Snowpack-forced browsing by deer onto Scots Pine (*Pinus sylvestris*): Selection of low volatile leaf terpenoid concentrations for browsing

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ABSTRACT

A comparison between heavily and lightly browsed Scots pine (*Pinus sylvestris*) trees revealed that the yield of volatile leaf oil and tree height were highly significantly different. Higher volatile oil yield was positively correlated with lightly browsed trees, and lower oil yield correlated with heavily browsed trees. Tree height was negatively correlated with terpenoid yield, indicating younger trees produced more oil, perhaps as a defense mechanisms. Methanol extractables were significantly higher in highly browsed trees, suggesting that sugars and carbohydrates might be attractive to browsing deer. Hexane extractables were not significantly different. Published on-line www.phytologia.org *Phytologia 96(2): 84-88 (April 1, 2014).*

KEY WORDS: *Juniperus pinchotii*, goats, deer, browsing, terpenes, fiber, condensed tannins, digestibility, diet selection.

Deer browsing of conifers is a common phenomenon. Two of the best documented cases are *Chamaecyparis nootkatensis* and *Thuja plicata* (Vourc'h, Russell and Martin, 2002). They found the concentrations of mono- sesqui-, and di-terpene to be high in un-browsed *C. nootkatensis* and low in browsed genets in a plot at Cowichan Lake Research Station. For *Thuja plicata*, they found monterpenes higher in lightly-browsed trees but di-terpene concentrations were not significantly different (*T. plicata* oil contains almost no sesquiterpenes). Schwartz et al. (1980a) observed deer browsing on *Juniperus scopulorum* when deep winter snows in northern Colorado prevented access to their regular browse species. They conducted feeding trials on confined deer using foliage of *J. deppeana*, *J. monosperma* and *J. scopulorum* and found the consumption of juniper foliage varied inversely with the total oil yields among these three species. Schwartz et al. (1980a) also found that 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 *J. 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 monoterpenes (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 (Feeny 1976, 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 (Estell, et al. 2008, Straka 1993; Bisson et al. 2001; Pritz et al. 1997), and feeding cessation (Dziba et al. 2006; Provenza 1995).

Recently, Adams et al. (2013a) reported on differences between browsed and non-browsed trees of *Juniperus ashei*. In that study, the yields of volatile leaf oil were found to be the major factor associated with browsed and non-browsed samples. The leaf oils of browsed trees were much lower (2.18% DM) than in non-browsed trees (3.47% DM). Associated with oil yields were 12 terpenoids, all of which were at a higher concentration (mg/g) in the non-browsed trees. However, the profile of terpene composition (% total oil) was not very effected, as only 3 terpenoids were significantly different ($P \leq 0.05$). In that study, it appears that selection was based mostly on the total oil yield rather than individual oil components. Of the other variables investigated: extractable condensed tannins (ECT); protein bound

CT (PCT); fiber bound CT; percent total condensed tannin (TCT); percent crude protein (CP); % neutral detergent fiber (NDF); acid detergent fiber (ADF); *in vitro* dry matter digestibility (IVDMD), only PCT and IVDMD showed significant differences between browsed and not-browsed trees.

However, in a related study of browsing on *J. pinchotii*, growing in the same population as *J. ashei* (above), Adams et al. (2013b) found no differences in the yields or composition of the leaf terpenes, tannins, or digestibility characteristics between browsed and un-browsed trees. They concluded that goats were using trees as social gathering points and continued meeting and an occasional bite led to the heavily browsed condition on some trees.

A study of deer browsing (Duncan, Hartley and Iason, 1994) on Sitka spruce (*Picea sitchensis*) found total monoterpene concentration in needles was a negative influence on browsing. Danell, Gref and Yazdani (2008) compared the incidence of moose browsing on Scots pine (*Pinus sylvestris*) with concentrations of leaf mono- and di-terpenes. No correlation was found between mono-terpene concentration and the degree of browsing, but a significant negative correlation was found between the di-terpene, pinifolic acid, and moose browsing.

