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Effects of *Juniperus* species and stage of maturity on nutritional, in vitro digestibility, and plant secondary compound characteristics

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ABSTACT: Rising feed costs and recurring feed shortages necessitate the investigation into alternative and underutilized feed resources. Nutritional characteristics of *Juniperus* species are either unknown or limited to leaves and ground material from small stems. Therefore, the objective was to quantify nutritional characteristics, 48-h true IVDMD (tIVDMD), microbial gas production, and secondary compound characteristics of entire woody plant material of 4 *Juniperus* species—*Juniperus pinchotii*, *Juniperus monosperma*, *Juniperus ashei*, and *Juniperus virginiana*—at immature and mature stages of growth. Immature plants had greater CP concentrations and lower NDF concentrations ($P < 0.001$) than mature plants regardless of species. Mature plants also had greater ($P < 0.001$) concentrations of ADF compared with immature plants with the exception of *J. virginiana*. In general, immature *J. pinchotii*, *J. monosperma*, and *J. ashei* had greater ($P < 0.02$) tIVDMD and total 48-h and asymptotic gas production than mature plants. Immature *J. monosperma* and *J. pinchotii* plants were more digested (tIVDMD; $P < 0.001$) than immature *J. virginiana* and *J. ashei*, but tIVDMD did not differ in mature plant material across species. Condensed tannins (CT) were greater ($P < 0.001$) in immature *J. pinchotii* and *J. ashei* than mature plants; differences in CT concentrations among immature species were also detected ($P < 0.04$). Volatile oil yields were similar across maturity and species with 1 exception: immature *J. pinchotii* yielded more ($P < 0.02$) volatile oil than mature material. Volatile oil composition across species varied and contained a range of 65 to 70 terpene compounds. The dominant terpenes across species were generally greater ($P < 0.05$) in immature vs. mature plant material with the exception of *J. virginiana*. Labdane acids were negligible in *J. pinchotii*, *J. ashei*, and *J. virginiana* and greater in *J. monosperma* ($P < 0.001$). Ground material from mature juniper species, although inferior in nutritional quality compared with immature plants, is comparable to traditional low-quality roughage ingredients. Given that *J. pinchotii* has been successfully fed in lamb feedlot diets, the similarities of *J. pinchotii*, *J. ashei* and *J. monosperma* suggest that all three species have potential to be effective roughage ingredients.

Key words: in vitro digestibility, juniper, nutritional quality, secondary compounds, sheep, woody plants

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INTRODUCTION

Rising feed costs and recurring feed shortages necessitate the investigation of alternative feed ingredients. Woody plants from the genus *Juniperus* have worldwide distributions (Hora, 1981) and cover over 50 million ha in the western United States (Van Auken and Smeins, 2008). *Juniperus virginiana* is the most common juniper species in the eastern United States and is found on all states east of the 100th meridian (Van Auken and Smeins, 2008). Leaves (Whitney and Muir, 2010) or leaves and small stems (Whitney et al.,

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2014) of these increasingly abundant plants can be used as a roughage ingredient in mixed diets for ruminant livestock. Use of woody biomass as a feed resource is not entirely novel nor is it limited to certain geographic regions; however, research with these nontraditional feeds receives greatest consideration during times of feed shortages and high feed costs but diminishes when feed related inputs and feed availability stabilize (NRC, 1983).

Secondary compounds such as volatile oil and condensed tannins (**CT**) present in *Juniperus* species can either positively or negatively affect animal performance and rumen function. Preliminary research regarding the feeding value of ground juniper plants is promising, but information is limited to ground *Juniperus pinchotii* leaves and small stems (<3.6 cm). Widespread use of juniper species in U.S. feeding systems requires approval by appropriate federal agencies based in part on baseline information regarding their nutritional, in vitro digestibility, and plant secondary compound characteristics. We hypothesized that plant biomass from immature plants (height of 1 to 1.8 m) will possess superior nutritional characteristics compared with mature plants (height of >3 m) whereas plant secondary compound characteristics would vary across species and maturity. The objective of this study was to quantify nutritional, in vitro digestibility, and plant secondary compound characteristics of *J. pinchotii*, *Juniperus monosperma*, *Juniperus ashei*, and *J. virginiana* at mature and immature growth stages to assess their suitability as a feed ingredient.

MATERIALS AND METHODS

Study Design and Harvesting Protocol

Juniper species were collected over a 4-wk period in March 2012 at 4 separate geographic locations. *Juniperus pinchotii* was collected in Tom Green County, TX (31°36′54.73″ N, 100°32′24.48″ W), on a Cho Association loamy, capionatic, thermic, shallow Petrocalcic Calciustolls site. *Juniperus ashei* was collected in Edwards County, TX (30°17′08.18″ N, 100°32′46.30″ W), on an Eckrant, clayey, skeletal, smectitic, thermic Lithic Haplustolls site. *Juniperus virginiana* was collected in Bastrop County, TX (29°55′18.69″ N, 97°15′3.28″ W), on a Silstid loamy, siliceous, semiactive, thermic Arenic Paleustalfs site. *Juniperus monosperma* was collected in Torrance County, NM (34°16′01.03″ N, 105°25′26.21″ W), on Pinon, loamy, mixed, super active, mesic Lithic Ustic Haplocalcids site. At each of the 4 sites, 4 plots, separated by a minimum of 165 m, were designated as harvest sites. Plots for each species were the experimental unit and were maintained separate throughout the study, as plot was the experimental unit.

One mature male and 1 mature female tree (height of >3 m) and 2 male and 2 female immature plants (height of 1 to 1.8 m) from each plot were mechanically harvested and transported to a central location and processed within 72 h. Immature trees were chipped using an ECHO Bear Cat chipper (ECHO Bear Cat, West Fargo, ND). Due to the large amount of biomass, mature plants were initially chipped through a coarse shredder (Vermeer X1500; Vermeer Corp., Pella, IA) and then a 90-kg random sample was chipped once more using the ECHO Bear Cat chipper. All chipped material from immature and mature plants was then subsampled, fine ground through a hammermill to pass a 4.76-mm sieve (Sentry, model 100; Mix-Mill Feed Processing Systems, Bluffton, IN), dried at 55°C in a forced-air oven for 48 h, ground through a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm screen, and stored at –20°C.

Laboratory Analyses

Dry matter of ground juniper subsamples was calculated by drying chipped material at 105°C in a forced-air oven for 24 h. For all other nutrient, in vitro digestibility, and plant secondary compound analyses, material dried at 55°C in a forced-air oven was used. Nitrogen was analyzed (Method 990.03; AOAC, 2006; Leco Corp., St. Joseph, MI) and CP was calculated as 6.25 × N. The NDF and ADF were sequentially analyzed according to Van Soest et al. (1991), modified for an ANKOM 2000 Fiber Analyzer (Ankom Technology Corp., Fairport, NY), correcting for residual ash, and using α-amylase and sodium sulfite according to Mertens (2002). Lignin was analyzed by a standard method (AOAC 973.18; AOAC, 2006). Ash was quantified (Method 942.05; AOAC, 2006) and minerals were analyzed by a Thermo Jarrell Ash IRIS Advantage (Thermo Jarrell Ash Corp., Franklin, MA) inductively coupled plasma radial spectrometer.

The protocol for collecting ruminal fluid was approved by the Texas A&M University Institutional Animal Care and Use Committee. Ruminal fluid from sheep (n = 4) fed a low-quality basal hay diet and 125 g of a 12% CP supplement daily was collected via oral lavage into a prewarmed thermos purged with CO_2, filtered through 4 layers of cheesecloth, combined, and continuously purged with CO_2 until added to prewarmed gas production modules. For each jar, 56 mL McDougal's buffer solution (1.0 g of urea/L) and 14 mL of ruminal fluid was added to 0.7 g of juniper material. Jars were flushed with CO_2 and ANKOM gas production modules were secured and then incubated for 48 h at 39°C. Recording of gas production commenced 15 min after ruminal fluid was added to jars. All species and maturities within plot were analyzed in duplicate; therefore, 4 separate gas produc-

tion runs were evaluated. In addition, 2 blanks that did not contain a feed substrate were used in each run.

