AN ABSTRACT FOR THE THESIS OF

Karen M. Lange for the degree of Master of Science in Forest Science presented on May 27, 1998. Title: Nutrient and Tannin Concentrations of Shrub Leaves in Managed and Unmanaged Forests of the Oregon Coast Range: Implications for Herbivores.

Abstract approved: Signature redacted for privacy.

Edward E. Starkey

Many herbivores of the Pacific Northwest rely on forest understory shrub leaves for a source of nitrogen, energy, and moisture. I measured nitrogen, protein-binding capacity, and condensed tannin concentration as indicators of available nitrogen; cell wall constituents and lignin as indicators of available energy; and moisture concentration in young and mature leaves of Oregon grape and salal, and in young leaves of sword fern, vine maple and ocean spray. In late summer I estimated lepidopteran larval herbivory of leaves from these species. This research was conducted during the summers of 1995 and 1996 in the Oregon Coast Range. My objectives were to determine whether insect herbivory and indicators of leaf nutritional quality differed 1) between clear-cut and forest stands (thinned and unthinned second-growth, and old-growth), 2) between thinned second-growth and unthinned second-growth forest, 3) between thinned second-growth and old-growth forest, and 4) between young and mature leaves of Oregon grape and salal. I also measured leaf area, specific leaf mass, and the proportion of available diffuse radiation reaching one meter above the forest floor. Most differences were found between clear-cuts and forest stands with leaves from clear-cuts tending to have less nitrogen concentration, greater protein-binding ability, greater condensed tannin concentration, lower proportions of cell wall constituents, decreased moisture concentration, smaller leaf area, and greater specific leaf mass than leaves from

forested stands. Comparisons between the forested stands exhibited few statistically significant differences. Leaf quality differences were likely due to differences in light exposure which was significantly greater in clear-cuts than in forest and differed little among forested stands. Herbivory by lepidopteran larvae tended to be lower in clear-cuts than in forested stands, but differences could not be related to leaf nutritional quality, because environmental variables associated with stand type also may have affected herbivory. Nutritional quality decreased with leaf age, leaves older than one year having more lignin, less nitrogen, greater protein-binding ability, more condensed tannins, and less moisture than young leaves. Herbivory of mature leaves decreased by one-half or more relative to young leaves, and is likely attributable to the low nutritional quality of mature leaves.

Nutrient and Tannin Concentrations of Shrub Leaves in Managed and Unmanaged Forests of the Oregon Coast Range: Implications for Herbivores

by

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INTRODUCTION

Forest understory shrubs of the Pacific Northwest support numerous vertebrate, invertebrate, and microbial herbivorous species. Herbivores are important components of natural ecosystems, fulfilling key roles in nutrient cycling and transport, food chain dynamics, and maintenance of plant species diversity (Huntley 1991). Whether herbivores consume fresh, senescing, or decaying leaf material, they are affected by fluctuations in leaf quality. Variation in digestible nitrogen, digestible energy, and water concentration of leaves, can have consequences for herbivore survival and reproduction (Robbins 1983).

Leaf nitrogen concentration is of major importance for herbivore development and reproduction (Robbins 1983; White 1984). Deer and elk feeding studies indicate that low nitrogen diets will not support lactation in females (Hanley et al. 1987). The amount of nitrogen available to herbivores generally decreases with leaf age (McNeil and Southwood 1978; Mattson 1980; and Scriber and Slansky 1981), and fluctuates with environmental conditions that alter the carbon to nitrogen (C/N) ratio in plants (Bryant et al. 1983; Larsson et al. 1986). When soil nitrogen is limiting, plant protein production decreases, increasing C/N. Elevated exposure to sunlight may increase C/N by causing an accumulation of carbon-based molecules in leaves.

Reduction in available nitrogen with age and light exposure is especially pronounced in slow-growing woody shrubs that produce carbon-based defense chemicals such as tannins (Perry 1994). Tannins bind with leaf protein (Feeny 1968; Feeny 1970) and digestive enzymes (Cooke et al. 1984), inhibiting protein digestion by herbivores. Some tannins may also have toxic effects (Mehansho et al. 1987a; Bryant et al. 1992; Clausen et al. 1992). Many herbivores therefore

grow and survive poorly on diets with high tannin concentration (Klein 1964, 1965; Verme and Ullrey 1972; Holter et al. 1979; Blair and Mitaru 1983; Wickstrom et al. 1984). Some host-specific herbivores, however, have evolved adaptations that reduce the binding of essential proteins by tannins. Simple proteins in the saliva of some mammals bind and inactivate tannins during mastication (Mehansho et al. 1983; Mehansho et al. 1987b; Robbins et al. 1987b; Austin et al. 1989; Robbins et al. 1991), and a high gut pH (Feeny 1970; Berenbaum 1980; Martin and Martin 1984) and surfactants (Feeny 1969; Martin and Martin 1984; Mole and Waterman 1985; Martin, et al. 1987) in the digestive tract of lepidopteran larvae may reduce or reverse binding activity.

Herbivores are less likely to be limited by energy content of their food than by nutrients (Perry 1994). Leaves have a fairly consistent gross energy content across species, averaging 4.2 kcal/g (Golley 1961). The amount of energy actually available to herbivores depends on the proportion of digestible cell materials: cytoplasmic protein, nonstructural carbohydrates and fats (Robbins 1983). Indigestible cell materials are largely tannin bound proteins and structural cell wall constituents (CWC) including cellulose, hemicellulose, lignin, suberin, and cutin (Robbins 1983). The relative quantities of digestible and indigestible cell materials can fluctuate with season (Robbins 1983), leaf age (Scriber and Slansky 1981), and environmental variables such as availability of soil nutrients, water, and sunlight (Bryant et al. 1992).

Some herbivores depend entirely on leaf moisture for their water requirements (Kimmins 1987). This may be true for lepidopteran larvae that are limited in mobility. The poor growth and survival of larvae feeding on mature leaves may be partially due to the low moisture concentration of mature leaves relative to young leaves (Hough and Pimentel 1978; Schroeder 1986; Ayres and MacLean 1987; Raupp et al. 1988). Exposure to full sun may reduce leaf moisture in young leaves as well, and affect feeding larvae.

Forest management practices affect understory leaf quality by altering light intensity, soil moisture, and soil nutrient availability (Kimmins 1987). For example, open clear-cuts are exposed to full light, whereas even-aged second-growth can be extremely light-limited resulting in leaf chemistry differences between stand types (Rose 1990). The relationship between leaf quality, light exposure, and soil nitrogen in managed and unmanaged stands has been investigated in Alaska for huckleberry (*Vaccinium alaskaense* Howell), bunchberry dogwood (*Cornus canadensis* L.), trailing bramble (*Rubus pedatus* J.E. Smith), skunk cabbage (*Lysichitum americanum* Hulten & St. John), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.)(Hanley et al. 1987; Van Horne et al. 1987; Rose 1990). Swordfern (*Polystichum munitum* (Kaulf.) Presl), vine maple (*Acer circinatum* Pursh), salmonberry (*Rubus spectabilis* Pursh), and huckleberry (*Vaccinium parvifolium* Smith) have been investigated in Washington (Happe et al. 1990). Most leaves grown in clear-cuts had less available nitrogen and moisture, but greater available energy than leaves grown in mature forest. Soil nitrogen fluctuations had little or no detectable effect on leaf chemistry.

The present study extends this research into the Coast Range of Oregon, measuring leaf quality for five common shrub species: vine maple, Oregon grape (*Berberis nervosa* Pursh), salal (*Gaultheria shallon* Pursh), ocean spray (*Holodiscus discolor* Maxim.), and sword fern. My objective was to determine whether insect herbivory and indicators of leaf nutritional quality (nitrogen, condensed tannins, and protein-binding capacity of tannins, cell wall constituents, lignin and moisture), differed between stand types: clear-cut, thinned second-growth forest, unthinned second-growth forest, and old-growth forest. Clear-cuts were compared with forest stands as a group to assess leaf quality and herbivory differences between two environments with very different light regimes. Thinned second-growth forest was compared with unthinned second-growth forest to determine if any differences were related to thinning.

Thinned second-growth forest was compared with old-growth forest to identify where similarities and differences exist between managed and natural forest. An additional objective was to determine how insect herbivory and indicators of leaf nutritional quality differed between young and mature leaves for two evergreen shrubs, Oregon grape and salal.

METHODS

Experimental Design

The study was structured in a randomized complete block design, with four treatments (stand types) and four replications (study sites)(Appendix A). The stand types were thinned and unthinned second-growth forest, old-growth forest, and clear-cut. Four 50 x 50 m plots were randomly located within each of the stand types at each study site. Within a 50 x 50 m plot, ten sampling points were located using a stratified-random sampling technique. Leaves and soil and diffuse radiation measurements were collected at each sampling point. The ten soil samples were placed into one bag per plot, and the leaves from one plot were placed into one bag for each species and age category.

Study Sites

The four study sites were located in the Oregon Coast Range on land managed by United States Department of the Interior (USDI) Bureau of Land Management (BLM)(Figure 1). The region is dominated by a wet, mild, maritime climate. Annual precipitation averages 1,500 to 3,000 mm, with only six to nine percent occurring in the summer. Mean annual temperature is 8° to 9° C, without extreme seasonal variation (Franklin and Dyrness 1973).

At each site thinned and unthinned second-growth stands had been part of the same unit prior to silvicultural treatment. Unthinned stands received no treatment. Old-growth stands contained trees up to approximately 200 years old or older. Management descriptions prior to 1950 are BLM estimates based on tree age and historical knowledge of the area (Table 1).

Figure 1. Site Locations (△) in the Oregon Coast Range, Western Oregon.

