Palatability of Douglasfir Foliage to Mule Deer in Relation to Chemical and Spatial Factors

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Highlight: Relative preference of Douglasfir needles from eight environmental conditions was determined in a feeding trial with penned tame deer of mixed age and sex. Relative preference (percent fresh weight ingested) varied between 0 and 74%, with significant differences between open (16%) and gully (9%) sites, between old (20%) and young (4%) trees, and between tops (16%) and bottoms (8%) of trees. Shading was found to be responsible for some difference in palatability, unshaded being preferred (22%) over shaded needles (4%). Relative preference was found to be correlated with moisture content ($r = -0.57$). Relative preference was also found to be correlated with chlorogenic acid ($r = 0.41$), a naturally occurring phenolic ester which was quantified by fluorometric scanning after being observed in a thin layer chromatography (TLC) screening experiment. Crude protein was not significantly correlated with preference.

Douglasfir (*Pseudotsuga menziesii* var. *glauca*) has recently been recognized as a major winter feed for mule deer (*Odocoileus hemionus hemionus*) in the dry Interior of British Columbia¹ (Lowe, 1974). In addition, the increased emphasis on whole-tree utilization (Keays and Barton, 1975) points to the use of Douglasfir foliage from felled trees as an alternate animal feed. It may be possible to coordinate time and place of logging operations with maximum probability of deer use. However, in earlier studies of deer foraging habits, it was noted that palatability of trees and tree parts was variable.¹

Willms¹ also tentatively concluded that old trees were more palatable than young and that tops of trees were more palatable than bottoms. These results agree with Lowe's observations on deer winter foraging habits in the Kamloops area which led him to hypothesize that the degree of shading has an effect on the acceptability of fir needles, shaded parts being less acceptable (personal communication).

The purpose of this study was to examine palatability differences of Douglasfir, to search for possible correlated chemical constituents, and to suggest how this information

could be used in formulating cutting plans that would best contribute to the mule deer winter feed.

The ultimate factors of proximate analysis have been classically related to palatability (Heady, 1964) and for this study Kjeldahl crude protein was used as an index of ultimate food quality, the correlation between relative preference and crude protein giving the relationship between palatability and food quality. On the other hand, animals may not exhibit nutritional wisdom but select foods on a proximate factor basis, where the deciding components do not necessarily have nutritive value. Screening of Douglasfir phenolic constituents by thin layer chromatography (TLC) was undertaken in this investigation of select non-nutritive secondary metabolites that could be related to relative preference. Other workers (Hanks et al., 1971; Hanks et al., 1975) have demonstrated a strong association between phenols and relative palatability of big sagebrush (*Artemisia tridentata*) strains and rubber rabbitbrush (*Chrysothamnus nauseosus*) subspecies by deer and livestock. The most obviously related compound was isolated and measured initially and other components will be investigated subsequently.

Materials and Methods

Feeding Trials

Three factors hypothesized as affecting palatability: age, part of tree, and site, were arranged into a 2³ factorial experiment. The levels of each treatment were designated young (diameter breast height [dbh] < 5 cm) or old (dbh > 25 cm); top (top 1/4 of tree) or bottom (bottom 1/4 of tree); and open (slight slope with southern exposure and associated species ponderosa pine [*Pinus ponderosa*], rubber rabbitbrush, and bluebunch wheatgrass [*Agropyron spicatum*]) or gully (moist draw with associated species trembling aspen [*Populus tremuloides*], Columbia maple [*Acer columbiana*], and wild rose [*Rosa* spp.]).

Eight tame mule deer were fed 34 kg pelleted ration Monday, Wednesday, and Friday. The feeding trial was conducted before the first feeding of the week to ensure that the animals had a hearty appetite. Trees were felled and enough meter-long branches were clipped from each treatment to yield about 50 kg fresh weight. Approximately 25 kg of each treatment (20 to 30 branches) were tied in a bundle, weighed, and presented to the deer and left about 2 hours. The bundles were laid on the ground in a circle with about 3 m between each. This was repeated 7 times with new trees for each trial, on the following dates: Dec. 9, Dec. 16, Jan. 6, Jan. 13, Jan. 20, Jan. 27, and Feb. 17, 1974-75. From the remainder of the samples, 500 g fresh weight of 5-cm-long twig ends were clipped from each treatment, placed in plastic bags, and kept frozen for chemical analysis.

