Differences in chemical composition between browsed and non-browsed *Juniperus ashei* Buch. Trees

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**Abstract**

Leaf secondary compounds were examined in browsed and non-browsed *Juniperus ashei* trees to identify selective browsing patterns by goats and deer. Analysis of volatile leaf oils (terpenoids) revealed that the browsed trees were lower in total oil than non-browsed trees (2.18 vs. 3.46%, DW basis). Extractable and fiber-bound condensed tannins (CT) were not different but protein-bound CT concentrations were greater in browsed trees. Among digestibility measures (NDF, ADF, IVMD), IVMD was greater in non-browsed leaves. Terpene components analyzed on a percent total oil basis had 3 differences versus 12 significant differences on a mg/g DW basis. The terpenoid components profile differed little between browsed and non-browsed trees. Total CT are negatively associated with oil yields. No association was found between crude protein and oil yields or between digestibility (IVMD, NDF, ADF) and oil yields. The question of whether individual plants or populations may invest less in CT when greater amounts of terpenes are produced (or vice-versa) may have evolutionary implications since individual browsers or populations may adapt to consuming or avoiding either CT or terpenes. The fact that browsed tree leaves were less digestible than non-browsed tree leaves may be a result of complex interactions between CT, terpenes, fiber, and nutrients.

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1. Introduction

The genus *Juniperus* contains species with a large array of terpenoids and other secondary compounds (Adams, 2011). It is common to find trees that have been browsed by deer (as well as domestic goats and sheep). Schwartz et al. (1980a) observed browsing by deer and then tested confined deer in feeding trials using foliage of *Juniperus deppeana*, *Juniperus monosperma* and *Juniperus scopulorum*. They found the consumption of juniper foliage varied inversely with the total oil yields among these three species. Schwartz et al. (1980a) also found the levels of oxygenated terpenoids were a greater feeding deterrent than the amount of hydrocarbon terpenoids. Recently, Marko et al. (2008) reported that leaf essential oil concentrations were lowest in *Juniperus communis* heavily browsed by sheep and rabbit and highest in non-browsed plants.

Juniper foliage intake by goats has been shown to be limited by the presence of monoterp enes (Riddle et al., 1996; Pritz et al., 1997). Monoterpenes have a clearly defined ecological defensive role as feeding deterrents in a variety of mammals and
insects (Gershenzon et al., 1992). Negative post-ingestive consequences experienced by large ungulates following consumption of high levels of monoterpenes include rumen microbial inhibition (Oh et al., 1967; Schwartz et al., 1980b), hepatic pathogenesis (Straka, 1993; Bisson et al., 2001; Pritz et al., 1997), and feeding cessation (Dziba et al., 2006). Furthermore, the presence of condensed tannins (CT) in plant leaves has long been associated with protection against herbivory (Feeny, 1976) by inducing post-ingestive feedback (Provenza, 1995). The strength or direction of this feedback depends on individual plant-herbivore characteristics and therefore requires individual situation testing (Stamp, 2003).

The objective of the present study was to correlate plant chemical and nutritive values in Juniperus ashei leaves with the incidence of browsing by goats (and to a much lesser extent, deer).

2. Material and methods

2.1. Study site

The study was conducted at the Texas AgriLife Research Station, Sonora, located on the southwestern edge of the Edwards Plateau (30°15.747′N, 100°34.164′W, 707 m). Annual precipitation averages approximately 600 mm; it is bimodal with largest amounts occurring in spring and fall. Soils in the study pasture are Tarrant silty clays and soil depth overlaying a fractured limestone substrate ranges from about 10 to 450 mm. Dominant herbaceous species include Hilaria belangeri (Steud.) Nash and Bouteloua curtipendula (Michx.) Torr. Dominant woody species include Quercus fusiformis Mill, Q. pungens Liebm var. vaseyana, J. ashei and Juniperus pinchotii. Fires within the area have not occurred for at least 100 years before data collection. From 1983 to 1993, stocking rates in the study pasture were maintained at a moderate level (i.e., 11.3 ha/animal). From 1994, stocking rates in the study pasture have varied from 18 to 10.4 ha/animal. A combination of cattle, sheep, and goats were grazed on the pasture until 2003 when all cattle were removed from the study area. The area is now grazed by goats and deer.