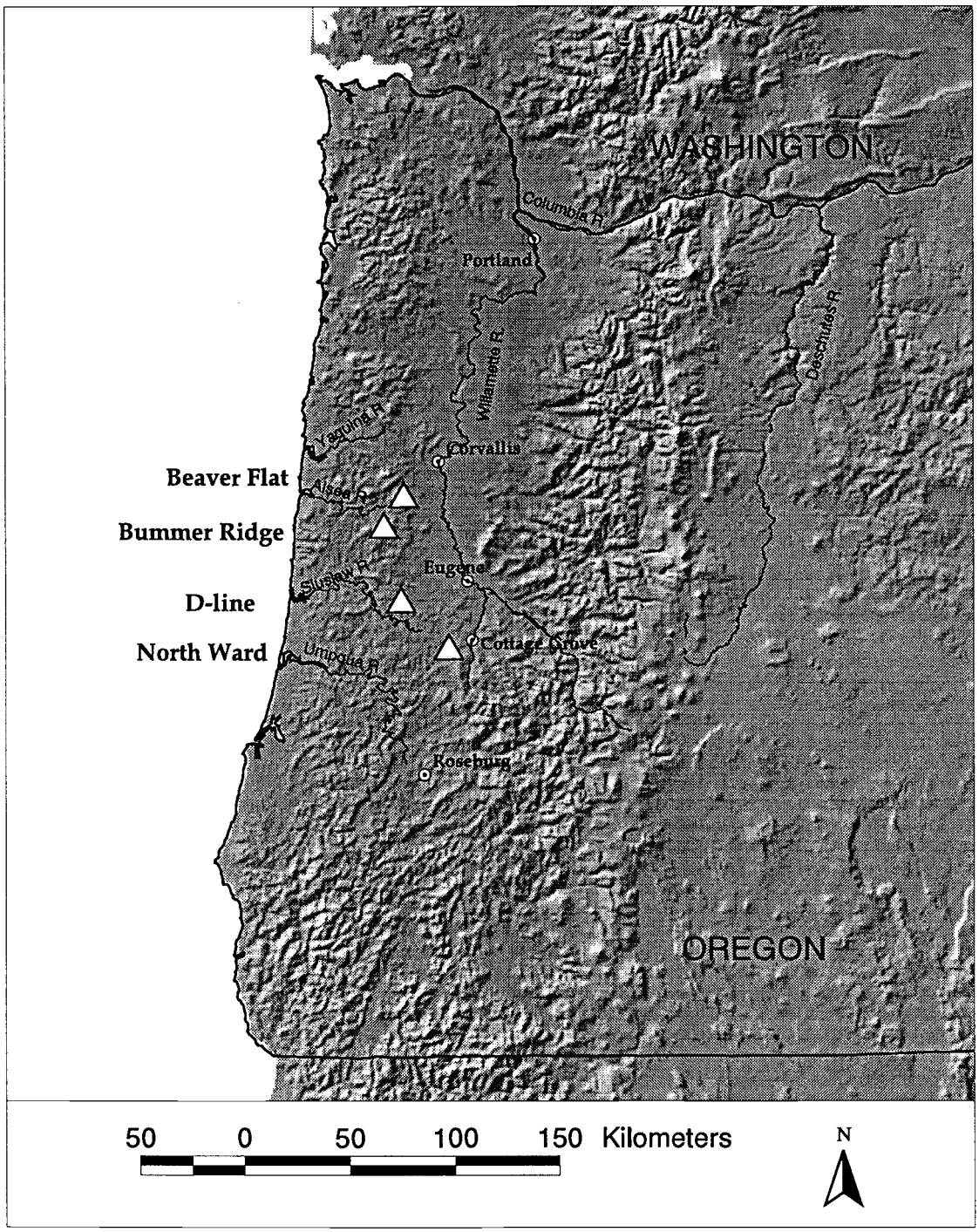

Table 1. Site T-R-S coordinates, stand age, year of thinning treatment, basal area in square feet per acre (BA), and tree density per acre (TA).

Site Location	Stand type[a]	Stand Age (years)	Thinned (year)	BA[b]	TA[b]
Beaver Flat	CC	5		-	-
T13S R6W Sec.19	ST	55	1974	140	61
	SU	55		222	131
	OG	400+		268	19
D-line	CC	6		-	-
T18S R7W Sec.25	ST	66	1972	180	74
	SU	66		210	157
	OG	200+		236	31
North Ward	CC	6		-	-
T21S R4W Sec.5	ST	55	1985	119	62
	SU	55		146	96
	OG	200+		236	22
Bummer Ridge	CC	6		-	-
T15S R7W Sec.6	ST	105	1975	185	45
	SU	105		-	-
	OG	196		-	-

[a]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth

[b]Measurements not available are indicated with dashes.

Beaver Flat. The Beaver Flat site is approximately 8.5 miles southwest of Philomath. Second-growth stands had been logged and allowed to naturally regenerate from about 1940. In 1974, 51% of tree volume was removed from a portion of the second-growth, creating the thinned stand. The clear-cut area was harvested and broadcast burned in 1988, and planted in 1990. The old-growth stand is located seven miles further west in the Mary's Peak watershed. Mean stand slope ranged from 24° to 43°. The clear-cut and second-growth stands had a northeasterly aspect, and the old-growth stand had a northwesterly aspect.

North Ward. The North Ward site lies further south, approximately 6.5 miles to the west of Cottage Grove. Second-growth stands had been logged and naturally regenerated from about 1940. In 1985, 50% of tree volume was removed from a portion of the second-growth, creating the thinned stand. The clear-cut was harvested in 1987, machine piled and burned, and then planted in 1989. The old-growth stand was located five miles northwest of the second-growth and clear-cut stands. Mean stand slope ranged from 22° to 39°. The clear-cut and second-growth stands had a northwesterly aspect, and the old-growth stand had a northeasterly aspect.

D-line. The D-line site is located approximately 15 miles west of Eugene. Both second-growth stands naturally regenerated after a medium to high volume removal in about 1930. In 1972, a light thinning of 12% was conducted on a portion of the second-growth stand, creating the thinned stand. The clear-cut was harvested in 1988, slash-piled and burned in 1989, and planted in 1990. All stands were on adjacent slopes ranging from 23° to 61°. The second-growth stands had a south to southwesterly aspect, and the clear-cut and old-growth stands had a northerly aspect.

Bummer Ridge. The Bummer Ridge site is located approximately six miles south of Alsea. The second-growth stands naturally regenerated after fire in about 1890. Approximately 50% of the tree volume was removed in 1975 from a portion of the second-growth, creating the thinned stand. The clear-cut was harvested and broadcast burned in 1986, and planted in 1990. The old-growth stand, was located one mile to the east. Slopes ranged from 26° to 42°, and stand aspects ranged from northeasterly to northwesterly.

Leaf Sampling and Chemical Analysis

Five shrub species were chosen for this study based on their abundance in Oregon Coast Range forest and their observed importance to herbivores: Oregon grape, salal, sword fern, vine maple, and ocean spray. During July and early August of 1995 (Beaver Flat and North Ward) and 1996 (D-line and Bummer Ridge), young leaves from the current season were collected for each species and placed into plastic bags. At Beaver Flat and North Ward, mature leaves (1-4 years old) of Oregon grape and salal were also collected and kept separate from young leaves. Leaves were taken from all sides and heights on randomly located plants to objectively characterize the leaf chemistry that occurs within a stand. The samples were either immediately transported to the laboratory where they were stored in a freezer at -10° C, or occasionally kept overnight on dry ice in a cooler (North Ward only) prior to placement in the laboratory freezer.

The leaves in each bag were mixed, and half were removed for freeze-drying, as required for tannin and protein-binding analyses. The other half were weighed, dried in a drying oven at 70°C to a constant weight , and weighed again. Leaf moisture was calculated from the weight change and is expressed as a percentage of dry leaf weight. Ten leaves of each species per plot were

randomly selected for measurement of leaf area (cm^2) with a LI-COR 3100 Area Meter. Specific leaf mass (g/cm^2) was calculated for vine maple, ocean spray and sword fern, from the average weight and area of ten randomly selected leaves (D-line and Bummer Ridge sites only). Both oven-dried and freeze-dried leaves were ground in a Wiley Mill to 1 mm mesh size.

Five grams per species per plot of oven-dried, ground leaves were analyzed by the Forage Analytical Service at Oregon State University for Kjeldahl nitrogen, neutral detergent fiber, and acid detergent lignin using methods described by Harris (1970). The Kjeldahl procedure measures the total amount of nitrogen per gram of leaf. Multiplying total nitrogen by 6.25 gives a crude estimate of leaf protein concentration, often referred to as crude protein (Robbins 1983). The neutral detergent fiber analysis (Goering and Van Soest 1970) determines the percentage of cell wall constituents (CWC) which consists of hemicellulose, cellulose, lignin, suberin and cutin. Acid detergent lignin (Goering and Van Soest 1970) is a measure of the acid indigestible residue (AIR lignin) in leaf tissue, which includes structurally bound tannins and cutin in addition to lignin (Preston et al. 1997). Kjeldahl nitrogen, fiber, and AIR lignin quantities were expressed as a percentage of dry leaf weight.

For measuring condensed tannin concentration and protein-binding capacity, tannins were extracted from freeze-dried and ground leaves with 70% aqueous acetone (Scalbert 1991). One gram of ground leaves was stirred with 10 ml of 70% acetone for 15 minutes, vacuum filtered, and then rinsed with another 20 ml of 70% aqueous acetone. This process was repeated three times, saving consecutive filtrates into one flask. Celite 545 was used as a filtration aid to keep the leaf material from clogging crucible pores. Acetone was evaporated from the filtrate with a rotary evaporator using a 30° C water bath. The resulting aqueous solution was rinsed twice with an equal portion of ethyl ether to remove low molecular weight phenolics. A rotary evaporator was used to remove ethyl ether remaining in the extract solution after separation. Distilled water was

added to the extract in a volumetric flask to raise the volume to 25 ml. Extracts were stored in sealed polypropylene tubes in the freezer at -10° C.