Winter snow-pack was extremely deep in the foothills around Salt Lake City, UT. Two to three feet of snow prevented deer from browsing on their preferred, shrubs and forbs. As the condition persisted, deer descended into the University of Utah Research Park on Wakara Way, Salt Lake City in search of browse. Several rows of planted Scots pine (*Pinus sylvestris*) from 5 to 11 ft tall were exposed above the snow-pack. Starting with the trees nearest the foothills, the deer began to browse on the lower limbs (up to about 4 ft where they could reach the foliage). However, it soon became obvious that deer were selecting to browse the taller trees. Thus, an opportunity presented itself to examine some phytochemical data from these browsed and lightly browsed trees. The purpose of the present paper is to present data from these phytochemical analyses. Unfortunately, in the intervening years since 1982, the volatile leaf oils have been lost during a lab fire, so no analysis of the oil compositions was possible. However, the phytochemical data is still valid and is examined in this study.

MATERIALS AND METHODS

Plant material - Leaves from six heavily browsed Scots pine (*Pinus sylvestris*) trees and six lightly-browsed trees were sampled on Wakara Way, Salt Lake City, UT.

Essential oils analysis - A portion (100 g FW) of the fresh foliage was kept cool (20ºC) and in the dark, then, within 24 hr, steam-distilled for 2 h using a modified circulatory Clevenger-type apparatus (Adams, 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20ºC. Steam distilled leaves were oven dried to a constant weight (48 hr, 100ºC) for the determination of oil yield as [oil wt./(oil wt. + oven dried extracted foliage wt.)].

Hexane, methanol extractions - Methods follow that of Adams et al. (1986). Fresh leaves were air dried, (48 h, 70° C) and ground in a Wiley mill to pass a 2 mm mesh screen. A plug of glass wool was placed in an empty Whatman paper thimble (33 mm x 94 mm) and oven dried (48 h, 70° C). To prevent rehydration before pre-weighing, the thimble (containing the glass wool plug) was placed in a desiccator and allowed to cool for 4 hr. Disposable aluminum pans (Kaiser, no. 1016) were used for evaporation of solvents from each extraction, but these were found to contain a volatile coating that would contribute a source of error. Therefore, the aluminum pans were pre-baked at 100° C for 24 h, placed in a desiccator, allowed to cool for 4 h, and then pre-weighed. The air-dried, ground plant material (15-20 g) was placed in the pre-weighed thimble and secured with the corresponding glass wool plug. The plant material in the thimble was extracted sequentially, first with hexane for 20 h in a Soxhlet extractor (followed by oven-drying for 4 h at 100° C to remove traces of hexane from the extracted plant material), and then with methanol for 20 h in the same extraction apparatus. The extracts were placed in pre-weighed aluminum pans, and the solvents were evaporated in an externally-vented oven. Hexane extracts were concentrated at 100°C for 24 hr before weighing. Methanol extracts were concentrated at 100°C for 48 h and then put in a desiccator and permitted to cool for 4 h before weighing. The extraction thimble, glass wood plug, and marc were oven dried at 100°C for 48 h and placed in a desiccator for 4 h before final weighing for gravimetric determination of yields.

Statistical analyses - Volatile leaf terpenoids (% total oil), % hexane extractables, % methanol extractables, total hexane plus methanol extractables and tree height were compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Principal Component Analysis (PCA) was performed as formulated by the Veldman (1967).

RESULTS AND DISCUSSION

ANOVA revealed (Table 1) a highly significant difference in the yields of volatile leaf terpenoids between heavily browsed trees (0.65%) and for lightly browsed trees (1.06%). No significant differences were found in % hexane extractables or % total hexane + % methanol extractables, but combined hexane + methanol extractables was significantly different (Table 1). As per the field observations, the heavily browsed trees were highly significantly taller (9.08 ft.) compared to the lightly browsed trees (6.00 ft.).