2.2. Plant material

Nine browsed J. ashei trees were randomly selected along a serpentine transect line of approximately 200 m. Trees showing severe browsing (e.g., all lower branches up to approximately 1 m were essentially defoliated) and had new, immature growth on the browsed branches were sampled as “browsed trees”. This corresponds to the ‘heavily browsed’ category of Marko et al. (2008, Fig. 2, right). Nine trees with no evidence of browsing were sampled along the transect as ‘non-browsed’ trees, corresponding to the ‘non-browsed’ category of Marko et al. (2008, Fig. 2, left). Trees with other levels of browsing were excluded from sampling. J. ashei has two kinds of leaves: whip- (decurrent) and scale-like leaves that remain functional for 4–6 years. The whip leaves are only found on the new growth (leaders). At the study site, due to drought, very

![Fig. 1. Principal components analysis (PCA) of terpenoids, condensed tannins, and crude protein concentrations. Variance explained by a component is indicated in parenthesis.](image-url)
few whip-leaves were observed and the occasional branch with whip-leaves was excluded from sampling as Adams and Hagerman (1976) found significant differences in the oils from whip- and scale-like leaves of *Juniperus*. No samples were taken from the branches with new, immature whip-leaves. Careful attention was given to sample at least 1 m above the browse line. As the browse line was up to 1 m above the ground, samples were taken at 2–2.5 m heights from both non-browsed and browsed trees from the south side of all trees. All trees were similar in size and height (3–6 m). Foliage was collected on May 14, 2010. Browsing occurred during the winter of 2010. Specimens collected: *J. ashei*, browsed trees: Adams 12251–12259 and non-browsed trees: Adams 12260–12268; herbarium vouchers are deposited in the herbarium, Baylor University (BAYLU).

2.3. Volatile oil analysis

A portion (200 g FW) of the fresh foliage was kept cool (20 °C) and in the dark, then, within 24 h, exhaustively steam-distilled for 24 h using a modified circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at –20 °C until analyzed. The steam distilled leaves were oven dried to a constant weight (48 h, 100 °C) for the determination of oil yield as (oil wt./oil wt. + oven dried extracted foliage wt.).

Extracted oils were analyzed on an HP5971 MSD mass spectrometer: 0.2 ul of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60°–246 °C at 3 °C/min(62 min), carrier gas He, flow 34.96 cm/s or 1.02 ml/min, injector 220 °C, detector 240 °C, scan time 1/second, directly coupled to an HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm × 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams, 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams, 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

2.4. Condensed tannin analysis

Condensed tannins in air dried (48 h, 42 °C) leaves were assayed for soluble, protein-bound, and fiber-bound fractions by methods described by Terrill et al. (1992). Samples were oven-dried and standards prepared from Ashe juniper as recommended by Wolfe et al. (2008).
2.5. Crude protein and in vitro dry matter digestibility analyses

A portion of each foliage sample was also air dried (48 h, 60 °C), ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen, and analyzed for N (AOAC, 2006); crude protein (CP) was calculated as 6.25 × N. An additional 0.35 g of the ground sample material was placed into separate F57 digestion bags and analyzed for 48-hr true in vitro dry matter digestibility (IVDMD) using an Ankom Daisy II incubator (Ankom Technol. Corp., Fairport, NY). Bags were placed into an incubation jar containing 400 mL of goat rumen fluid (donors fed Tifton 85 hay) and 1600 mL of McDougal’s buffer solution (1.064 g urea L−1). After anaerobic incubation at 39 °C, all bags were gently rinsed under cold water and washed by hand until water was clear. Bags were subjected to the neutral detergent fiber procedure according to Van Soest et al. (1991), modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) without correcting for residual ash and using α-amylase and Na sulfite. Bags were then rinsed in acetone and dried at 55 °C in a forced-air oven for 48 h and weighed.