After 48 h, undigested feed material was rinsed out of each jar and analyzed by NDF procedures according to Van Soest et al. (1991) with adaptations according to Mertens (2002) without adding sodium sulfite. Filter papers (541 ashless; Whatman International Ltd., Kent, ME) and undigested residue were dried at 55°C for 48 h and weighed. Percentage of 48-h true IVDMD (**tIVDMD**) was calculated as 100 × [(initial sample dry weight – residue – blank)/initial sample dry weight]. In addition, undigested material was subsequently analyzed for N to determine NDIN (Mass et al., 1999). All samples were analyzed for soluble, protein-bound, and fiber-bound CT as described by Terrill et al. (1992). Species-specific standards were created for each juniper species analyzed (Wolfe et al., 2008) using CT extracts purified on a Sephadex LH-20 (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) and lyophilized to recover purified CT. Ground juniper samples were steam distilled to determine total volatile oil yield and individual terpene profile as adapted by Adams (1991) and Koedam and Looman (1980). Isocupressic acid (**ICA**), agathic acid (**AGA**), imbricatoloic acid (**IMB**), and dihydroagathic acid (**DHAA**) were analyzed at the USDA-ARS Poisonous Plants Research Center, Logan, UT, according to methods described in Gardner and James (1999).

Statistical Analyses

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Dry matter, ash, NDF, ADF, lignin, CP, tIVDMD, NDIN, volatile oil, CT (extractable, protein bound, fiber bound, and total), and individual labdane acids were analyzed using a model that included species, maturity and species × maturity interaction. The experimental unit was plot. Covariance structures (variance components, autoregressive order 1, compound symmetry, and heterogeneous autoregressive order 1) were compared to determine the most appropriate structure for each model and variance components was chosen. Data are reported as least squares means with greatest SE.

Kinetic analysis of the cumulative gas production was evaluated using several nonlinear functions (Schofield and Pell, 1995). The nonlinear fitting was performed using GasFit (http://nutritionmodels.com/gasfit.html) as previously described (Williams et al., 2010). Results indicated the Gompertz 2-pool nonlinear function had the lowest sum of square of errors for most of the variables and therefore was chosen:

$$Y = a \times \exp\{-\exp[1 + b \times (c - t)]\} + d \times \{-\exp[1 + e \times (c - t)]\},$$

in which Y is gas produced in milliliters, a is the asymptote in milliliters, b is the fractional degradation rate per hour, t is time in hours, c is lag time in hours, d is the asymptote of the second pool (assumed to be fiber) in milliliters, and e is the fractional degradation rate of the second pool per hour. Parameters calculated from GasFit were analyzed using the PROC MIXED procedure with species, maturity, and species × maturity used in the model. Run was the random variable and each plot was evaluated on separate days; therefore, in vitro run = plot. Correlations among nutritional characteristics and gas production characteristics were evaluated by species and maturity using Pearson correlation.

RESULTS AND DISCUSSION

Crude Protein and NDIN

Crude protein and NDIN are summarized in Table 1. No species × maturity interaction was observed; however, an effect ($P < 0.001$) of maturity on CP was observed in *J. pinchotii* and *J. monosperma* but not in *J. ashei* or *J. virginiana*. The greater CP concentration in immature vs. mature plants was likely due to a greater proportion of vegetative material in immature plants. In the current study, CP concentrations of both mature and immature plants were comparable to other low-quality roughage sources, that is, corn stover (5%; NRC, 2007), cottonseed hulls (5%; NRC, 2007), wheat straw (3%; NRC, 2007), and pine bark (1%; Min et al., 2012). No differences ($P = 0.22$) in NDIN were observed among species or maturities. However, NDIN in the current study was similar to 1.5% NDIN of low-nutritive-value mixed meadow hay (Mass et al., 1999).

Neutral Detergent Fiber, ADF, and ADL

Fiber components are summarized in Table 1. A species × maturity interaction was observed ($P < 0.047$) for NDF, ADF, and ADL. Neutral detergent fiber concentrations were greater ($P < 0.001$) in mature vs. immature plant material. Mature plants also had greater ADF ($P < 0.001$) concentrations compared with immature plants with the exception of *J. virginiana*. Maturity ($P = 0.04$) affected ADL content of *J. pinchotii* and *J. monosperma* but not *J. ashei* and *J. virginiana*. Differences in fiber components among immature species found both *J. monosperma* and *J. pinchotii* to have 6% less NDF and 9% less ADF than *J. ashei* and 19% less NDF and 24% less ADF than *J. virginiana*. In contrast, no differences ($P = 0.35$) in NDF and ADF were detected among species for mature plants.

Use of woody biomass as a feed ingredient has been accomplished with other tree species including *Populus*

Table 1. Effects of plant stage of maturity on nutritional and mineral composition (DM basis) of *Juniperus pinchotii*, *J. monosperma*, *J. ashei*, and *J. virginiana*[1]

Item,[2]	*J. pinchotii*		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled
%	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	SEM
DM	68.1^{a}	69.1^{a}	66.7^{a}	65.8^{ab}	64.9^{abc}	65.4^{abc}	61.5^{c}	62.2^{bc}	0.92
CP	4.7^{a}	3.6^{c}	4.6^{ab}	3.6^{c}	4.1^{abc}	3.4^{c}	4.6^{ab}	3.7^{bc}	0.19
NDIN	1.54	1.44	1.80	1.67	1.60	1.35	1.56	1.64	0.23
NDF	50.1^{c}	66.9^{ab}	50.0^{c}	64.6^{ab}	54.4^{c}	67.4^{ab}	62.6^{b}	68.5^{a}	1.32
ADF	40.7^{b}	56.2^{a}	40.0^{b}	54.0^{a}	44.2^{b}	55.5^{a}	52.9^{a}	58.0^{a}	1.46
ADL	21.1^{e}	25.0^{cd}	23.1^{de}	26.3^{bc}	29.4^{ab}	29.8^{a}	27.9^{abc}	29.1^{ab}	0.66
Ash	5.7^{a}	4.3^{cd}	5.4^{ab}	4.4^{c}	5.9^{a}	4.7^{bc}	4.1^{cd}	3.6^{d}	0.30
Ca	1.69^{ab}	1.25^{cde}	1.59^{ab}	1.36^{bcd}	1.89^{a}	1.57^{abc}	1.10^{de}	0.99^{e}	0.08
P	0.04^{bc}	0.03^{d}	0.05^{a}	0.04^{bcd}	0.04^{bc}	0.03^{cd}	0.05^{ab}	0.04^{bcd}	0.003
K	0.26^{a}	0.16^{d}	0.24^{ab}	0.18^{cd}	0.23^{abc}	0.19^{cd}	0.25^{a}	0.19^{bcd}	0.01
Mg	0.11^{a}	0.07^{bcd}	0.07^{cd}	0.05^{cd}	0.07^{cd}	0.04^{d}	0.10^{ab}	0.07^{bc}	0.01
S	0.07^{ab}	0.05^{c}	0.07^{a}	0.05^{c}	0.07^{ab}	0.05^{c}	0.06^{bc}	0.05^{c}	0.002
Fe	129.0^{a}	118.3^{a}	149.5^{a}	114.5^{a}	98.8^{a}	113.8^{a}	185.3^{a}	166.5^{a}	37.4
Cu	1.9^{b}	1.9^{b}	2.3^{ab}	2.3^{ab}	2.2^{b}	2.0^{b}	2.5^{a}	2.1^{ab}	0.10
Mn	18.9^{bc}	13.3^{c}	15.4^{c}	11.5^{c}	23.9^{bc}	13.10^{c}	172.7^{a}	130.4^{ab}	24.0
Zn	9.7^{ab}	4.7^{c}	5.7^{bc}	4.6^{c}	6.9^{bc}	4.8^{c}	12.3^{a}	5.9^{bc}	1.03
Al	145.8^{ab}	128.7^{ab}	176.8^{a}	137.3^{ab}	120.0^{ab}	135.0^{ab}	121.6^{ab}	91.1^{b}	15.3
B	9.5^{a}	7.8^{b}	9.8^{a}	7.8^{b}	9.4^{a}	7.7^{b}	9.0^{ab}	7.7^{b}	0.31
Ba	34.8^{a}	16.7^{a}	24.4^{a}	15.3^{a}	18.6^{a}	16.3^{a}	46.1^{a}	42.8^{a}	7.99

$^{a–d}$Within row means without a common superscript differ ($P < 0.05$), according to a LSD. Juniper species × stage of maturity.