The capacity of tannins to bind with protein was determined using the radial diffusion assay (Hagerman 1987)(Appendix B), and the blue-dye labeled BSA method (Asquith and Butler 1985)(Appendix C). Both methods measure binding activity between tannins and the standard laboratory protein, bovine serum albumin (BSA). In the radial diffusion assay, protein-binding was visible as an opaque ring radiating from 4 mm injection wells in agar/BSA petri plates. After four days incubation, the ring diameter was proportional to the binding capacity of a fixed quantity of leaf extract. Comparisons between extract ring diameter and ring diameter produced by known quantities of tannic acid allowed protein-binding to be expressed as milligrams of tannic acid equivalents (TAE) per gram dry leaf tissue. The blue-dye labeled BSA method was used in addition to the radial diffusion method for vine maple leaf extracts. Remazol brilliant blue R, mixed in a buffer solution with BSA, covalently bonds with the protein (Waterman and Mole 1994). When combined with leaf extracts containing tannin, the blue-dye bound BSA formed a tannin-protein precipitate. The precipitate was resuspended in a detergent solution, and the absorbance was read at 590 nm with a spectrophotometer. The amount of tannin-bound BSA was calculated by multiplying the mg of BSA in a 100% solution by the ratio of sample absorbance to the absorbance of a 100% BSA solution. Results are expressed as milligrams BSA precipitated per milligram dry leaf tissue. It was essential that the optimum concentration of leaf extract be used for this method; protein precipitation capacity decreased when tannin concentration was only slightly above or below the optimum ratio with BSA.

Condensed tannin concentration was measured using the vanillin assay as outlined by Broadhurst and Jones (1978), with modifications from Swain and Hillis (1959) (Appendix D). The aromatic aldehyde on vanillin reacts with condensed tannin under acidic conditions and creates a red adduct that can be

measured as absorbance at 500 nm wavelength with a spectrophotometer. Using a standard curve of absorbance versus known quantities of the commercial condensed tannin, catechin, leaf extract results could be expressed as catechin equivalents, or milligrams catechin per gram dry leaf tissue.

Estimation of Insect Herbivory

At the end of the growing season I assessed insect herbivory of shrubs at each plot by estimating the percent of total leaf area consumed from leaves on randomly selected branches. I focused on consumption by lepidopteran larvae, disregarding occasional leaf miner and deer herbivory. Accuracy was determined to be within 10% by comparing assimilated herbivory estimates to measurements made with the LI-COR leaf area meter. Because polyphenols have been shown to increase in leaves as a result of damage (Baldwin and Schultz 1983; Hartley 1988), care was taken to gather estimates from shrubs not sampled during leaf collection. Estimates were averaged over each plot and expressed as a percentage of total leaf area. Young leaf values assess the total herbivory that occurred in the first growing season, whereas mature leaf values assess the total herbivory that occurred during one to four growing seasons.

Measurement of Diffuse Radiation

Diffuse radiation was measured using a pair of calibrated LI-COR LAI-2000 light meters. One light meter was used to take measurements at sampling points beneath the canopy, 1 m above ground level. The second light meter was placed in a nearby clearing so that understory readings could be adjusted for fluctuating light conditions. The data were downloaded with ASCII-based

communication software supplied by LI-COR, Lincoln, NE, USA. The understory and clearing files were merged and values of diffuse radiation were calculated with LAI-2000 software (LI-COR, Lincoln, NE, USA). Diffuse radiation was expressed as a value between zero and one, indicating the proportion of available light that may penetrate the canopy to the location of measurement (Welles and Norman 1991).

Soil Sampling and Nitrogen Analysis

At each sampling point soil was extracted with a bulb planter to a depth of 4 inches and placed into one bag per plot. The soil was transported back to the laboratory, where it was air-dried, mixed, partitioned, and sifted to remove rocks and wood debris. One gram of soil per plot was sent to the Department of Forest Science Analytical Service Laboratory for analysis of total nitrogen. The soil was ground to 60 mesh and total nitrogen was obtained by high temperature combustion (Nelson and Sommers 1972) and gas chromatography using a Carlo-Erba CNS analyzer (model 1500). Total soil nitrogen is expressed as a percent of soil dry weight.

Statistical Analyses

Statistical analyses were performed using SAS (release 6.12, 1989). Response variables analyzed were leaf nitrogen, condensed tannin concentration, protein-binding capacity, cell wall constituents, lignin, leaf moisture, and insect herbivory of leaves for five shrub species, as well as soil nitrogen and diffuse radiation. The data were analyzed using a randomized complete block statistical model in PROC MIXED to test for differences between

stand types. Stand level responses were calculated from averaged plot values. Comparisons between stands were limited to three orthogonal linear contrasts, the number allowed by the experimental design, to minimize the possibility of making a type I error (detecting a difference where there is none). I tested for differences between the following stand type means: thinned second-growth vs. old-growth; thinned second-growth vs. unthinned second-growth; and clear-cut vs. forest stands. The forest stands consisted of thinned second-growth, unthinned second-growth, and old-growth. Mature and young leaves from salal and Oregon grape were treated as a split-plot factor and analyzed using PROC MIXED and a split-plot statistical model. The significance level was set at $P < 0.10$ for all statistical tests.

RESULTS

Leaf Nitrogen

Nitrogen concentration was lower in leaves from clear-cuts than in leaves from forest stands for vine maple ($P = 0.061$), and ocean spray ($P = 0.042$; Table 2; Figure 2a). Other species showed no significant difference between clear-cuts and forest stands ($P \geq 0.157$). Nitrogen concentration of leaves did not differ between forest stands ($P \geq 0.256$). Vine maple and ocean spray leaves tended to have greater nitrogen concentration than Oregon grape, salal, or sword fern. Mature Oregon grape and salal leaves had significantly less nitrogen than young leaves ($P = 0.048$, and 0.008, respectively; Table 3).

Protein-binding Capacity

Extracts of leaves from clear-cuts tended to have greater protein-binding capacity than those from forest stands for sword fern ($P = 0.017$), ocean spray ($P = 0.015$), and salal ($P = 0.083$), as measured with the radial diffusion assay (Table 2; Figure 2b). Vine maple did not significantly differ between stands when measured with the radial diffusion assay ($P \geq 0.124$), but when analyzed with the blue-dye BSA method protein-binding capacity was greater in clear-cuts than in forest stands ($P = 0.021$). Most species showed no differences between forest stand types ($P \geq 0.228$), but ocean spray leaf extracts from thinned second-growth precipitated more protein than those from unthinned second-growth ($P = 0.024$). Overall, vine maple had much greater protein-binding capacity than the other species. Mature salal leaves had significantly greater protein-binding capacity (27.98) than young salal leaves (7.98; $P = 0.010$).

TABLE 2. Nitrogen concentration (percent dry weight), protein-binding capacity[a], and condensed tannin concentration (mg catechin equivalent/g dry weight) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[b]	Stand Type[c]	% Nitrogen Mean	% Nitrogen SE	Radial diffusion Mean	Radial diffusion SE	Blue-dye Mean	Blue-dye SE	C. tannin Mean	C. tannin SE	Stand Type[b] Comparison	% Nitrogen p-value	Rad. diff. p-value	Blue-dye p-value	C.tannin p-value
BENE	CC	1.05	0.15	-	-	-	-	-	-	CC vs. FO	0.157	-	-	-
	ST	1.35								ST vs. SU	0.430			
	SU	1.20								OG vs. ST	0.256			
	OG	1.38												
GASH	CC	1.16	0.15	14.16	2.51	-	-	47.12	6.78	CC vs. FO	0.756	**0.083**	-	0.114
	ST	1.28		8.54				38.50		ST vs. SU	0.287	0.954		0.405
	SU	1.12		8.36				31.03		OG vs. ST	0.534	0.924		0.348
	OG	1.19		8.25				32.66						
POMU	CC	1.35	0.07	18.51	3.11	-	-	42.50	2.65	CC vs. FO	0.305	**0.017**	-	**0.008**
	ST	1.51		8.16				30.43		ST vs. SU	0.315	0.538		0.632
	SU	1.40		6.14				29.14		OG vs. ST	0.309	0.621		0.825
	OG	1.40		6.56				31.01						
ACCI	CC	1.64	0.1	134.63	12.7	0.59	0.04	3.37	0.3	CC vs. FO	**0.061**	0.124	**0.021**	**0.007**
	ST	1.92		108.75		0.39		1.11		ST vs. SU	0.921	0.944	0.696	0.692
	SU	2.12		107.50		0.37		1.29		OG vs. ST	0.256	0.707	0.521	0.954
	OG	1.93		101.94		0.43		1.09						
HODI	CC	1.44	0.09	42.00	6.26	-	-	16.08	2.3	CC vs. FO	**0.042**	**0.015**	-	**0.026**
	ST	1.92		25.72				6.26		ST vs. SU	0.279	**0.024**		0.509
	SU	1.97		-4.77				3.83		OG vs. ST	0.645	0.228		0.860
	OG	1.75		14.80				5.63						

[a]Expressed as mg tannic acid equivalent/g dry leaf tissue for radial diffusion technique and mg BSA precipitated/mg dry leaf tissue for Blue-dye BSA technique.
[b]BENE = Oregon grape, GASH = salal, POMU = sword fern, ACCI = vine maple, HODI = ocean spray
[c]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth, FO = average of ST, SU, and OG means