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Dry matter was determined from duplicate oven-dried (55°C, 48 hours) subsamples which were then ground for crude protein analysis, using the micro-Kjeldahl procedure (AOAC, 1975).

Extraction and TLC Screening

The procedure for exhaustive ethanolic extraction of fresh-frozen plant material has been described previously (Majak et al., 1974). The evaporated filtrate (obtained from 10 g fresh-frozen Douglasfir twigs) was taken up in warm 50% ethanol and transferred to a 25-ml volumetric flask for volume adjustment at room temperature. After cooling overnight at 4°C, the suspension was centrifuged at 27,000 × g for 5 minutes at 1°C and the supernatant stored in tightly capped 1-oz bottles at 4°C. The supernatant was the starting point for TLC screening and subsequent isolation and purification of phenolic constituents. TLC screening was performed as follows: Aliquots (20 µl) from each trial (8 treatments) were applied to a 1-mm Avicel microcrystalline cellulose plate (20 × 20 cm) and chromatographic development proceeded in butanol-ethanol-water (4:1:5, upper phase). Phenolic compounds were visualized with ultraviolet light and ammonia fumes and qualitatively assessed by the intensity of their fluorescence.

Fractionation, Fluorometric Scanning, and Identification of Chlorogenic Acid

Five milliliters of the above supernatant were applied to a polyamide-CC6 (Machery, Nagel and Co.) column (2.5 × 8.0 cm) equilibrated in distilled water containing 0.1% mercaptoethanol. The column was washed with the following sequence of solvents which were purged with nitrogen prior to use: 0.1% mercaptoethanol in water (100 ml); 50% ethanol (100 ml); 90% acetone (150 ml); 5% formic acid in acetone (50 ml). Chlorogenic acid was then eluted with an additional 250 ml of the final solvent. The eluate was evaporated to dryness in a rotary evaporator and redissolved and evaporated again to remove the last traces of formic acid. The dried eluate was dissolved in 2 ml ethanol and stored in securely capped vials at 4°C. Standard chlorogenic acid was recovered in 95% yield using the above procedure.

Duplicate 1 µl aliquots from the above 2 ml solution were applied to a precoated cellulose TLC plate (100 µ thickness, EM Laboratories Cat. No. 5757) and the plate was developed in butanol-acetic acid-water (4:1:2). Fluorometric scanning conditions for TLC were similar to those described by Ibrahim (1969) with the following modifications: The developed plate was irradiated with UV light (Black-Ray Model UVL-56 placed on top of the chlorogenic acid spots for 30 seconds) to stabilize peak area readings prior to scanning and a set screw was inserted in the upper right hand corner of the Camag scanner to level the TLC plate (MacNeil and Hikichi, 1974).

Chlorogenic acid was identified by its UV spectrum, fluorescence under UV light, color reaction with buffered diazotized para-

nitroaniline (Swain, 1953) and by co-chromatography with the authentic standard in five solvent systems.

Statistical Analysis

Relative preference was defined as the weight taken from one treatment divided by the weight taken from all eight treatments and was assumed to be directly related to palatability because there was equal choice of approximately equal volume of each treatment. A transformation $\ln(x+1)$ suggested by Elliot (1966) was used to normalize the relative preference data.

Crude protein was expressed as a percent of dry matter, a transformation of $\sqrt{x}$ (Elliot, 1966) being required to normalize data. Chlorogenic acid was expressed as percent of dry matter, a transformation of $\sqrt{x+0.01}$ being required for normalization (Elliot, 1966). Moisture was expressed as percent of fresh weight.

Analysis of variance of the 2³ (site × age × part) factorial experiment was conducted once for each of the above chemical components as dependent variable with site, age, and tree part as independent variables. Each feeding trial was considered a replication of the experiment, yielding 6 observations per cell or 48 observations total. Included in the analysis of variance for percent relative preference was an individual degree of freedom (Li, 1966) to test the hypothesis that shaded treatments had the same palatability as unshaded treatments.