[1]Imm = immature growth stage (height of 1 to 1.8 m); Mat = mature growth stage (height of >3 m).

[2]Fe, Cu, Mn, Zn, Al, B, and Ba are expressed in milligrams per kilogram DM. Minimal detected levels were observed for Na (<500 mg/kg), Se (<10 mg/kg), and Co (<0.50 mg/kg).

and *Pinus* genera (NRC, 1983). However, the current study is novel in that it specifically evaluates nutritional characteristics of *Juniperus* species using the entire plant biomass. Differences in fiber components between plant species and stages of maturity found in the current trial may be the result of greater leaf to stem ratio and the horizontal (shrublike) vegetative growth structure of *J. monosperma* and *J. pinchotii* in contrast to *J. ashei* and *J. virginiana*. A greater proportion of ADL measured in *J. ashei* and *J. virginiana* would support these observational differences in phenotypic characteristics. Whitney and Muir (2010) reported 38% NDF and 31% ADF in *J. pinchotii* leaves, but these fiber fractions increased to 40% NDF and 37% ADF when ground leaves and stems <3.6 cm diameter were reported (Whitney et al., 2014). Inclusion of stems led to tIVDMD decreasing from 67 (leaves) to 55% (leaves and stems) in those studies, approaching the tIVDMD values (49%) observed in the current study with immature *J. pinchotii* plant material. Extrapolating fiber and in vitro digestibility characteristics from feeding trials conducted with *J. pinchotii* (Whitney et al., 2014) suggests that immature *J. monosperma* and *J. ashei* could also be used as effective roughage ingredient alternatives.

Average fiber components of mature juniper species in the current study (NDF = 66% and ADF = 55%), although greater than those of immature juniper species, are similar to NDF and ADF of other low-quality roughage ingredients, for example, corn stover (NDF = 70% and ADF = 44%; NRC, 2007), cottonseed hulls (NDF = 87% and ADF = 68%; NRC, 2007), wheat straw (NDF = 81% and ADF = 58%; NRC, 2007), and pine bark (NDF = 78% and ADF = 72%; Min et al., 2012).

Although NDF and ADF values provide valuable information regarding fiber digestibility, comparing suitability of woody roughage ingredients based solely on NDF and ADF is inadequate when choosing between low-nutritive-value roughage ingredients. For example, growing kid goats consuming mixed diets of 30% pine bark (*Pinus taeda*; NDF = 78% and ADF = 72%) experienced increased growth performance and enhanced rumen fermentation compared with goats consuming 0 and 15% pine bark diets with lower ADF values (Min et al., 2012). Also, it is probable that woody plant material is best used in nutrient-rich mixed diets (Marion et al., 1957; Min et al., 2012; Whitney et al., 2014) compared with inclusion in forage-based diets (Bas et al., 1985). Juniper's unique physical characteristics with respect to particle density and buoyancy characteristics may be advantageous in the rumen environment by contributing to a more uniform distribution throughout stratified rumen layers (Whitney et al., 2014), potentially reducing pH fluctuations and latent acidosis (Bryant, 1964; Huntington, 1988).

Table 2. Effects of plant stage of maturity on in vitro fermentation dynamics of *Juniperus pinchotii*, *J. monosperma*, *J. ashei,* and *J. virginiana*[1]

	J. pinchotii		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled
Item[2]	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	SEM
tIVDMD, %	49.8[a]	29.7[b]	49.4[a]	33.0[b]	43.6[a]	30.0[b]	33.6[b]	29.1[b]	1.67
Total, mL	44.5[a]	25.7[b]	45.2[a]	25.7[b]	43.2[a]	31.0[b]	32.3[b]	24.9[b]	2.16
a, mL	26.8[a]	10.6[b]	23.7[ab]	17.7[ab]	27.6[a]	17.1[ab]	15.5[ab]	16.9[ab]	2.82
b, per h	0.17	0.46	0.31	0.16	0.14	0.15	0.17	0.15	0.07
c, h	0.08	0.10	0.10	0.23	0.19	0.30	0.09	0.02	0.08
d, mL	20.7[ab]	21.6[ab]	21.9[a]	10.2[b]	19.9[ab]	18.8[ab]	18.8[ab]	14.9[ab]	2.41
e, per h	0.30	0.18	0.32	1.5	0.12	0.89	0.21	0.15	0.38

[a,b]Within row means without a common superscript differ ($P < 0.05$) according to a LSD. Juniper species × stage of maturity.

[1]Imm = immature growth stage (height of 1 to 1.8 m); Mat = mature growth stage (height of >3 m).

[2]tIVDMD = 48-h true IVDMD; Total = 48-h cumulative gas production (mL/g substrate DM); a = asymptote (mL/g substrate DM); b = fractional degradation rate; c = lag time; d = asymptote of second pool (mL/g substrate DM); e = fractional degradation rate of second pool.

Mineral Composition

Mineral profiles of *Juniperus* species are presented in Table 1. Immature plants contained greater percent ash than mature plants ($P < 0.03$). *Juniperus virginiana* had the least amount of ash compared with the other *Juniperus* species and its ash and mineral composition was not affected by stage of maturity ($P > 0.05$). This supports the assumption that the greater proportion of leaves in the other species compared with *J. virginiana* and the greater amount of ash generally found in vegetative biomass (Oregon Department of Energy, 2003) accounted for this difference in ash content and, in some instances, mineral composition. Immature *J. pinchotii* and *J. monosperma* contained greater ($P < 0.05$) P concentrations than mature plants of the same species In general, P concentrations are similar to that in cottonseed hulls but lower than values reported for corn stover (NRC, 2007). Potassium concentrations were greatest ($P < 0.05$) in immature plants compared with mature plants, with the exception being *J. ashei*. Iron concentrations in the current study are greater than those found in lower nutritive value ingredients (e.g., cottonseed hulls; Whitney and Muir, 2010; wheat straw; Min et al., 2012) but lower than concentrations detected in immature *J. pinchotii* or pine bark (Whitney and Muir, 2010; Min et al., 2012). The antagonistic relationship of Fe concentrations (250–1,200 mg/kg) with reducing Cu status in ruminants (Prabowo et al., 1988; Spears, 2003) might be considered when feeding ground juniper; however, no clinical signs of iron antagonism resulting in Cu deficiency have been reported in feeding trials (Whitney and Muir, 2010; Whitney et al., 2014).

Gas Production and 48-h True IVDMD

Immature *J. pinchotii*, *J. monosperma*, and *J. ashei* produced greater total gas than mature plants of the same species ($P = 0.028$; Table 2), whereas *J. virginiana* did not. In vitro 48-h gas production data can provide valuable information in regards to forage digestibility (Schofield and Pell, 1995; Blümmel et al., 1997). Blümmel and Ørskov (1993) found the gas production techniques highly correlated with *in vivo* parameters of DMI ($r = 0.88$), digestible DMI ($r = 0.94$), and growth rate ($r = 0.94$), validating its use as a reliable in vitro measure for low nutritive value ingredients (e.g., straw). Gas production results from the current study agree with Cornou et al. (2013), who found that the most repeatable and reproducible measures using ANKOM gas production modules were asymptotic and 48-h gas production.

Total gas production was positively correlated with tIVDMD across all species ($r = 0.95$, $P = 0.001$) and negatively correlated with NDF ($r = –0.97$, $P = 0.01$) and ADF ($r = –0.97$, $P = 0.001$), with correlations most pronounced among immature *Juniperus* species. The agreement between gas production values and tIVDMD allow a more confident prediction that immature *J. monosperma*, followed by *J. ashei*, would have fermentation characteristics similar to immature *J. pinchotii*, which has been successfully fed in mixed lamb diets (Whitney et al., 2014).