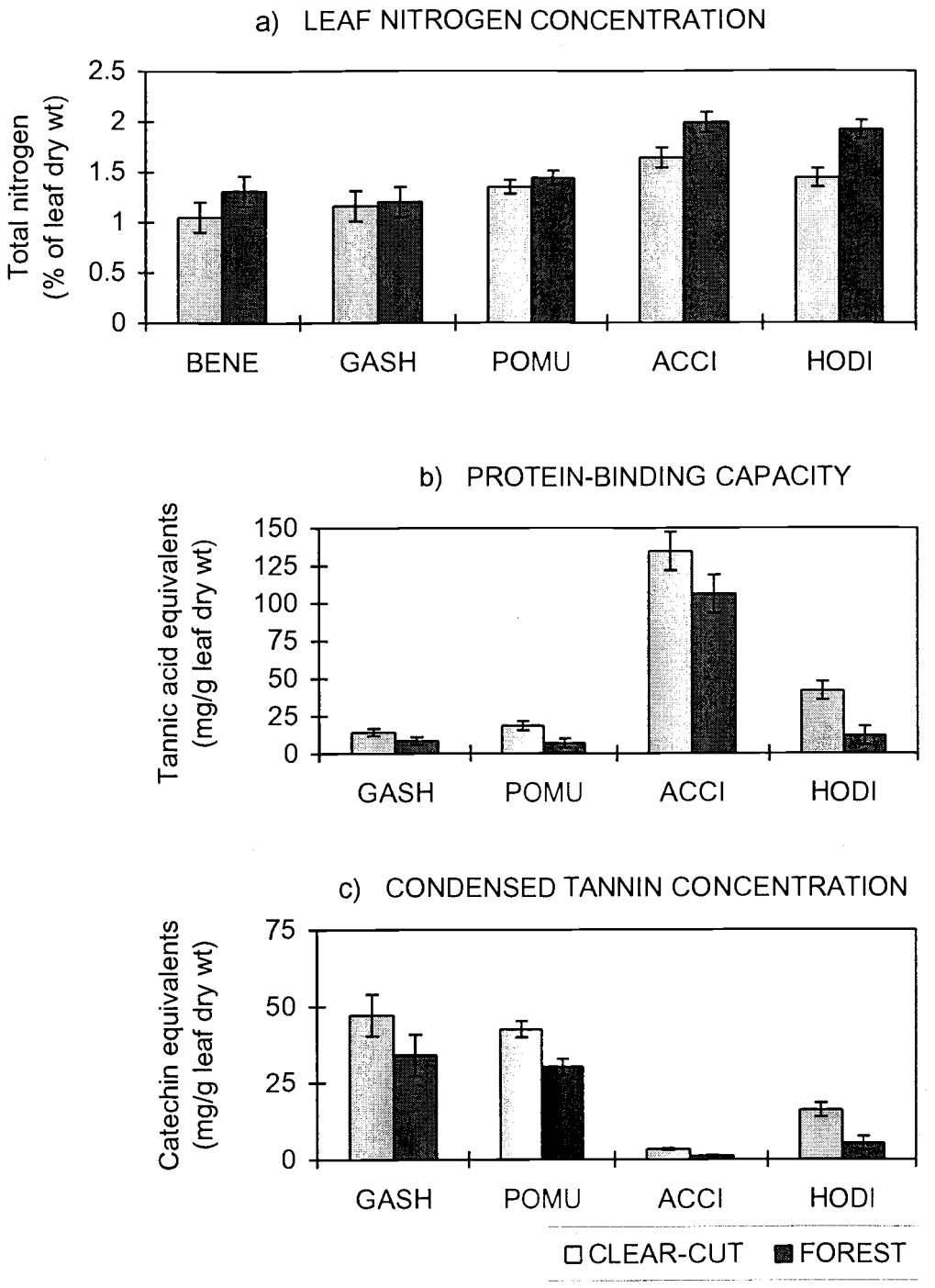

Figure 2. Nitrogen concentration (a), protein-binding capacity (b), and condensed tannin concentration (c) in clear-cuts and forest for young (Y) and mature (M) leaves of Oregon grape (BENE), and salal (GASH), and for young leaves of sword fern (POMU), vine maple (ACCI), and ocean spray (HODI). Error bars are standard errors.

TABLE 3. Comparisons between young and mature leaves for nitrogen concentration (percent dry weight), protein binding capacity (mg tannic acid equivalent/g dry weight), condensed tannin concentration (mg catechin equivalent/ g dry weight), CWC (percent dry weight), lignin (percent dry weight), moisture (percent dry weight), and herbivory (percent leaf area consumed) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[a]		Leaf Age[b]	Nitrogen %	Protein binding	Cond. tannin	CWC %	Lignin %	Moisture %	Herbivory
BENE	Mean	Y	1.11	-	-	72.03	14.66	120	6.09
		M	0.96	-	-	70.50	14.77	75	9.48
	SE		0.05	-	-	1.25	0.94	13	0.53
	p		**0.048**			0.437	0.914	0.055	**0.011**
GASH	Mean	Y	1.05	7.98	34.80	43.10	14.04	229	2.87
		M	0.79	27.98	63.03	43.72	16.74	108	3.24
	SE		0.09	3.04	6.58	1.84	0.47	39	0.76
	p		**0.008**	**0.010**	**0.039**	0.823	**0.001**	0.058	0.520

[a]BENE = Oregon grape, GASH = salal
[b]Y = young, M = mature

Condensed Tannins

Concentrations of condensed tannins in sword fern, vine maple, and ocean spray were greater in clear-cuts than in forest stands ($P = 0.008$, 0.007, and 0.026, respectively; Table 2; Figure 2c). Forest stand comparisons were not significant ($P \geq 0.114$). Salal and sword fern leaves had the highest condensed tannin concentration, while ocean spray and vine maple had relatively low condensed tannin concentration. Mature salal leaves had significantly more condensed tannin than young salal leaves ($P = 0.039$). Oregon grape was not analyzed for condensed tannin concentration since preliminary analysis showed values to be negligible.

Cell Wall Constituents

Leaves of salal, sword fern, and vine maple had lower mean % CWC in clear-cuts than in forest stands ($P = 0.089$, 0.081, and 0.021, respectively; Table 4; Figure 3a). There were no differences between forest stands ($P \geq 0.103$). Oregon grape and sword fern had greater CWC than salal, vine maple, and ocean spray. Differences in CWC between mature and young leaves were insignificant ($P \geq 0.437$).

Lignin

There were no significant differences in leaf lignin concentration between stand types and an overall pattern of means was not apparent ($P \geq 0.114$; Table 4; Figure 3b). Sword fern had the highest mean lignin concentration, followed in decreasing order by Oregon grape, salal, vine maple and ocean spray. Age did

TABLE 4. Cell wall constituents (CWC)(percent dry weight), and lignin (percent dry weight) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[a]	Stand Type[b]	% CWC Mean	% CWC SE	% Lignin Mean	% Lignin SE	Stand Type[b] Comparison	% CWC p-value	% Lignin p-value
BENE	CC	71.82	4.36	15.25	1.45	CC vs. FO	0.445	0.494
	ST	70.65		14.04		ST vs. SU	0.103	0.390
	SU	80.51		14.61		OG vs. ST	0.556	0.499
	OG	73.46		16.38				
GASH	CC	40.55	3.07	12.45	0.88	CC vs. FO	**0.089**	0.305
	ST	44.31		12.59		ST vs. SU	0.284	0.114
	SU	47.64		14.65		OG vs. ST	0.743	0.730
	OG	45.23		12.94				
POMU	CC	67.82	2.6	23.74	1.49	CC vs. FO	**0.081**	0.420
	ST	73.84		21.49		ST vs. SU	0.820	0.919
	SU	74.56		21.72		OG vs. ST	0.882	0.401
	OG	73.38		23.44				
ACCI	CC	28.14	1.64	5.82	1.16	CC vs. FO	**0.021**	0.143
	ST	32.21		7.02		ST vs. SU	0.254	0.548
	SU	33.99		7.83		OG vs. ST	0.886	0.325
	OG	32.01		8.43				
HODI	CC	35.56	1.92	6.17	0.74	CC vs. FO	0.567	0.658
	ST	35.91		6.66		ST vs. SU	0.518	0.280
	SU	37.65		5.34		OG vs. ST	0.720	0.271
	OG	36.85		5.30				

[a]BENE = Oregon grape, GASH = salal, POMU = sword fern, ACCI = vine maple, HODI = ocean spray
[b]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth, FO = average of ST, SU, and OG means