2.6. Statistical analyses

Terpenoids (as percentage of total oil and as mg per g dry foliage weight) compared among the samples by ANOVA and SNK (Student–Newman–Keuls) analyses as described by Steele and Torrie (1960), Gower or Manhattan metric (Gower, 1971; Adams, 1975) were computed among all populations using character weighting of F-1 (F from ANOVA). Principal Component Analysis (PCA) was performed to examine the patterns of association among individual terpenes, oil yield, crude tannins, ADF, NDF, CP and digestibility (formulation of Gower, 1966 and Veldman, 1967).

3. Results and discussion

A comprehensive analysis of the leaf oil of J. ashei is shown in Table 1. The most principal result was that percentage of total volatile leaf oil yields were greater (F = 19.4*** in non-browsed trees (3.47%, DM-basis) than browsed trees (2.18%, DM-basis; Table 1). This result parallels the findings of Marko et al. (2008) who found that sheep and rabbit-browsed J. communis trees in Hungary had lower total volatile leaf oils than non-browsed trees.

| Variable | Browsed % yield (g/100 g DW) | Not browsed % yield (g/100 g DW) | F signific. | Browsed % extractable CT | Not browsed % extractable CT | F signific. | Browsed % protein bound CT | Not browsed % protein bound CT | F signific. | Browsed % fiber bound CT | Not browsed % fiber bound CT | F signific. | Browsed % total CT | Not browsed % total CT | F signific. | Browsed % CP | Not browsed % CP | F signific. | Browsed % NDF | Not browsed % NDF | F signific. | Browsed % ADF | Not browsed % ADF | F signific. | Browsed % IVDMD | Not browsed % IVDMD | F signific. |
|----------|-----------------------------|----------------------------------|-------------|---------------------------|----------------------------|-------------|---------------------------|-----------------------------|-------------|---------------------------|-----------------------------|-------------|-------------------|-------------------|-------------|-----------------|-------------------|-------------|-----------------|-------------------|-------------|-------------------|-------------------|-------------|
| KI Compound | Dry Matter basis, mg/g DW | Percentage total oil basis |
| 921 Tricyclic (mg/g DW extracted) | 0.76 | 1.33 | 10.8 P < 0.01 | 3.47 | 3.76 | 0.7 ns | 932 α-pinene | 0.32 | 0.59 | 7.4 P < 0.05 | 1.46 | 1.66 | 0.9 ns | 946 Camphene | 0.69 | 1.24 | 14.7 P < 0.01 | 3.21 | 3.53 | 1.3 ns | 969 Sabine | t | t | nt | t | t | nt | 974 β-pinene | 0.03 | 0.04 | 0.9 P < 0.05 | 0.64 | 0.62 | 0.9 ns | 988 Myrcene | 0.42 | 0.93 | 7.9 P < 0.05 | 1.87 | 2.60 | 3.1 ns | 1001 δ-2-carene | t | t | nt | t | t | nt | 1002 α-phellandrene | t | t | nt | t | t | nt | 1008 δ-3-carene | 0.10 | 0.04 | 1.4 ns | 0.64 | 0.12 | 3.4 ns | 1014 α-terpine | t | t | nt | t | t | nt | 1020 p-cymene | 0.57 | 0.75 | 7.7 P < 0.05 | 2.66 | 2.18 | 7.1, P < 0.05 | 1024 Limonene | 1.84 | 3.59 | 11.2 P < 0.01 | 8.31 | 10.41 | 5.8 P < 0.05 | 1025 β-phellandrene | t | t | nt | t | t | nt | 1054 γ-terpine | 0.14 | 0.30 | 5.4 P < 0.05 | 0.59 | 0.84 | 2.4 ns | 1067 cis-linalool oxide (furanoid) | t | t | nt | t | t | nt | 1078 Camphenilone | t | t | nt | t | t | nt | 1083 Fenchone | t | t | nt | t | t | nt | 1084 Trans-linalool oxide (furanoid) | t | t | nt | t | t | nt | 1086 Terpinolene | 0.13 | 0.21 | 4.7 P < 0.05 | 0.59 | 0.60 | 0.02 ns | 1089 p-cymene | t | t | nt | t | t | nt | 1095 Linalool | t | t | nt | t | t | nt | 1102 Isopentyl-isovalerate | 0.08 | 0.12 | 1.4 ns | 0.34 | 0.32 | 0.04 ns | 1112 3-methyl-3-butenyl butanoate, 3-methyl- | t | t | nt | t | t | nt | 1136 Trans-p-methyl-2-en-1-ol | t | t | nt | t | t | nt |
Table 1 (continued)

