relation between plant morphology, age, and terpenoid profiles of junipers is needed.

MANAGEMENT IMPLICATIONS

Our results suggest that the length of storage period of one-seed juniper sapling branches should not exceed 3 weeks and that storage refrigeration temperatures should be kept below 8°C to prevent significant terpenoid alterations. Use of this relatively simple handling protocol should allow researchers to minimize the risk of promoting significant changes in terpenoid profiles when conducting feeding trials that require transporting and storing plant materials harvested at distant sites. Within the limitations of this study, sapling size appears to be a poor predictor of terpenoid content and composition in one-seed juniper. The magnitude of plant-to-plant variation in individual terpenoid content observed in this study suggests that researchers may need to explicitly address this source of variation when designing pen feeding trials.

Table 3. Suite of minor terpenoids able to discriminate one-seed juniper sapling samples into significantly different groups on the basis of plant handling protocol. Protocols were: a) *Control:* placed on dry ice after clipping and after 5 h frozen at −80°C for 3 wk; b) *Frozen after 24 h:* kept at ambient temperature for the first 24 h and then frozen as control samples; and c) *Refrigerated after 24 h:* kept at ambient temperature for the first 24 h, and then stored at 8°C for 3 wk.

				Treatment[2]		
Stepwise iteration	Compound	Partial R^2	*P* value[1]	Control (mg·g^{-1} DM)	Frozen after 24 h (mg·g^{-1} DM)	Refrigerated after 24 h (mg·g^{-1} DM)
1	8-α-11-elemodiol	0.52	< 0.01	0.189 ± 0.039	0.019 ± 0.003	0.016 ± 0.002
2	α-selinene	0.43	< 0.01	0.007 ± 0.001	0.003 ± 0.000	0.004 ± 0.001
3	(z)-β-ocimene	0.15	0.01	0.008 ± 0.001	0.008 ± 0.001	0.006 ± 0.001
4	terpin-4-ol	0.18	< 0.01	0.011 ± 0.001	0.014 ± 0.002	0.014 ± 0.002
5	trans-carveol	0.18	< 0.01	0.008 ± 0.001	0.002 ± 0.000	0.003 ± 0.001
6	pinocarvone	0.19	< 0.01	0.006 ± 0.001	0.004 ± 0.001	0.004 ± 0.001
7	meta-cymen-8-ol	0.11	0.06	0.006 ± 0.001	0.005 ± 0.001	0.004 ± 0.001
8	verbenone	0.11	0.05	0.022 ± 0.003	0.015 ± 0.002	0.014 ± 0.002
9	α-humulene	0.10	0.09	0.031 ± 0.005	0.029 ± 0.005	0.023 ± 0.003
10	α-terpinene	0.13	0.04	0.007 ± 0.000	0.004 ± 0.000	0.005 ± 0.001

[1]Significance for entry into the discriminant function.
[2]Values are means ± SEM (standard error of the means).

ACKNOWLEDGMENTS

Authors thank Stephanie Lopez for her collaboration in the laboratory. We would like to thank two anonymous reviewers and the editor for their insightful comments and suggestions that helped to improve an earlier version of the manuscript.

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