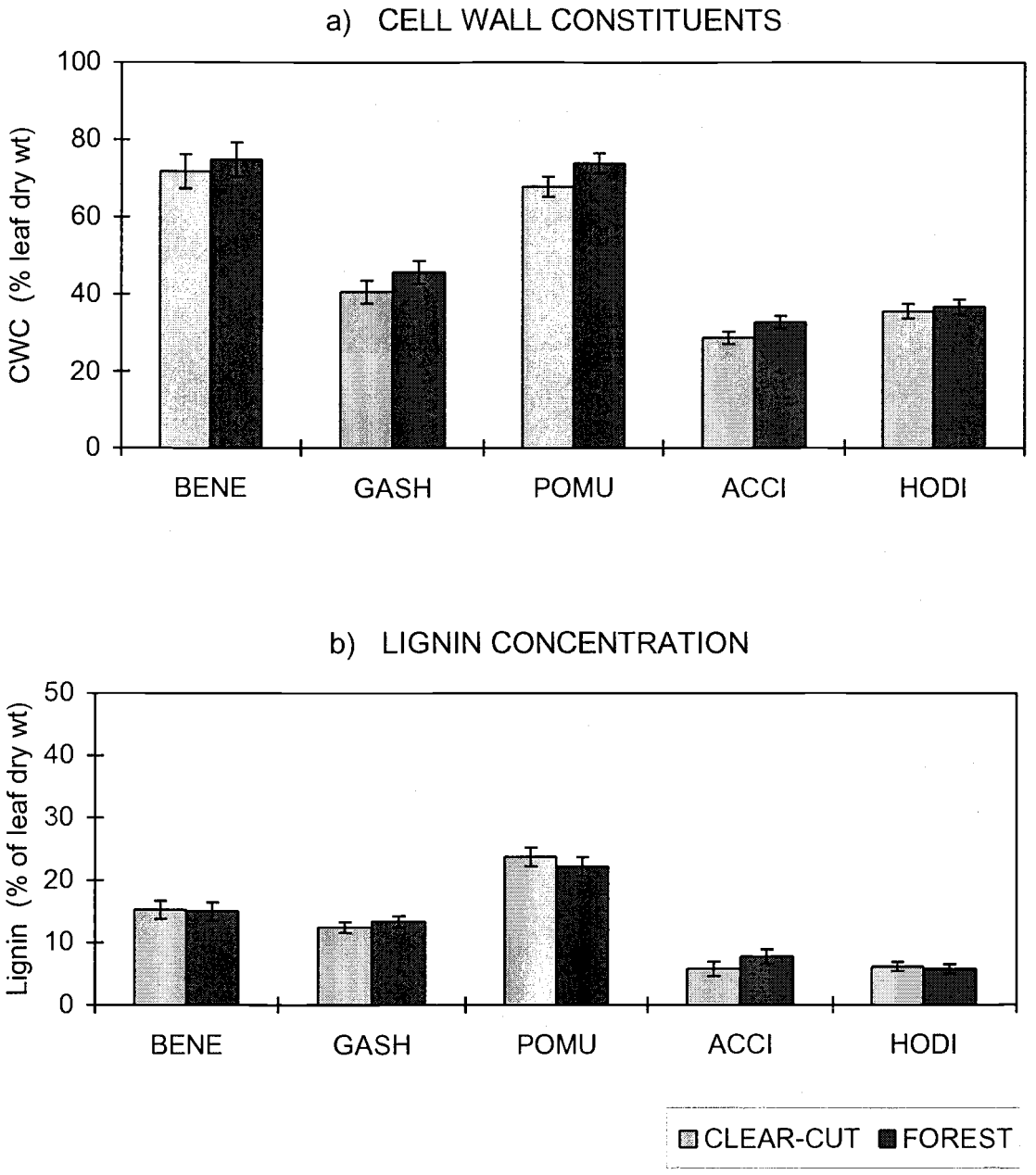

Figure 3. Percent cell wall constituents (a), and percent lignin (b) in clear-cuts and forest for young (Y) and mature (M) leaves of Oregon grape (BENE), and salal (GASH), and in young leaves of sword fern (POMU), vine maple (ACCI), and ocean spray (HODI). Error bars are standard errors.

not have a significant effect on lignin concentration in Oregon grape leaves ($P = 0.914$); however, mature leaves of salal had higher lignin concentrations than young leaves ($P = 0.001$).

Leaf Moisture

Leaves collected from clear-cuts had lower mean moisture concentration than leaves collected from forest stands for vine maple and ocean spray ($P = 0.023$, and 0.072, respectively; Table 5; Figure 4). Oregon grape young leaves had a higher moisture concentration (120%) than mature leaves (75%) ($P = 0.055$), and salal young leaves had a higher moisture concentration (229%) than mature leaves (108%) ($P = 0.058$).

Herbivory

Leaves in clear-cuts received less herbivory than leaves in forest stands for Oregon grape, and sword fern ($P = 0.087$, 0.018, and 0.023; Table 6; Figure 5). Though herbivory of salal, vine maple, and ocean spray did not significantly differ between clear-cuts and forest ($P \geq 0.121$), mean values were lowest in clear-cuts. Herbivory of vine maple was greater in unthinned second-growth than in thinned second-growth ($P = 0.099$). Differences in herbivory between forest stands were not detectable for other species ($P \geq 0.167$). Herbivory was greatest in vine maple, Oregon grape, and ocean spray, followed by salal and sword fern. There was no difference in total herbivory between mature and young salal leaves ($P = 0.52$), despite the greater exposure time for mature leaves (1~4 years) relative to young leaves (< 1 year). Mature leaves of Oregon grape had half again greater total herbivory than young leaves ($P = 0.011$).

Table 5. Moisture concentration (percent dry weight) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[a]	Stand Type[b]	% Moisture Mean	SE	Stand Type[b] Comparison	P-value
BENE	CC	135	43	CC vs. FO	0.290
	ST	223		ST vs. SU	0.118
	SU	133		OG vs. ST	0.361
	OG	179			
GASH	CC	215	60	CC vs. FO	0.235
	ST	336		ST vs. SU	0.271
	SU	251		OG vs. ST	0.480
	OG	285			
POMU	CC	148	34	CC vs. FO	0.716
	ST	165		ST vs. SU	0.596
	SU	152		OG vs. ST	0.543
	OG	150			
ACCI	CC	113	29	CC vs. FO	**0.023**
	ST	190		ST vs. SU	0.518
	SU	205		OG vs. ST	0.264
	OG	162			
HODI	CC	109	25	CC vs. FO	**0.072**
	ST	161		ST vs. SU	0.866
	SU	165		OG vs. ST	0.165
	OG	127			

[a]BENE = Oregon grape, GASH = salal, POMU = sword fern, ACCI = vine maple, HODI = ocean spray

[b]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth, FO = average of ST, SU, and OG means

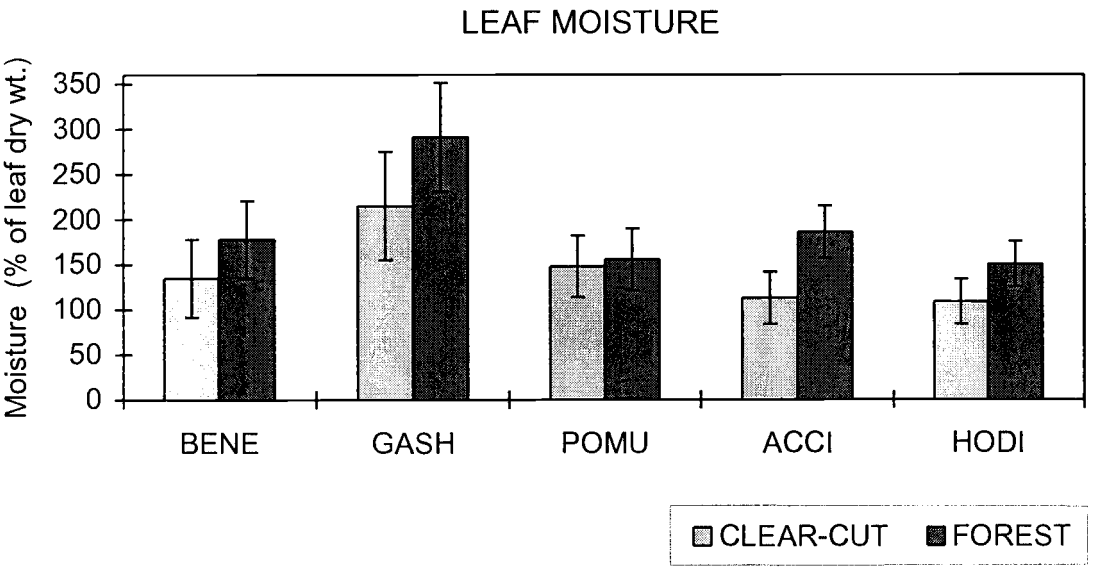

Figure 4. Moisture concentrations in clear-cuts and forest for young (Y) and mature (M) leaves of Oregon grape (BENE) and salal (GASH), and for young leaves of sword fern (POMU), vine maple (ACCI), and ocean spray (HODI). Error bars are standard errors.

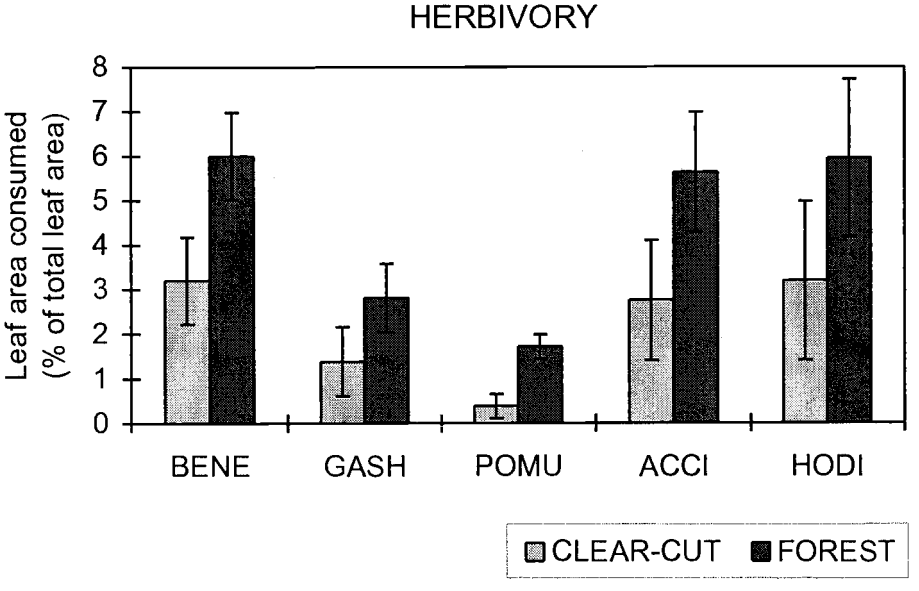

Figure 5. Herbivory in clear-cuts and forest for young (Y) and mature (M) leaves of Oregon grape (BENE) and salal (GASH), and for young leaves of sword fern (POMU), vine maple (ACCI), and ocean spray (HODI). Error bars are standard errors.

Table 6. Herbivory (percent leaf area consumed) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[a]	Stand Type[b]	% Herbivory Mean	SE	Stand Type[b] Comparison	P-value
BENE	CC	3.20	0.98	CC vs. FO	0.087
	ST	5.08		ST vs. SU	0.855
	SU	5.35		OG vs. ST	0.167
	OG	7.56			
GASH	CC	1.38	0.77	CC vs. FO	0.195
	ST	2.78		ST vs. SU	0.751
	SU	3.15		OG vs. ST	0.799
	OG	2.49			
POMU	CC	0.38	0.27	CC vs. FO	0.023
	ST	1.46		ST vs. SU	0.287
	SU	1.95		OG vs. ST	0.551
	OG	1.71			
ACCI	CC	2.75	1.35	CC vs. FO	0.121
	ST	3.65		ST vs. SU	0.099
	SU	7.77		OG vs. ST	0.385
	OG	5.48			
HODI	CC	3.19	1.77	CC vs. FO	0.178
	ST	6.24		ST vs. SU	0.765
	SU	5.61		OG vs. ST	0.903
	OG	5.98			

[a]BENE = Oregon grape, GASH = salal, POMU = sword fern, ACCI = vine maple, HODI = ocean spray

[b]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth, FO = average of ST, SU, and OG means

Leaf Area and Specific Leaf Mass

For all species, young leaves were smaller in clear-cuts than in forest stands (Oregon grape P = 0.018; salal P = 0.005; sword fern P = 0.004; vine maple P = 0.021; ocean spray P = 0.011; Table 7; Figure 6a). There were no differences in leaf area between forest stands, or between young and mature leaves.