The yield of methanol extractable material was significantly higher in heavily browsed trees (22.73% vs. 20.50%) suggesting that sugars and methanol soluble carbohydrates might be attractive to deer.

Table 1. Comparison of five factors from heavily browsed and lightly browsed trees of *Pinus sylvestris.* Significance, $P \leq 0.05$ = *; $P \leq 0.01$ = **, ns = non significant.

Factor tested	heavily browsed	lightly browsed	F ratio	P, signifance
% volatile leaf terpenoids yield (% ODW basis)	0.65	1.06	21.664	0.001, **
% hexane extractables	10.56	10.02	1.001	0.343, ns
% methanol extractables	22.73	20.50	6.704	0.025, *
% total hexane + methanol extractables	33.19	30.68	3.796	0.080, ns
tree height (ft)	9.08	6.00	16.106	0.003, **

The correlation among factors (Table 2) showed a high correlation between % methanol extractables and total extractables which was likely due to the large contribution of methanol to the total extractables. Tree height had a fairly high negative correlation with % oil yield (-0.75). This is not surprising as the smaller trees had higher oil yields (Table 1), suggestive that younger trees produced more oil as a defense.

Table 2. Pearson product correlations among factors in this study.

	% hexane	% methanol	total ext.	tree height	% oil yield
% hexane	1.00	0.54	0.81	0.33	-0.06
% methanol	0.54	1.00	0.92	0.71	-0.43
total ext.	0.81	0.92	1.00	0.59	-0.28
tree height	0.33	0.71	0.59	1.00	-0.75
% oil yield	-0.06	-0.43	-0.28	-0.75	1.00

Factoring the correlation matrix resulted in 3 eigenroots that accounted for 64.7, 24.6 and 7.1% (96.4%) of the variance among the samples. Ordination of the samples revealed eigenroot 1 (correlated with % old yield) is largely responsible for the separation of lightly and heavily browsed trees (Fig. 1).

Finally, it should be noted that after these samples were taken, the deep snow-pack continued and even the smaller, lightly browsed trees became targets of the deer. Browsing continued on all these 12 trees until all the foliage was removed up to about 6 ft into the trees, because the deer stood on their hind legs to reach higher branches. Nearly all the Scots pine trees died in the summer.

So, although a higher yield of leaf terpenoids served as defense, it was not a perfect defense. Because the deer were driven deeper into hunger, they consumed all the leaves available, regardless of oil content or tree height.

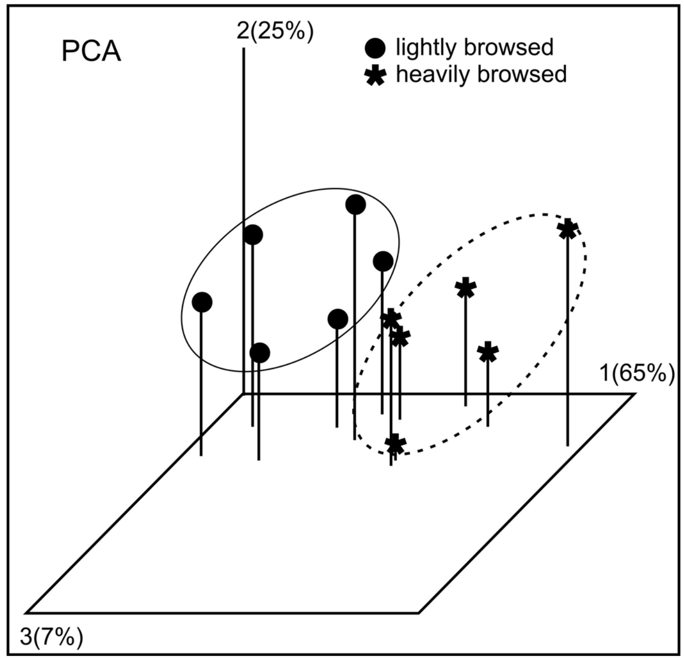

Figure 1. PCA ordination of the heavily and lightly browsed trees.

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