Simple regression was conducted to examine relationships between relative preference, chlorogenic acid, percent crude protein, and percent moisture.

Results and Discussion

Spatial Determinants of Palatability

The treatment effects in the factorial experiment (Table 1) indicate that trees in the open are more palatable than trees in the gully, old trees are more palatable than young trees, and tops of trees are more palatable than bottoms of trees. An analysis of variance (Table 1) showed that these differences within factors are slight.

The high level of significance of the three-way interaction term indicates that the effects of factors are not independent and therefore not additive.

The comparison between shaded and unshaded needles showed that unshaded needles were more palatable than shaded, the individual degree of freedom test (Q^2 , Table 1) indicating a highly significant difference. This Q^2 is not orthogonal to and therefore not independent of the analysis of variance tests of the factorial experiment.

From the above results, it is obvious that Douglasfir needles

Table 1. Direction, magnitude, and level of significance of effects of site, age, and part of tree of Douglasfir foliage on relative preference and content of chlorogenic acid, moisture, and crude protein.

Source of variation	Relative preference			Chlorogenic acid			Moisture			Crude protein		
	Magnitude			Magnitude			Magnitude			Magnitude		
	Direction	(%) ³	<i>p</i>	Direction	(%) ⁴	<i>p</i>	Direction	(%) ³	<i>p</i>	Direction	(%) ⁴	<i>p</i>
Site (A)	O>G ¹	3.5 ⁵	<.005	O>G	.02	<.005	G>O	.53	NS	G>O	.01	NS
Age (B)	o>y	8.1	<.005	y>o	.0009	NS	y>o	1.78	<.005	o>y	.0005	NS
Part (C)	T>B	4.2	<.05	T>B	.01	<.05	T>B	.73	NS	T>B	.005	NS
A × B		NS			NS			NS			NS	
A × C		NS			NS			NS			NS	
B × C		NS			NS			NS			NS	
A × B × C		<.005			NS			NS			NS	
Mean		12.5			.12			53.11			6.1	
Shading (Q^2)	S<U ²	9.1	<.005									

¹(O) open, (G) gully, (o) old, (y) young, (T) top, (B) bottom, (S) shaded, (U) unshaded.

²(S) shaded treatments (GYB, GoB, GYT), (U) unshaded treatments (OoT), (OYT), (GoT); treatments not included (OoB, OYB).

³Percent of fresh weight.

⁴Percent of dry matter.

⁵Means and treatment effect were detransformed where applicable.

exhibit variable palatability which can be attributed to some degree to spatial factors. Part of the spatial effect may be attributed to degree of shading. One would assume that there are a number of factors involved in determining palatability and therefore a number of environmental variables affecting it.

The large *F* value for the three-way interaction term implies that each factor effect is modified by the condition of the other two. Age has the largest average treatment effect (8.1%), but its effect is reduced if the other two factors are set to restrict palatability. For example, in comparing treatment gully-young-bottom to gully-old-bottom, a change of 8.1% is expected but a change of only 5.0% - 4.5% = 0.5% was observed. On the other hand, the effect is enhanced when comparing gully-old-top and gully-young-top, where the observed change was 24.2%.

Examination of treatment effects on relative preference showed that direction is consistent for two factors, site and age. When comparing site or age differences, therefore, the direction of effect on palatability can be predicted but not the exact magnitude. The appearance of the interaction term complicates interpretation of the data. In general, age has a greater effect on palatability than site or part, but not in all cases.