Inclusion of low-quality roughage ingredients in mixed diets is partially influenced by their digestibility. Giacomini et al. (2006) determined that feeding 25% *J. monosperma* leaves in a low-quality forage diet increased NDF digestibility (65%) in sheep when compared with a low-quality forage diet (54%). Ground *J. pinchotii* leaves and stems <3.6 mm (48-h tIVDMD; 55%) can effectively replace oat hay (tIVDMD = 57%) as the roughage source in lamb feedlot diets as measured by animal growth performance (Whitney et al., 2014). Use of the entire plant biomass in both immature and mature *J. pinchotii* in the current study resulted in an expected decrease in tIVDMD compared with *J. pinchotii* leaves (tIVDMD = 67%; Whitney and Muir, 2010) and leaves and small stems (48-h tIVDMD; 55%; Whitney et al., 2014).

Table 3. Effects of plant stage of maturity on plant secondary compound characteristics (DM basis) of *Juniperus pinchotii*, *J. monosperma*, *J. ashei*, and *J. virginiana*[1]

	J. pinchotii		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled
Item,[2] %	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	SEM
ECT	5.5^{a}	3.1^{bc}	4.1^{b}	2.5^{c}	3.8^{bc}	2.7^{c}	2.8^{bc}	2.7^{c}	0.29
FCT	1.2	0.59	0.71	0.59	1.8	0.82	1.1	1.3	0.39
PCT	1.6^{b}	1.1^{b}	1.4^{b}	1.1^{b}	3.5^{a}	2.2^{ab}	1.7^{b}	1.5^{b}	0.37
TCT	8.4^{ab}	4.7^{c}	6.3^{abc}	4.2^{c}	9.0^{a}	5.7^{bc}	5.6^{bc}	5.5^{bc}	0.72
Oil	1.20^{a}	0.63^{b}	1.05^{ab}	0.73^{ab}	0.57^{b}	0.61^{b}	0.70^{ab}	1.07^{ab}	0.11
ICA	0.018^{c}	0.020^{c}	0.273^{a}	0.163^{b}	0.0^{c}	0.005^{c}	0.015^{c}	0.035^{c}	0.04
AGA	0.063^{c}	0.035^{cd}	0.228^{a}	0.160^{b}	0.018^{d}	0.020^{d}	0.008^{d}	0.030^{cd}	0.013
IMB	0.003^{c}	0.0^{c}	0.195^{a}	0.090^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.005^{c}	0.005
DHAA	0.0^{c}	0.0^{c}	1.41^{a}	0.713^{b}	0.003^{c}	0.013^{c}	0.0^{c}	0.0^{c}	0.040
Total	0.083^{cd}	0.055^{d}	2.11^{a}	1.13^{b}	0.025^{d}	0.055^{d}	0.203^{c}	0.215^{c}	0.049

$^{a–d}$Within row means without a common superscript differ ($P < 0.05$), according to a LSD. Juniper species × stage of maturity.

[1]Imm = immature growth stage (height of 1 to 1.8 m); Mat = mature growth stage (height of >3 m).

[w]ECT = extractable condensed tannins; PCT = protein-bound condensed tannins; FCT = fiber-bound condensed tannins; TCT = total condensed tannins. Oil is total volatile oil. ICA = isocupressic acid; AGA = agathic acid; IMB = imbricatoloic acid; DHAA = dihydroagathic acid; total acids is the combined concentration of measured labdane acids. Total acids for *J. virginiana* reflect an additional labdane acid (0.162% DM) closely related to ICA but not identified in the other species.

In the current study, tIVDMD ranged from 33 to 49% for immature plants and 29 to 33% for mature plants. Digestibility of ground juniper material in this study was similar to values observed for cottonseed hulls (21 to 32%; Torrent et al., 1994; Whitney and Muir, 2010) and wheat straw (27 to 41%; Braman and Abe, 1977; Haddad et al., 1994).

Secondary Compounds

A species × maturity interaction was detected ($P = 0.047$) for CT. Specifically, immature *J. pinchotii* and *J. ashei* had greater ($P = 0.012$) CT than mature plants whereas this was not observed in *J. monosperma* and *J. virginiana*. Mature plants across all species did not differ in total CT concentration. Condensed tannin and volatile oil concentrations are summarized in Table 3. Secondary plant compounds (e.g., CT and volatile oil) can either enhance or reduce animal performance, health, metabolism, end products, or rumen microbial function (Waghorn et al., 1994; Terrill et al., 2007; Min et al., 2012). The effects vary depending on the ruminant species, dietary CT concentration, chemical structure, bioactivity of the secondary compounds, and dietary nutrient composition and intake (Calsamiglia et al., 2007; Patra and Saxena, 2011). Total CT for immature *J. pinchotii* was 8%, which is slightly greater than the 6% CT found in ground leaves and stems (Whitney et al., 2014) as well as greater ($P < 0.05$) than the 4.7% CT in mature *J. pinchotii* in the current study. These CT concentrations are less than pine bark (10%; Min et al., 2012) and less than or equal to the 6.4 to 12.4% measured in varieties of *Lespedeza cuneata* (Solaiman et al., 2010; Acharya et al., 2015).

Research results measuring the effects of CT on rumen function have varied depending on plants containing the CT and dietary composition of the basal diet (concentrate-mixed diet vs. grazing-based diets). Discrepancies in these research findings may be best attributed to the wide variation in chemical characteristics of CT across the different plants that contain them (Schofield et al., 2001; Naumann et al., 2013). A variety of monomer subunits, for example, profisetinidins (quebracho tannins), prodelphinidins (*L. cuneata*), probinetidins, and proguibortinidins, can vary in polymerization, molecular weight, and stereochemistry to form many diverse chemical structures with different levels of bioactivity (Patra and Saxena, 2011; Naumann et al., 2013; Mechineni et al., 2014).

The protein precipitation capacity of CT with dietary proteins can reduce protein degradation in the rumen through the formation of CT–protein complexes. In the rumen, hydrogen bonds are formed between the phenolic group of CT and the carboxyl groups of aliphatic and aromatic side chains of proteins, and that bound protein is unavailable to proteolytic bacteria (Patra and Saxena, 2011). The binding strength of the CT–protein complex determines its availability in the abomasum, and the complex generally disassociates at a pH of <3.5 (Kariuki and Norton, 2008). Increased RUP via CT–protein precipitation may increase the quality and quantity of protein flow to the small intestine (Perez-Maldonado and Norton, 1996; Kariuki and Norton, 2008) under circumstances when dietary AA (lysine and methionine) may be limited. It is unknown whether CT in ground juniper would exert the same degree of bioactivity as other plant species; however, Min et al. (2012) fed increasing amounts of ground pine bark (10% CT) to growing kid

goats and observed greater growth performance, a linear decrease in the acetate:propionate ratio, and a reduction in rumen NH_3 concentrations.

Results from studies with goats consuming *L. cuneata* (6.4% CT or 1.03 g CT/kg BW; Solaiman et al., 2010) or in mixed diets containing pine bark (10% CT or 1.4 g CT/kg BW; Min et al., 2012) suggest the beneficial range for animal performance occurs from 2 to 4% CT of diet DM (Min et al., 2003, 2012). Similarly, results from Whitney et al. (2014) suggested an optimal dietary CT concentration exists, as enhanced growth occurred in lambs consuming a range of 6 to 15 g CT/d, or 0.143 to 0.345 g CT/kg BW, in a mixed diet of mainly dried distillers grains with solubles. Total CT concentrations in the 4 *Juniperus* species in the current study suggested a diet containing 30% ground juniper would provide approximately 17 to 25 g CT/kg DM from immature plants and 13 to 17 g CT/kg DM from mature juniper plants. Given the competitively smaller plant biomass available from harvested immature juniper plants, a mixture of immature and mature plants might facilitate an optimal concentration of CT in mixed diets.