Sword fern, vine maple, and ocean spray were analyzed for specific leaf mass (g/cm^2) at two sites: D-line and Bummer Ridge. Specific leaf mass was greater in clear-cut areas than in forest areas for all three species (P = 0.001, 0.002, and 0.014, respectively; Figure 6b). Specific leaf mass did not differ between forest stand types ($P \geq 0.489$).

Diffuse Radiation

Diffuse radiation was higher in clear-cuts (0.720) than in forest stands (0.072)(P = 0.002; Figure 7). Significant differences in diffuse radiation were not found between forest stands ($P \geq 0.325$). However, mean diffuse radiation in thinned second-growth (0.104) was almost double that in old-growth (0.053) and unthinned second-growth (0.059).

Soil Nitrogen

The weight percent total soil nitrogen did not differ between stand types (P = 0.617). A measure of mineralizable nitrogen may have detected differences.

TABLE 7. Leaf area (cm^2), and specific leaf weight (g/cm^2) of shrub leaves in clear-cuts and forest stands of the Oregon Coast Range.

Species[a]	Stand Type[b]	Leaf Area Mean	Leaf Area SE	SLW Mean	SLW SE	Stand Type[b] Comparison	Leaf Area P-value	SLW P-value
BENE	CC	9.56	0.74	-	-	CC vs. FO	0.018	-
	ST	13.85				ST vs. SU	0.518	
	SU	13.08				OG vs. ST	0.967	
	OG	13.90						
GASH	CC	20.37	2.07	-	-	CC vs. FO	0.005	-
	ST	35.70				ST vs. SU	0.554	
	SU	34.09				OG vs. ST	0.953	
	OG	35.55						
POMU	CC	3.29	0.28	0.0139	0.0004	CC vs. FO	0.004	0.001
	ST	5.51		0.0077		ST vs. SU	0.778	0.606
	SU	5.62		0.0073		OG vs. ST	0.622	0.489
	OG	5.70		0.0072				
ACCI	CC	22.63	1.97	0.0052	0.0002	CC vs. FO	0.021	0.002
	ST	29.45		0.0031		ST vs. SU	0.613	0.776
	SU	30.60		0.0030		OG vs. ST	0.787	0.543
	OG	30.05		0.0028				
HODI	CC	5.81	1.43	0.0079	0.0089	CC vs. FO	0.011	0.014
	ST	12.39		0.0035		ST vs. SU	0.699	0.797
	SU	13.06		0.0032		OG vs. ST	0.387	0.897
	OG	13.97		0.0034				

[a]BENE = Oregon grape, GASH = salal, POMU = sword fern, ACCI = vine maple, HODI = ocean spray
[b]CC = clear-cut, ST = thinned, SU = unthinned, OG = old-growth, FO = average of ST, SU, and OG means

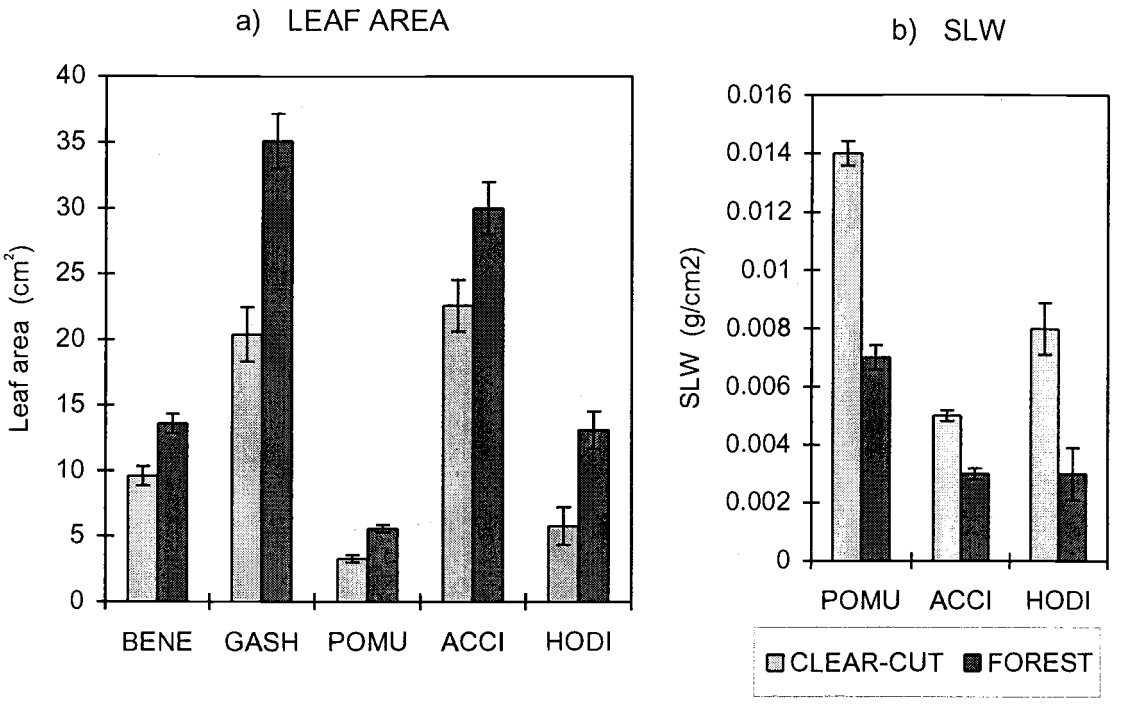

Figure 6. Leaf area (a) in clear-cut vs. forest for young (Y) and mature (M) leaves of Oregon grape (BENE) and salal (GASH), and for young leaves of sword fern (POMU), vine maple (ACCI), and ocean spray (HODI). Specific leaf weight (SLW) (b) for sword fern, vine maple, and ocean spray. Error bars are standard errors.

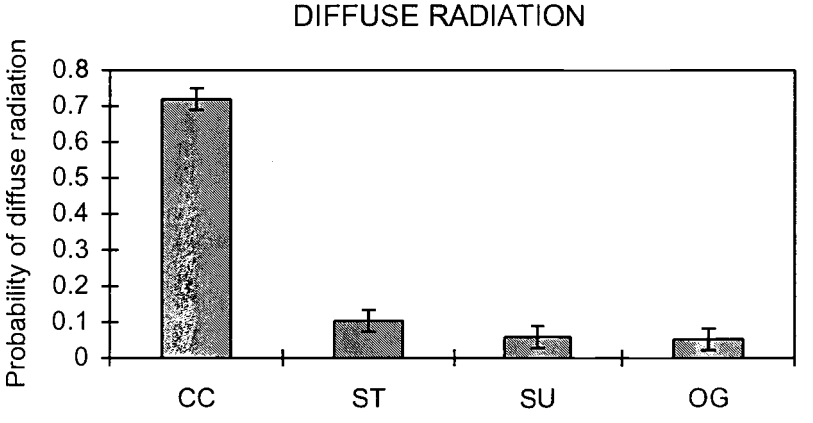

Figure 7. The proportion of available diffuse radiation reaching average shrub height (1 m) in clear-cuts (CC), thinned second-growth (ST), unthinned second-growth (SU), and old-growth (OG). Error bars are standard errors.

DISCUSSION

Nutritional and structural characteristics of leaves, amount of leaf herbivory, and probability of diffuse radiation reaching understory levels differed between clear-cuts and forest. Few differences were found in pair-wise comparisons between forest stands. The lack of canopy cover clearly differentiated clear-cuts from forest stands, and greater levels of diffuse radiation in clear-cuts likely contributed to many observed differences. Similar diffuse radiation conditions may explain the lack of difference in leaf quality and herbivory between forested stands. Canopy coverage also affects other variables such as temperature, wind speed, photosynthetic rates, evapotranspiration, soil moisture, and rates of decomposition and nutrient cycling (Waring and Schlesinger 1985). Further research is needed to assess the relative responsibility of these variables for observed differences in leaf chemistry and herbivory between clear-cuts and forest stands.

Available Nitrogen

Nitrogen availability is likely to be less in clear-cuts than in forest stands for some herbivores. Ocean spray and vine maple leaves from clear-cuts had lower nitrogen concentrations than those from forest stands. Also, protein-binding capacity of tannins was highest in clear-cuts for leaves of all species, which may result in decreased availability of nitrogen for herbivores (Robbins et al. 1987). Condensed tannin concentrations were greater in leaves from clear-cuts than in leaves from forest, and may partially explain the protein-binding differences between stands. Vine maple had a relatively high protein-binding capacity despite low condensed tannin values, probably because vine maple has

significant levels of hydrolyzable tannins (Happe 1993). Hydrolyzable tannin was not measured in this study, but like condensed tannin, it binds with protein and affects forage digestion (Hanley et al. 1992).

Other studies, in Washington and Alaska, also found less nitrogen, greater protein-binding capacity, and greater tannin concentration in leaves from clear-cuts relative to forest stands (Hanley et al. 1987; Van Horne et al. 1987; Happe et al. 1990; Rose 1990). Low nitrogen and high condensed tannin concentration of leaves in clear-cuts are consistent with the carbon/nutrient balance hypothesis (Bryant et al. 1983). When nitrogen becomes limited relative to carbon, carbohydrate production is increased relative to protein production and carbohydrates not used for growth are used to produce carbon-based secondary metabolites such as tannins. Intense light conditions are likely to increase photosynthetic rates and CO_2 fixation (Salisbury and Ross 1992). Since total soil nitrogen did not differ between stand types, and carbon assimilation by leaves was likely greatest in clear-cuts, leaf tannin/nitrogen ratios in clear-cuts were expected to exceed those of forest stands.