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<td>0.06</td>
<td>0.09</td>
<td>2.7 ns</td>
<td>0.26</td>
<td>0.27</td>
<td>0.01 ns</td>
</tr>
</tbody>
</table>

Kl = Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). The four most prevalent ions are listed for each unknown compound. The base ion (100%) is underlined. Unidentified components less than 0.5% are not reported.

Protein-bound CT concentrations differed (F = 6.2*, Table 1) between browsed and non-browsed trees. Crude protein, ADF, and NDF were similar between browsed and non-browsed trees. However, browsed trees had lower IVDMD (71.56%) than non-browsed trees (74.32%; F = 12.62**, Table 1).

Components computed on a dry matter basis (mg/g DW) varied more between browsed and non-browsed trees (Table 1) than components computed on a percentage of total oil basis. In fact, for components computed on a percentage of total oil basis, there were only three components with significant differences (P = 0.05) between browsed and non-browsed trees. In contrast, there are 12 components that differed (6 at P = 0.05, 6 at P = 0.01) when analyzed on a mg/g DW basis. Marko et al. (2008) found the percentage of α-pinene and γ-terpinene were larger in browsed J. communis trees, whereas the percentage of δ-3-carene, myrcene, and unknown (RT12.22) were larger in non-browsed trees. Marko et al. (2008) did not analyze their oil components on a mg/g basis. Estell et al. (2008) found that the addition of sesquiterpenes to alfalfa pellets tended to decrease the intake of feed, but cis-β-ocimene and cis-sabinene hydrate did not appear to effect feed consumption.
Principal Components Analysis (PCA) revealed (Fig. 1) that the percentage of oil yield was loaded to the far right on the PCA 1 (31.2% of the variance) and CT, in general, loaded very low on PCA 1, far from percentage of oil yield. In general, CT were grouped (except percent fiber-bound, which had very small values, and often zero). Monoterpenes hydrocarbons were tightly grouped (Fig. 1) except for 3-carene and p-cymene. Likewise, the sesquiterpene and diterpene alcohols showed patterns that are related to their structural similarities. However, it should be noted that compressing the variables into two dimensions only gives the major trends, and individual associations may appear quite distorted. It would be of agronomic interest to closely examine statistical correlations between percentage of oil yield, CT, CP, and IVDM and but that was beyond the scope of this study.

Ordination of browsed and non-browsed trees on the basis of CT and leaf essential oils (mg/g DM-basis data), showed a slight separation (Fig. 2). Because PCA 1 is associated with the percentage of oil yield, it is apparent that there is some overlap in browsing among the intermediate oil-yielding trees (Fig. 2). It is possible that a few trees were avoided (non-browsed) for completely unrelated reasons. An observational or feeding trial would give an even more clear-cut classification.

The examination of the negative correlation between crude tannins (CT) and oil yields in J. ashei merits further study. The question of whether individual plants or populations may invest less in CT when terpenes are produced (or vice-versa) may have management implications since individual browsers or populations may adapt to consuming or avoiding either CT or terpenes. If this occurs, it begs the question whether plants then reverse their investment in CT or terpenes to adjust to the additional browsing pressure. Browsed tree leaves being less digestible than non-browsed tree leaves may be a result of complex interactions between CT, terpenes, fiber, and nutrients, and warrants further investigation.

Acknowledgments

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References