A species × maturity interaction was detected ($P = 0.003$) for volatile oil yields. Immature *J. pinchotii* had a greater ($P < 0.03$) volatile oil yield than its mature counterpart, but no differences in maturity were observed for the other 3 species. Volatile oil concentrations in mechanically dried ground juniper material in the current study are less than those measured in fresh leaf material from *J. ashei* (0.6 vs. 2.5% oil; Adams et al., 2013), *J. virginiana* (0.7 vs. 2.3%; Animut et al., 2004), and *J. monosperma* (0.8 vs. 1.8%; Estell et al., 2014). However, volatile oil yields in the current study are similar to oil yields of dried *J. pinchotii* leaves and stems (Whitney et al., 2014). The reduction in volatile oil yields from processed plant material is positive in terms of *Juniperus* as feed ingredient. Direct comparisons with previous studies that fed fresh *Juniperus* foliage are of limited value given the reduction in secondary compound composition. Drying of intact foliage and the mechanical rupture of oil glands on both the leaf surface and in woody material accentuates volatilization of oil and reduces total yield and can alter profile and composition (Adams, 2010, 2013).

Losses up to 80% of the volatile oil in vegetative material are estimated due to mastication, rumination, and absorption (Welch and Pederson, 1981; Cluff et al., 1982; White et al., 1982). Consumption of volatile oil by browsing goats has ranged from 0.719 g oil/d (0.031 g oil/kg BW) with *J. virginiana* (Animut et al., 2004) to 0.53 g oil/d (0.01 g/kg BW) with *J. ashei* (Riddle et al., 1996), without negatively affecting growth performance or health. Whitney and Muir, (2010) fed ground *J. pinchotii* (leaves) to lambs in mixed diets with total volatile oil intake of 1.86 to 3.6 (0.06 to 0.075 g oil/kg BW) and 1.12 g oil/d (0.037 g oil/kg BW) without negatively affecting growth performance (Whitney et al., 2014).

Complete volatile oil composition data are presented in Appendix 1. In summary, volatile oil from *J. pinchotii* consisted of 75 compounds: 26% monoterpenes, 55% sesquiterpenes, and 18% diterpenes. The dominant compounds in this profile were elemol (18% of total volatile oil) and camphor (8% of total volatile oil) and were similar across stage of maturity. Volatile oil from *J. monosperma* consisted of 71 compounds (14% monoterpenes, 80% sesquiterpenes, and 6% diterpenes). Primary constituents were oxygenated sesquiterpene (24% of total volatile oil) similar to that reported by Utsumi et al. (2009), α-eudesmol (11% of total volatile oil), and α-pinene (7% of total volatile oil yield). α-Pinene was greater ($P < 0.001$) in immature *J. monosperma* than mature plants but less than that previously reported (Adams et al., 1981; Dearing et al., 2000; Utsumi et al., 2006). Volatile oil from *J. ashei* consisted of 65 compounds: 48% monoterpenes, 38% sesquiterpenes, and 14% diterpenes. The dominant compounds in this profile were camphor (34% of total volatile oil; greater [$P < 0.001$] in immature plants compared with mature plants) and cedrol (16% of total volatile oil; greater [$P < 0.001$] in mature plants). *Juniperus virginiana* consisted of 80 compounds: 19% monoterpenes, 68% sesquiterpenes, and 13% diterpenes. The dominant compounds in this profile were elemol (11%) and cedrol (11%). Widdrol, *cis*-thujopsenol, methyl eugenol, and safrole made up an additional 20% of the total volatile oil concentration, consistent with Adams (1991). Cedrol and widdrol were greatest ($P < 0.001$) in mature plant material whereas safrole was greatest ($P < 0.001$) in immature plant material.

Monoterpenes represent a significant component of volatile oils in foliage material; therefore, a greater proportion of monoterpenes present in immature vs. mature plants is to be expected. With the exception of *J. virginiana*, immature plants in the other species contained greater ($P < 0.05$) monoterpene concentrations compared with their mature contemporaries. Declining monoterpene concentrations has been observed when comparing immature species to that measured in fresh foliage. For example, 11 and 7 mg/g of α-pinene were present in fresh *J. monosperma* (Utsumi et al., 2009) and bark (Estell et al., 2014), compared with 1.2 and 0.14 mg/g in immature and mature material, respectively, in the current study. Sabinene concentrations in immature *J. virginiana* (0.1 mg/g) were much less than that measured in foliage (2.5 mg/g) by Animut et al. (2004), whereas safrole and elemol in the current study approximated concentrations detected by Adams and Hogge (1983). Camphor and sabinene concentrations in *J. pinchotii* leaf oil show significant decline from 24 to 48 h when dried at 42 to 45°C (Adams et al., 2013). Values in

the current study were slightly less than those measured by Whitney and Muir (2010).

Unique to the study in question is the volatile oil composition of woody components. This was reflected by the greater proportion of sesquiterpenes in the mature plant material compared with immature plant material or fresh *Juniperus* foliage. Compounds common in heartwood oil (cedrol, widdrol, *cis*-thujopsenol, and nookatone; Adams, 1991) account for some of this increase as a result of a decreasing leaf to stem ratio from immature to mature plants. Greater woody biomass compared with other species and increased heartwood oil contents are of interest as they have limited insecticidal and termiticidal properties (Zhu et al., 2001). However, these antimicrobial or antifungal characteristics are not well understood (Clark et al., 1990) and knowledge of these effects on rumen microflora is unknown. Cedrol was present in greater concentrations in mature *J. virginiana* and *J. ashei* than in mature *J. pinchotii* and *J. monosperma*. It is possible that a 14% increase in tIVDMD for immature *J. pinchotii* and *J. monosperma* compared with *J. ashei* could be partly due to its greater cedrol concentrations, although *J. ashei* also contained 9% greater ADF concentration. Widdrol was a major compound in mature *J. virginiana* and to a lesser degree in mature *J. monosperma*, but not in the other species. Nookatone was measured only in *J. virginiana* and contributed to its diverse volatile oil composition. Elemol represented a major terpene constituent across the 4 species and to a greater extent in *J. pinchotii* and *J. monosperma* than that previously measured (Utsumi et al., 2009; Whitney and Muir, 2010). This difference could be attributed to oxidation during processing and storage (Adams, 2013), chemical decomposition during distillation (Adams, 2011), or variability among plants and locations (Von Rudloff, 1975). Comparisons of individual terpene concentrations across species should be judiciously interpreted as different volatile oil extraction methods (steam distillation vs. solvent extraction) can result in terpene rearrangement and decomposition (Adams, 1991; Koedam and Looman, 1980).

Modification and biosynthesis of terpenoids by lactic acid bacteria (Belviso et al., 2011, 2014), rumen microorganisms in vitro (Broudiscou et al., 2007), and rapid absorption when intraruminally dosed (Dziba et al., 2006) point to the extensive biotransformation of terpenes. Malecky et al. (2012) reported less extensive rumen degradation of oxygenated monoterpenes (0% linalool and 10% 4-terpinenol) compared with monoterpene hydrocarbons (97% α-phellandrene and 84% α-terpinene) in continuous-culture fermentation. These monoterpenes represent a minor component of volatile oil across the 4 species in the current study; however, degradation of 78% of the sesquiterpene β-cedrene (Malecky et al., 2012) has particular relevance to *J. virginiana* and *J. ashei* in the current study. Whether the more prominent oxygenated sesquiterpenes observed (e.g., cedrol, widdrol, thujopsenol) are similarly degraded by rumen microorganisms is unknown.

In the current study, volatile oil percentages, especially heartwood oil components (Appendix 1; cedrol, α-cedrene, thujopsene, and nookatone), might overestimate biological availability. Methods used to extract and quantify the volatile oil components (steam distillation vs. solvent extraction) impart an imperfect estimation of a ruminant's ability to extract 100% of the volatile oil in a woody plant particle. In the current study, volatile oil yield and composition of tIVDMD residue was not analyzed; however, considering the limited digestibility (29 to 33%) of woody material from mature plants across species and poor water solubility of terpenes and terpenoids due to adsorption to plant particles (Weidenhamer et al., 1993; Malecky et al., 2012), it is possible that a major fraction of the less volatile sesquiterpenes remained bound to the indigestible woody plant material.