Mean nitrogen concentrations of swordfern, salal, and Oregon grape leaves did not differ significantly between clear-cuts and forest stands. Larsson et al. (1986) found percent dry weight of nitrogen to be similar for willow (*Salix dasyclados)* leaves from both high and low light environments if nutrients are not limiting. Differences between species responses may be due to the varying ability of each species to acquire nitrogen (Barbour et al. 1987), or to differences in soil N beneath different shrub types (Horner et al. 1988). Measuring available soil nitrogen is difficult, because it can change across small distances. I used total N as an indicator of differences over large N gradients (Pearcy et al. 1989). Mineralizable N should be considered when taking a closer look at the relationships between soil and leaf nitrogen concentration; mineralizable N correlates well with plant uptake in agricultural fields, and with foliar nutrient levels and fertilization response in forests (Pearcy et al. 1989).

Mature leaves of Oregon grape and salal had lower nitrogen concentration than young leaves, and mature salal leaves had higher protein-binding capacity and condensed tannin concentrations than young leaves. Consequently, nitrogen available to herbivores was greater in young leaves than mature leaves. Leaf nitrogen concentration decreases in maturing leaves of other species (Hough et al. 1978; Scriber and Slansky 1981; Schroeder 1986; Ayres and MacLean 1987; Happe et al. 1990), though some increases in leaf nitrogen with age are known for tropical plants (Cooke et al. 1984). Tannin concentration increases with seasonal maturation for oak (Feeny 1970; Schultz et al. 1982) and *Pinus serotina* leaves (Schroeder 1986), but decreases with seasonal maturation for vine maple, salmon berry, and sword fern (Happe et al. 1990), and many tropical species (Coley 1983; Cooke et al. 1984).

Both leaf nitrogen concentration and protein-binding capacity of tannins must be considered when assessing availability of nitrogen to herbivores, because some herbivores cannot digest tannin-bound protein. Digestible protein in Alaska blueberry and bunchberry dogwood from clear-cuts falls below requirements of lactating does due to tannin activity (Hanley et al. 1987). Deer selected forest leaves over leaves from clear-cuts in feeding trials, perhaps to avoid tannin (Hanley et al. 1987). In African rainforests, ruminant digestion of protein was negatively correlated with condensed tannin from leaves and seeds, potentially affecting resident colobine monkeys (Waterman et al. 1980). Robbins et al. (1987) developed a predictive equation, based on results of feeding trials with deer and elk, which calculates digestible protein as a function of crude protein concentration and protein-binding capacity of tannins measured with the BSA method (Martin and Martin 1982). Using this predictive equation, Happe et al. (1990) found that for salmonberry, sword fern and vine maple, tannins had the capacity to precipitate more protein than was present in leaves. In my study, vine maple had the highest protein concentration, but leaf extracts precipitated an average of 4 to 13 times more protein than extracts of other species. Here

the equations of Robbins et al. (1987) indicate that vine maple leaves from clear-cuts would have a negative protein digestibility (-1.3 percent), and thus support the findings of Happe et al. (1990). Results from the blue-dye BSA method used in this study, may vary slightly from the BSA (non-blue-dye) method used to create the predictive equation (Robbins et al. 1987), however the equation should still exemplify the effect of protein-binding on protein availability.

The effects of tannin on invertebrates are difficult to predict. Tannin can slow development at various life stages, decrease pupal weight (Bourchier and Nealis 1993; Mallampalli et al. 1996), and possibly reduce pupal survival (Feeny 1968). Some phytophagous insects, however, are able to ingest tannin without harmful effects (Fox and Macauley 1977; Martin et al. 1987; Lederhouse et al. 1992), and small quantities of tannin have been reported to act as a feeding stimulant for some phytophagous insects (Bernays 1981). Some lepidopteran larvae are able to reverse protein-binding action of tannins by the presence of surfactants (Feeny 1969; Martin and Martin 1984; Mole and Waterman 1985; Martin et al. 1987) and high pH in their digestive system (Feeny 1970; Berenbaum 1980; Martin and Martin 1984). Larvae may also increase leaf consumption to make up for reduced protein intake (Slansky 1982; Wheeler and Slansky 1991), or avoid tannin while feeding (Reese et al., 1982; Manuwoto et al. 1985). Some tannin may actually be beneficial to herbivores as defense against microbial pathogens (Hedin et al. 1978; Koike et al. 1979; Felton and Duffey 1990; Schultz and Keating 1991; Schultz et al. 1992). The effect of tannin on larvae seems to be species dependent. Larvae adapted to feeding on tannin rich leaves will thrive, as long as their particular host species is available and leaf phenology is synchronized with development (Mitter et al. 1979; Schroeder 1986; Carroll and Quiring 1994).

Fiber and Digestible Energy

The mean fiber concentration (percent cell wall constituents) in leaves of all species was least from clear-cuts, and significantly different from forest stands for vine maple. This suggests that the digestible energy available to herbivores may be greater in clear-cuts. Furthermore, leaves from clear-cuts had larger specific leaf mass than leaves from forest stands which may increase the amount of energy available to an herbivore with each bite effort (Robbins et al. 1987). This does not take into consideration the affect that tannin may have on digestible energy, however. Hanley et al. (1987) and Happe et al. (1990) reported no difference between stand types of in vitro digestible dry matter (IVDDM), a predictor of digestible energy for deer that incorporates tannin activity, despite having less CWC in clear-cuts. In vine maple, salmonberry, huckleberry (Happe 1990), and *Prunus serotina* (Schroeder 1986) leaf fiber increased with seasonal maturation, and IVDDM decreased. Mature and young leaves of Oregon grape and salal did not differ in fiber concentration, thus digestible energy may have been similar in both age groups, but only when tannin does not affect digestion.

Leaf area was greater in forest stands than in clear-cuts for all species, perhaps because large leaves in a relatively dim forest environment would be able to intercept more light than small leaves (Perry 1994). These large, relatively thin leaves may require more fiber for structural support than small, thick leaves found in clear-cuts. The extreme toughness of leaves in high light environments (Dudt and Shure 1994; personal observation 1995 and 1996), and the theorized increase in production of carbon-based molecules with light exposure (Bryant et al. 1983) might seem to indicate that these leaves would have more fiber. Clear-cut leaves had less fiber, however, so perhaps a low proportion of fiber may still contribute to exterior leaf toughness in small thick

leaves. Furthermore, if nonstructural carbohydrates are more abundant in leaves from clear-cuts, then carbohydrate production may simply be allocated to nonstructural rather than structural components.

In addition to lignin, the acid detergent lignin analysis detects cutin and structural condensed tannins that are bound to lignin (Preston et al. 1997). Thus for some shrub leaves, lignin may actually be a very small component of the analysis value. High lignin concentrations in Oregon grape, salal, and sword fern may be due to increased amounts of cutin, a common protective compound on long-lived evergreen leaves (Bryant et al. 1992). Vine maple leaves, on the other hand, with low lignin values and low condensed tannin concentration, may more closely reflect actual lignin amounts than those in evergreen shrubs.

Leaf Moisture

Mean moisture concentration of young leaves was lowest in clear-cuts, probably due to decreased soil moisture availability and increased rates of evapotranspiration. Summer soil moisture in Douglas-fir forests of Oregon has been found to be lower in four-year-old clear-cuts than in adjacent forest stands (Adams et al. 1991); and rates of evaporation will be greater on surfaces exposed to light and wind than on overstory protected surfaces.

Young leaves had higher moisture concentrations than mature leaves for salal and Oregon grape. This is consistent with other studies comparing young and mature leaves (Hough and Pimental 1978; Scriber and Slansky 1981; Schroeder 1986; Ayres and MacLean 1987).

Herbivory

Mean herbivory values were consistently low in clear-cuts for all shrubs studied, though only significantly so for Oregon grape and sword fern. Vine maple showed significantly higher herbivory in unthinned than in thinned second-growth forest. A concurrent study conducted at Beaver Flat, North Ward, and D-line, found that number and species of lepidopteran adults were lower in clear-cuts than in forest stands (J. Miller pers. com.), but larval abundance was not measured. Feeding behavior of lepidopteran larvae and presence of adults may be influenced by a number of variables including leaf nutritional quality, shrub biomass, light, temperature, wind, and predatory exposure, all of which may vary between stand types. Therefore comparisons across stand types are of limited value in assessing the influence of nutritional quality on herbivory.

Comparisons of herbivory and nutritional quality between mature and young leaves that occur together in the same stands may provide insight into insect food preferences. In this study the total herbivory of mature salal leaves did not differ from that of young leaves, even though mature leaves had been exposed to herbivores for approximately one to three years longer than young leaves. Oregon grape mature leaves received one-half again as much herbivory as young leaves. Little additional herbivory was received after the first growing season, indicating that herbivores prefer young leaves. Mature leaves of Oregon grape and salal had lower nitrogen and moisture concentrations, and greater condensed tannin concentration and protein-binding capacity (salal only), relative to young leaves, but no significant differences in fiber. Leaf nitrogen and water concentration is positively correlated with larval preference and performance, while fiber, leaf toughness, and occasionally tannins, are negatively correlated (Feeny 1970; Coley 1983). Thus lepidopteran larvae may have been selectively feeding on young leaves because they had greater

moisture and nitrogen, and less tannin and protein-binding capacity than mature leaves.