Chemical Determinants of Palatability

Moisture

Moisture content of Douglasfir foliage varied between 49% and 57% with an average of 53%. Analysis of variance of the three factor feeding trial indicated that age alone had a significant effect, young trees having a higher moisture (54%) than old (52%). Moisture was found to be negatively associated with relative preference ($r = -0.57$) by simple linear regression (Table 2). The majority of studies suggest that moisture is generally positively associated with palatability of range and domestic forages to a variety of herbivores (Radwan and Crouch, 1974; Marten, 1970; Heady, 1964). This evidence is usually based on comparison between different forage species and cause is generally attributed to free water content affecting succulence. Radwan (1972), though, found a negative relationship between moisture and palatability between samples of different genotypes of Douglasfir, which agrees with present results. It is possible that this relationship is due to variable quantities of essential oils (Bonner and Varner, 1965; von Rudloff, 1971) that have been found to be negatively associated with palatability of Douglasfir to blacktailed deer (*Odocoileus hemionus columbianus*) (Radwan and Ellis, 1975; Radwan, 1972).

Crude Protein

Percent crude protein of Douglasfir foliage varied between 3.8% and 9.2%, with an average of 6.1%. Analysis of variance of the three factors feeding trial indicated that protein varied randomly and was not related to any of the factors. Protein was not significantly associated with relative preference ($r = -0.25$).

Table 2. Correlation matrix for 4 variables showing correlation coefficients, *r*.

	R.P.	CGA	Moisture	C.P.
Relative preference (P.R.)	1			
Chlorogenic acid (CGA)	.41 ¹	1		
Moisture	-.57 ¹	-.39 ¹	1	
Crude protein (C.P.)	-.25 ²	.04 ²	.18 ²	1

¹Denotes $p < .01$.

²Denotes $p > .05$.

Chlorogenic Acid

The TLC screening procedure enabled one to readily visualize relative concentrations of phenolic conjugates in Douglasfir samples of each preference trial. Accordingly, chlorogenic acid (3-O-caffeoylquinic acid) was detected as the most obvious component which exhibited significant variation in concentration and a positive association with Douglasfir preference. Similarly, Radwan (1972) reported that blacktailed deer resisted clones of coastal Douglasfir (*Pseudotsuga menziesii* var. *menziesii*) with lower levels of chlorogenic acid. The four clones which he examined yielded values of .39 to .0075% chlorogenic acid in fresh foliage and this corresponded to a decreasing order of preference.

Percent chlorogenic acid varied between 0 and 0.4% of dry matter with an average of 0.12%. Analysis of variance of the feeding trial (Table 1) indicated that site and part of tree had significant and independent effects on chlorogenic acid content, open sites having greater amounts (0.14%) than gully sites (0.10%), and tops of trees having greater amounts (0.13%) than bottoms (0.11%). Chlorogenic acid was found to be positively associated with relative preference ($r = 0.41$). The relationship of chlorogenic acid to palatability is still obscure, but one can speculate on activity of its hydrolysis products: caffeic acid, which possesses antioxidant properties (Daniels et al., 1963), and quinic acid, which has a strong acid taste (Stecher, 1968) and which in water solutions has been found to be preferred over tap water by deer (Rice et al., 1974). It is conceivable that a product of chlorogenic acid metabolism such as lignin (Taylor and Zucker, 1966; Taylor, 1968) may be more directly involved with palatability.

The complexity of palatability in general was demonstrated in the differences in relative preference found between samples of needles from the same tree. Part of the variation may be due to gross differences in needles associated with functional differences within the foliage crown, which may be caused or modified by environmental gradients along the height of the tree, such as shading, air temperature and moisture, wind speed, and distance to water tables. If this is true, then foliage should not be examined as varying between trees but as varying between levels of above-ground profiles. Between profiles palatability differences should be predictable if the right variables are monitored, which can only be discovered by further research. Chlorogenic acid seems to be one possibility; but ambiguous variables such as moisture content may be more useful if broken into components, which if taken individually may yield more information than the proximate variable.

It is evident that the palatability of Douglasfir needles is variable, the most palatable needles coming from trees exposed to sunlight or growing on dry exposed slopes. When efficient utilization of tree foliage for deer feed is considered in the selection of logging sites, palatability of tree parts must also be considered. If hauling of foliage to penned livestock is practiced, palatability will become even more important.

In the application of the above results to timber harvesting operations, the effect of elapsed time after harvest on the palatability of Douglasfir needles to deer remains to be determined. If this relationship can be established, it often may be possible to recommend modifications in timber harvesting to accommodate the deer in areas where integrated timber-deer management programs are desirable.

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