Total volatile oil concentrations in *Juniperus* species can have an aversive effect on herbivory of these plants (Langenheim, 1994; Markó et al., 2008). Individual compounds and their relationship to herbivory (Utsumi et al., 2009) are less effective predictors than total volatile oil concentrations (Estell et al., 2014). For example, α-pinene was negatively related to intake of *J. ashei* (Riddle et al., 1996; Adams et al., 2013) but unrelated to intake in *J. monosperma* (Utsumi et al., 2009), possibly due to the seasonal and plant chemical variation with *J. monosperma*. However, more consistently, oxygenated monoterpenes and sesquiterpenes deter herbivory (Utsumi et al., 2009; Estell et al., 2014). At times, clearance of terpenes by hepatic phase I and II enzymes may occur when the concentration in the organism rises above a threshold (Torregrossa and Dearing, 2009). Based on low volatile oil yields in the current study, under hypothetical circumstances of maximal ground juniper consumption, terpene concentrations ingested would still be less than concentrations administered and or consumed (Villalba et al., 2006; Dziba and Provenza, 2008; Malecky et al., 2009) where no clinical health issues were observed.

Dietary terpenoids can increase microbial efficiency and microbial protein synthesis and reduce methane production and acetate:propionate ratio (reviewed by Calsamiglia et al., 2007). Specifically, Yang et al. (2007) fed 2 g/d of oil from *Juniperus communis* (35% α-pinene) to Holstein cows and observed marginally improved ruminal digestibility compared with controls not receiving volatile oil. Villalba et al. (2006) fed predominant terpenes in big sage brush and reported increased in vivo digestibility of DM, NDF, and ADF. Addition of *J. monosperma* leaves to sheep and goat di-

ets was associated with increased total VFA (Utsumi et al., 2013) and greater total tract digestibility (Giacomini et al., 2006), which suggests a potential stimulatory effect of α-pinene on rumen function. It is unclear whether the reduced volatile oil concentrations in ground plant material would have any stimulatory due to low concentrations remaining compared with fresh material.

Considering the similarity of volatile oil yields and composition to those found in feeding trials conducted with *J. pinchotii* (Whitney et al., 2014) and considering in vitro digestibility and gas production characteristics in the current study, it is probable that immature *J. monosperma* and *J. pinchotii* would have a more similar feeding value than *J. ashei* and *J. virginiana*. Limited digestibility of woody material and low volatile oil composition of processed plant material reduce the biological relevance of volatile oil. Although mature species had similar tIVDMD, the volatile oil characteristics (quantity and composition) in mature *J. virginiana*, divergent from those detected in other mature species, may warrant attention in future feeding applications.

Labdane Acids (Isocupressic Acid, Agathic Acid, Imbricatoloic Acid, and Dihydroagathic Acid)

The labdane resin acid ICA and similar related labdane acids including AGA, IMB, and DHAA were analyzed in the 4 *Juniperus* species and presented as a percentage of DM (Table 3). Concentrations of ICA in ponderosa pine and its related metabolite AGA found in *Juniperus osteosperma* and *Juniperus occidentalis* are associated with late-term abortions in cattle (Gardner and James, 1999; Welch et al., 2012) but not in pregnant sheep (Short et al., 1995). Labdane acid concentrations >0.5% DM are potentially abortifacient, with potentially greatest risk at >1.0% DM as discussed by Gardner et al. (1994) and Welch et al. (2013). Similarly, IMB and DHAA are suspected to be biologically active abortifacient compounds (Welch et al., 2012, 2013). Negligible amounts of ICA, IMB, and DHAA (≤0.03% DM) were measured for *J. ashei*, *J. virginiana*, and *J. pinchotii*. Minor concentrations of AGA were also measured in *J. ashei* (≤0.02% DM), *J. virginiana* (≤0.03% DM), and *J. pinchotii* (≤0.06% DM) with no differences ($P = 0.14$) between immature and mature plants, suggesting *J. ashei*, *J. virginiana*, and *J. pinchotii* pose little abortifacient risk.

In contrast, *J. monosperma* had greater ($P < 0.05$) concentrations of ICA, AGA, IMB, and DHAA compared with *J. ashei*, *J. virginiana*, and *J. pinchotii*. Furthermore, immature *J. monosperma* plants vs. mature plants had greater ($P < 0.05$) concentrations of ICA (0.27 vs. 0.16% DM), IMB (0.20 vs. 0.01% DM), DHAA (1.4 vs. 0.71% DM), and AGA (0.23 vs. 0.16% DM). Total combined labdane acid concentrations (ICA, AGA, IMB, and DHAA) in *J. monosperma* exceed the recommended conservative threshold of 0.5% DM, indicating a potential risk for late-term abortions in cattle (K. D. Welch, USDA-ARS, Logan, UT, personal communication). This concern is substantiated by related research with ICA in ponderosa pine (Gardner et al., 1994) and AGA in *J. osteosperma* (Gardner et al., 2010). It is noteworthy that the DHAA, which makes up 66% of total labdane acids in *J. monosperma*, although a related metabolite of ICA, has not been definitively proven to be an abortifacient metabolite. Welch et al. (2012) have hypothesized that ICA is metabolized to AGA and subsequently to DHAA and tetrahydroagathic acid but recognize that additional work is needed to determine if the DHAA found in *J. monosperma* possesses abortifacient properties. No documented cases of abortion in cattle have been directly implicated to consumption of *J. monosperma* (S. Cox, New Mexico State University, Corona, NM, personal communication).

The broad objectives of the current study were to identify baseline nutritional, in vitro digestibility, and plant secondary compound characteristics. Findings indicate the nutritional and in vitro digestibility of ground whole immature and mature juniper trees for the 4 juniper species studied were equal to or better than other commonly used and approved roughage sources. Concentrations of plant secondary compounds when juniper is dried, ground, and mixed with other feed ingredients should cause no adverse effects to ruminants and could provide some health and digestibility benefits. The analysis conducted in this study indicates that *Juniperus* species can be used as a roughage source in ruminant diets.

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Appendix 1. Effects of plant stage of maturity on volatile oil composition (mg/g DM) of *Juniperus pinchotii*, *J. monosperma*, *J. ashei*, and *J. virginiana*[1]

Item[2]	*J. pinchotii*		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled SEM
	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	
Tricyclene	0.004^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.053^{a}	0.016^{b}	0.0^{b}	0.0^{b}	0.005
α-Thujene	0.035^{a}	0.002^{b}	0.00^{b}	0.00^{b}	0.015^{ab}	0.00^{b}	0.015^{b}	0.00^{b}	0.001
α-Pinene	0.071^{b}	0.016^{b}	1.20^{a}	0.147^{b}	0.026^{b}	0.026^{db}	0.024^{b}	0.037^{b}	0.103
Camphene	0.006^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.056^{a}	0.021^{b}	0.00^{b}	0.00^{b}	0.007
Sabinene	0.547^{a}	0.010^{b}	0.027^{b}	0.002^{b}	0.0^{b}	0.012^{b}	0.129^{b}	0.078^{b}	0.023
Myrcene	0.083^{a}	0.022^{bc}	0.032^{b}	0.004^{bc}	0.007^{bc}	0.002^{c}	0.004^{bc}	0.002^{c}	0.006
α-Phellandrene	0.0^{b}	0.0^{b}	0.021^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.001
δ 3-Carene	0.007^{b}	0.0^{b}	0.095^{a}	0.004^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.01
α-Terpinene	0.045^{a}	0.017^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.003
p-Cymene	0.015^{bc}	0.005^{bc}	0.011^{bc}	0.0^{c}	0.061^{a}	0.028^{b}	0.0^{c}	0.0^{c}	0.006
Limonene	0.080^{a}	0.031^{bcd}	0.064^{abc}	0.001^{d}	0.072^{ab}	0.028^{bcd}	0.020^{cd}	0.013^{d}	0.010
β-Phellandrene	0.063^{b}	0.024^{b}	0.195^{a}	0.020^{b}	0.0^{b}	0.0^{b}	0.014^{b}	0.010^{b}	0.016
γ-Terpinene	0.091^{a}	0.035^{b}	0.019^{bc}	0.004^{c}	0.0^{c}	0.0^{c}	0.006^{bc}	0.002^{c}	0.006
cis-Sabinene hydrate	0.177^{a}	0.060^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.013^{b}	0.011^{b}	0.013
Terpinolene	0.048^{a}	0.021^{b}	0.040^{a}	0.004^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.003
Linalool	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.015^{ab}	0.009^{ab}	0.028^{a}	0.012^{ab}	0.005
trans-Sabinene hydrate	0.131^{a}	0.048^{b}	0.0^{c}	0.003^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.009
Isopentyl isovalerate	0.0	0.0	0.0	0.0	0.008	0.0	0.0	0.0	0.002
cis-p-Menth-2-en-1-ol	0.024^{a}	0.006^{b}	0.030^{a}	0.004^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.002
trans-p-Mentha-2,8-dien-ol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.014^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.001
trans-Pinocarveol	0.0^{c}	0.0^{c}	0.027^{a}	0.013^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.002^{c}	0.002
trans-Verbenol	0.0^{c}	0.0^{c}	0.059^{a}	0.035^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.004
Camphor	0.804^{bc}	0.553^{c}	0.0^{d}	0.0^{d}	2.53^{a}	1.15^{b}	0.024^{d}	0.058^{d}	0.103
Camphene hydrate	0.033^{b}	0.024^{b}	0.0^{c}	0.0^{c}	0.062^{a}	0.028^{b}	0.0^{c}	0.0^{c}	0.002
Citronellal	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.001
Isoborneol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.012^{a}	0.001^{b}	0.0^{b}	0.0^{b}	0.001
Borneol	0.105^{b}	0.066^{bc}	0.011^{cd}	0.015^{cd}	0.177^{a}	0.056^{bcd}	0.002^{d}	0.004^{d}	0.013
Coahuilensol	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.008	0.002
Terpinen-4-ol	0.309^{a}	0.161^{b}	0.035^{c}	0.013^{c}	0.011^{c}	0.033^{c}	0.034^{c}	0.027^{c}	0.023
p-Cymen-8-ol	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.023^{a}	0.012^{b}	0.0^{c}	0.0^{c}	0.001
α-Terpineol	0.004^{a}	0.005^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.002
trans-p-Mentha-1(7), 8-dien-2-ol	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.013^{a}	0.007^{b}	0.0^{c}	0.0^{c}	0.001
Methyl chavicol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.009^{ab}	0.002^{b}	0.011^{ab}	0.018^{a}	0.003
Myrtenol	0.0^{b}	0.0^{b}	0.022^{a}	0.013^{a}	0.016^{a}	0.017^{a}	0.0^{b}	0.0^{b}	0.003

Appendix 1. continued

Appendix 1. (cont.)

Item[2]	*J. pinchotii*		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled SEM
	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	
Verbenone + *trans*-piperitol	0.0^{b}	0.0^{b}	0.030^{a}	0.018^{ab}	0.019^{ab}	0.019^{ab}	0.009^{bc}	0.017^{ab}	0.003
43,79,91,152,terpene	0.0^{b}	0.0^{b}	0.0^{b}	0.032^{a}	0.035^{a}	0.025^{a}	0.0^{b}	0.0^{b}	0.004
Coahuilensol, methyl ether	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.111^{a}	0.088^{ab}	0.022
Citronellol	0.285^{a}	0.171^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.021^{b}	0.011^{b}	0.027
cis-p-Mentha-1(7),8dien 2-ol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.013^{ab}	0.027^{a}	0.0^{b}	0.0^{b}	0.005
Carvone thymol, methyl ether	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.042^{a}	0.038^{a}	0.0^{b}	0.0^{b}	0.003
Carvacrol, methyl ether	0.007^{b}	0.030^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.003
Piperitone	0.0^{b}	0.0^{b}	0.016^{a}	0.005^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.002
trans-Myrtanol	0.0^{b}	0.0^{b}	0.008^{a}	0.001^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.001
152,123,91,77, sesquiterpene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.022^{a}	0.004^{b}	0.002
151,166,95,135,phenolic	0.0^{b}	0.039^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.006
Pregeijerene	0.0^{b}	0.0^{b}	0.160^{a}	0.030^{b}	0.0^{b}	0.0^{b}	0.035^{b}	0.012^{b}	0.011
Bornyl acetate	0.137^{b}	0.098^{bc}	0.032^{cde}	0.028^{de}	0.358^{a}	0.080^{bcd}	0.0^{e}	0.0^{e}	0.015
Safrole	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.492^{a}	0.219^{b}	0.039
Carvacrol	0.0	0.0	0.0	0.0	0.001	0.003	0.0	0.0	0.001
α-Cubebene	0.026^{a}	0.005^{ab}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.002^{b}	0.002^{b}	0.005
α-Copaene	0.045^{a}	0.006^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.016^{ab}	0.013^{b}	0.007
β-Bourbonene	0.041^{b}	0.013^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.010^{b}	0.0^{b}	0.002
β-Cubebene	0.0	0.0	0.0	0.0	0.0	0.0	0.009	0.004	0.002
β-Elemene	0.028^{a}	0.009^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.003
Methyl eugenol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.429^{a}	0.700^{a}	0.080
α-Cedrene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.009^{b}	0.027^{b}	0.019^{b}	0.200^{a}	0.026
(E)-caryophyllene	0.130^{a}	0.037^{cd}	0.089^{ab}	0.048^{bc}	0.0^{d}	0.0^{d}	0.036^{cd}	0.011^{cd}	0.010
β-Cedrene	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.018^{bc}	0.026^{b}	0.049^{b}	0.128^{a}	0.011
cis-Thujopsene	0.0^{c}	0.0^{c}	0.247^{abc}	0.554^{a}	0.136^{bc}	0.501^{a}	0.143^{bc}	0.377^{ab}	0.069
cis-Muurola-3,5-diene	0.119^{a}	0.034^{bc}	0.065^{b}	0.025^{bc}	0.0^{c}	0.0^{c}	0.034^{bc}	0.027^{bc}	0.011
α-Humulene	0.161^{a}	0.032^{bc}	0.052^{b}	0.014^{bc}	0.0^{c}	0.0^{c}	0.026^{bc}	0.014^{bc}	0.009
cis-Muurola-4(14),5-diene	0.036^{a}	0.007^{b}	0.016^{ab}	0.011^{b}	0.0^{b}	0.0^{b}	0.009^{b}	0.002^{b}	0.005
trans-Cadina-1(6),4-diene	0.137^{a}	0.039^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.017^{b}	0.019^{b}	0.009
γ-Muurolene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.021^{a}	0.012^{a}	0.002
Germacrene D	0.061^{a}	0.023^{bc}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.051^{ab}	0.050^{ab}	0.006
β-Selinene	0.0^{c}	0.0^{c}	0.024^{a}	0.011^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.001
cis-*β*-Guaiene	0.0^{b}	0.0^{b}	0.050^{a}	0.011^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.007
trans-Muurola-4(14),15-	0.372^{a}	0.100^{b}	0.059^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.070^{b}	0.073^{b}	0.038
epi-Cubebol	0.104^{a}	0.047^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.044^{b}	0.040^{bc}	0.009
Valencene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.009^{ab}	0.016^{a}	0.0^{b}	0.0^{b}	0.002
β-Himachalene	0.066^{a}	0.021^{bc}	0.009^{c}	0.019^{c}	0.0^{c}	0.0^{c}	0.055^{ab}	0.074^{a}	0.008
α-Cuprenene	0.0^{c}	0.0^{c}	0.004^{bc}	0.019^{b}	0.0^{c}	0.019^{b}	0.013^{bc}	0.049^{a}	0.004
Cuparene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.001^{b}	0.019^{a}	0.0^{b}	0.0^{b}	0.001
γ-Cadinene	0.452^{a}	0.129^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.034
Cubebol	0.449^{a}	0.129^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.032
Nootkatene	0.0^{a}	0.0^{a}	0.179^{a}	0.027^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.045
trans-Calamenene	0.236^{a}	0.075^{b}	0.017^{b}	0.004^{b}	0.0^{b}	0.0^{b}	0.081^{b}	0.082^{b}	0.021
Zonarene	0.090^{a}	0.032^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.007
trans-Cadina-1,4-diene	0.044^{a}	0.007^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.014^{b}	0.047^{a}	0.005
Elemicin	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.212^{a}	0.171^{a}	0.018
α-Copaen-11-ol	0.0^{c}	0.0^{c}	0.067^{a}	0.030^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.003
Elemol	2.05^{a}	1.06^{ab}	0.752^{b}	0.443^{b}	0.059^{b}	0.183^{b}	0.857^{b}	0.809^{b}	0.213
Germacrene B	0.190^{a}	0.095^{ab}	0.205^{a}	0.109^{ab}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.024
(E)-nerolidol	0.026^{ab}	0.007^{cd}	0.035^{a}	0.018^{bc}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.003
Germacrene D-4-ol	0.030^{b}	0.011^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.317^{a}	0.191^{a}	0.028
Caryophyllene oxide	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.041^{a}	0.035^{a}	0.003
Thujopsan-2-*α*-ol	0.0^{b}	0.0^{b}	0.045^{a}	0.030^{a}	0.025^{ab}	0.046^{a}	0.0^{b}	0.0^{b}	0.006
trans-Muurol-5-en-4-*α*-ol	0.376^{a}	0.108^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.151^{b}	0.181^{ab}	0.045

Appendix 1. continued

Appendix 1. (cont.)

Item[2]	*J. pinchotii*		*J. monosperma*		*J. ashei*		*J. virginiana*		Pooled SEM
	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	
allo-Cedrol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.002^{b}	0.055^{a}	0.0^{b}	0.0^{b}	0.005
Widdrol	0.0^{b}	0.0^{b}	0.050^{b}	0.191^{b}	0.0^{b}	0.0^{b}	0.186^{b}	0.765^{a}	0.091
Cedrol	0.0^{b}	0.011^{b}	0.050^{b}	0.114^{b}	0.313^{b}	1.39^{a}	0.376^{b}	1.53^{a}	0.206
β-Oplopenone	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.059^{b}	0.729^{a}	0.104
1-epi-Cubenol	0.399^{a}	0.145^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.133^{b}	0.154^{b}	0.026
Eremoligenol	0.0^{b}	0.0^{b}	0.179^{a}	0.253^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.029
γ-Eudesmol	0.167^{bc}	0.119^{bc}	0.783^{a}	0.426^{b}	0.0^{b}	0.0^{b}	0.058^{c}	0.058^{c}	0.072
α-Acorenol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.041^{b}	0.179^{a}	0.0^{b}	0.0^{b}	0.010
β-acorenol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.017^{b}	0.049^{a}	0.0^{b}	0.0^{b}	0.005
Hinesol	0.047^{cd}	0.030^{de}	0.074^{bc}	0.058^{bcd}	0.0^{e}	0.0^{e}	0.092^{ab}	0.112^{a}	0.010
43,119,204,222,sesquiterpene	0.474^{bc}	0.344^{c}	2.57^{a}	1.48^{b}	0.012^{c}	0.074^{c}	0.170^{c}	0.201^{c}	0.218
β-Eudesmol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.038^{ab}	0.089^{a}	0.0^{b}	0.0^{b}	0.012
α-Eudesmol	0.412^{bc}	0.278^{cd}	1.05^{a}	0.708^{ab}	0.038^{d}	0.094^{cd}	0.209^{cd}	0.227^{cd}	0.075
selin-11-en-4-a-ol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.013^{b}	0.058^{a}	0.0^{b}	0.0^{b}	0.007
14-Hydroxy-9-epi-caryphyllene	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.005^{b}	0.018^{a}	0.0^{b}	0.0^{b}	0.002
Elemol acetate	0.0^{c}	0.0^{c}	0.061^{a}	0.044^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.004
epi-a-Bisabolol	0.0^{b}	0.0^{b}	0.024^{b}	0.056^{ab}	0.0^{b}	0.0^{b}	0.023^{b}	0.159^{a}	0.028
Shyobunol	0.0^{b}	0.0^{b}	0.053^{ab}	0.105^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.015
cis-Thujopsenol	0.0^{b}	0.0^{b}	0.119^{b}	0.284^{ab}	0.063^{b}	0.259^{ab}	0.132^{ab}	0.416^{a}	0.064
cis-Thujopsenal	0.0^{b}	0.0^{b}	0.013^{a}	0.045^{a}	0.008^{a}	0.035^{a}	0.068^{a}	0.049^{a}	0.023
(Z)-nuciferol	0.0^{b}	0.0^{b}	0.011^{b}	0.065^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.008
8-α-11-Elemodiol	0.0^{b}	0.0^{b}	0.175^{a}	0.157^{a}	0.0^{b}	0.0^{b}	0.044^{b}	0.026^{b}	0.022
41,109,138,202, sesquiterpene	0.0^{c}	0.0^{c}	0.061^{a}	0.037^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.004
8-α-Acetoxyelemol	0.0^{b}	0.0^{b}	0.106^{a}	0.072^{a}	0.0^{b}	0.0^{b}	0.070^{a}	0.086^{a}	0.011
Nootkatone	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.028^{bc}	0.061^{bc}	0.198^{b}	0.446^{a}	0.037
Oplopanonyl acetate	0.0	0.0	0.0	0.0	0.0	0.0	0.013	0.003	0.003
Manoyl oxide	0.329^{a}	0.218^{ab}	0.065^{bc}	0.057^{bc}	0.210^{abc}	0.157^{abc}	0.013^{bc}	0.006^{c}	0.045
Abietatriene	0.021	0.016	0.010	0.018	0.023	0.017	0.010	0.020	0.004
Abietadiene	0.164^{a}	0.100^{ab}	0.0^{c}	0.0^{c}	0.051^{bc}	0.028^{bc}	0.057^{bc}	0.074^{bc}	0.019
41,69,255,298,diterpene	0.073^{abc}	0.0454^{bc}	0.092^{abc}	0.066^{abc}	0.0^{c}	0.0^{c}	0.155^{a}	0.098^{ab}	0.021
Sandaracopimarinal	0.075	0.076	0.024	0.056	0.038	0.062	0.030	0.042	0.014
41,81,286,300,diterpene	0.0^{c}	0.0^{c}	0.027^{b}	0.051^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.003
91,133,187,286,diterpene	0.031^{a}	0.036^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.005
43, 91,271	0.031^{a}	0.049^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.005
41, 91, 157	0.039^{a}	0.045^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.006
227,269,185,284,diterpene	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.078^{a}	0.057^{b}	0.0^{c}	0.0^{c}	0.004
42,227,269,284, diterpene	0.033^{c}	0.035^{bc}	0.083^{a}	0.081^{ab}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.101
41,69,255,298,diterpene	0.0^{b}	0.0^{b}	0.056^{a}	0.045^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.005
Sempervirol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.050^{a}	0.032^{a}	0.0^{b}	0.0^{b}	0.005
4-epi-Abietal	0.316^{a}	0.359^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.052^{b}	0.193^{ab}	0.207^{ab}	0.051
Abieta-7,13-dien-3-one	0.156^{b}	0.165^{b}	0.040^{b}	0.052^{b}	0.500^{a}	0.023^{b}	0.034^{b}	0.054^{b}	0.050
Abietal	0.152^{a}	0.167^{a}	0.024^{b}	0.076^{ab}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.007
trans-Ferruginol	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.044^{ab}	0.055^{a}	0.008^{cd}	0.028^{bc}	0.005
4-epi-Abietol	0.019^{ab}	0.018^{ab}	0.0^{b}	0.0^{b}	0.028^{a}	0.008^{ab}	0.0^{b}	0.0^{b}	0.005
135,91,187,286, diterpene	0.031^{a}	0.041^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.005
Abietol	0.023^{ab}	0.036^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.021^{ab}	0.015^{ab}	0.007

$^{a–d}$Within row means without a common superscript differ ($P < 0.05$) according to a LSD. Juniper species × stage of maturity.

[1]Imm = immature growth stage (height of 1 to 1.8 m); Mat = mature growth stage (height of >3 m).

[2]Compositional values <0.001 milligrams per gram DM are denoted as 0.0 as they were detected in trace amounts.