A change in nutrient availability due to early phenological advancement, such as occurs in clear-cuts, may affect larval performance and survival (Raupp et al. 1988). Most larvae feed during the spring and summer, when soft young leaves unfold and provide a diet that is rich in nitrogen and water and low in fiber (Feeny 1970; 1976). As leaves mature, they decrease in water and nitrogen, become more tough, and some species increase in tannin concentration (Hough and Pimental 1978; Mitter et al. 1979). Laboratory feeding trials have shown that larvae are less likely to survive when fed only mature leaves of their host species (Raupp, et al. 1988; Hough and Pimental 1978). For example, *Malacosoma americanum*, a spring-feeding macrolepidopteran larvae, and *Hyalophora cecropia*, a summer-feeder, both perform poorly on fall leaves (Schroeder 1986). The spring-feeder, *M. americanum,* also performs poorly on summer leaves to which *H. cecropia* is adapted. However, both larvae perform best on spring leaves. Larvae must develop while host leaves are nutritious and palatable.

Low herbivory in mature leaves is likely due to low nutritional quality. Leaves collected from clear-cuts were also of lower quality than forest leaves, having less nitrogen and water, and greater condensed tannin and protein-binding capacity, but less fiber. The low herbivory in clear-cuts could be, among other factors, due to decreased leaf nutritional quality associated with accelerated phenological advancement. Greater herbivory in unthinned second-growth compared to thinned second-growth may be due to the low shrub biomass in unthinned stands (Bailey 1996).

Similarity in Forest Stands

Few significant differences in leaf quality or herbivory were found among second-growth thinned, second-growth unthinned, and old-growth. There was also no statistical difference in diffuse radiation between these stands. The thinned second-growth stand at North Ward was the only exception, having greater diffuse radiation than old-growth and unthinned second-growth, probably because North Ward was thinned more recently than the other sites, and the canopy had not yet closed. Thus time since treatment may have a marked effect on understory diffuse radiation. Similar light conditions are likely caused by similarities in stand structure. The age of second-growth ranged from 55 to 105 years, thus these stands were well established and had an elevated overstory canopy similar to 200 year old-growth. Some natural thinning of trees had occurred in unthinned second-growth; and thinned second-growth, treated 10 and 20 years prior to sampling, had likely undergone canopy closure. The old-growth stands may have developed yet a third way. Competition dynamics between trees may be creating similar understory light conditions in different types of older stands, if enough time has elapsed since treatment. Similar light conditions in turn correspond with similar leaf quality.

When different developmental stages are represented, the forest appears less homogeneous. Stands in the stem exclusion stage (~15-35 years) were not sampled for this study. They may provide nitrogen-rich understory shrub leaves, but biomass may be significantly reduced due to shading. The reduced nutritional quality of leaves from clear-cuts suggests that in recently harvested or heavily thinned stands of the Coast Range shrubs may be a poor food source for herbivores. An increase in herbaceous plants commonly associated with early succession may offer an alternative low-tannin food source for generalist herbivores, but species-specific herbivores (ie. most lepidopteran larvae) are affected by nutrient changes in their host plant.

CONCLUSIONS

Indicators of leaf nutritional quality were found to be lower in clear-cuts than in forest stands for at least one variable per species except Oregon grape. Vine maple and ocean spray which had low nitrogen concentration, high tannin concentration and protein-binding capacity, and low moisture concentration in clear-cuts, had the highest number of differences in leaf quality between clear-cuts and forest. Fiber on the other hand was the one indicator of low nutritional quality that was less in clear-cuts than forest stands for all species except ocean spray. Leaf toughness may have been a valuable additional variable to measure. Leaf quality differences are suspected to be a result of differences in understory light exposure. Herbivory by lepidopteran larvae tended to be low in clear-cuts, with a significant difference from forest stands for two species: Oregon grape and sword fern. Herbivory could not be confidently related to leaf nutritional quality across stand types in this study, however, because of too many confounding environmental variables.

Leaf nutritional quality was not related to thinning treatment, except in Ocean spray leaves which had higher protein-binding capacity in thinned than in unthinned second-growth stands. Similarity in leaf chemistry between these stands was probably a result of similar light conditions. Herbivory was greater in unthinned than in thinned stands for vine maple, but this may be related to the low shrub biomass in unthinned stands (Bailey 1996), or some other confounding factor. Thinned second-growth stands did not differ from old-growth stands in nutritional quality and herbivory of young leaves.

Nutritional quality decreased with leaf age for Oregon grape and salal. Mature leaves of Oregon grape had lower nitrogen and moisture concentrations than young leaves. Mature salal leaves had lower nitrogen concentration, greater protein-binding ability and condensed tannin concentration, higher lignin

concentration, and lower moisture concentration than young leaves. Herbivory of mature leaves was decreased by one-half or more relative to young leaves, and is probably a result of herbivore preference for the more nutritional young leaves.

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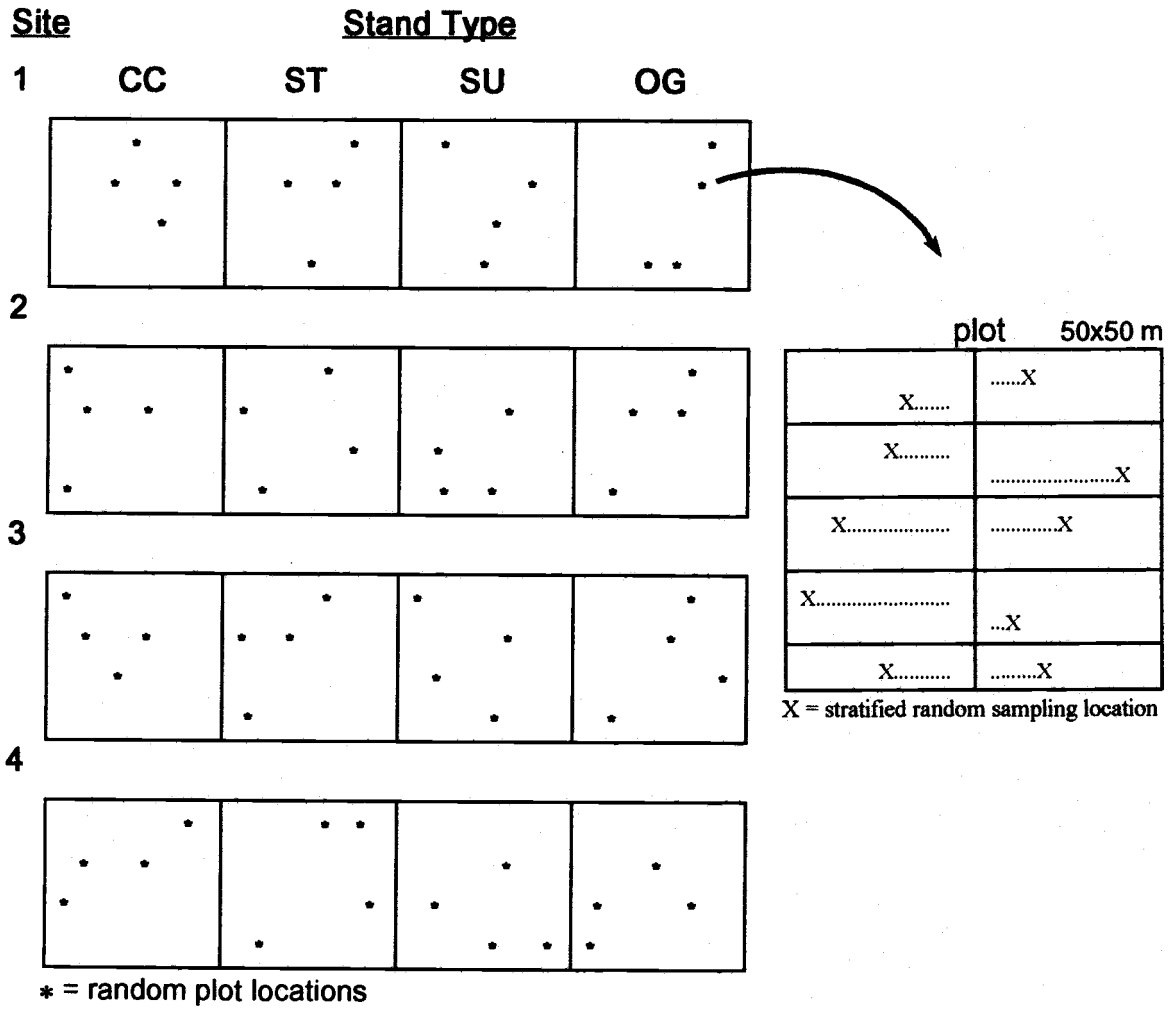
APPENDICES

Appendix A Experimental Design



Stand Type	Shrub species	Leaf characteristics	Environment
clear-cut thinned second-growth unthinned second-growth old-growth	Oregon grape salal sword fern vine maple ocean spray	nitrogen protein-binding condensed tannin CWC & lignin moisture herbivory leaf area specific leaf weight	diffuse radiation soil nitrogen

Appendix B Radial Diffusion Assay

(Hagerman 1987)

Materials:

agarose (type I)
BSA (fatty acid-free fraction V)
tannic acid (gallotannic acid - Baker Chemical Co.)
acetic acid
ascorbic acid
sodium hydroxide

parafilm
8.5 cm plastic petri dishes
a level surface

1 L beaker
1 L Ehrlenmeyer flask
Hamilton microsyringe (10 uL)
wide mouth 10 ml pipet
4 mm hole punch
(small diameter cork borer)
hot plate/stirring plate
pH meter
45° C water bath

Preparation:

Acetate buffer: Add 2.85 ml acetic acid and 10.6 mg ascorbic acid to 800 ml distilled water in a 1 L beaker. Using a stirring plate and pH meter, add 2 N NaOH until the pH reaches 5.0. Pour the buffer into a 1 L Ehrlenmeyer flask and fill to one liter with distilled water.

Agar plates: Pour 1.0 g agarose into a 150 ml beaker with 100 ml of buffer. Heat while stirring until the solution is completely clear and bubbles are beginning to form. Place in a 45°C water bath, and stir occasionally while the solution is cooling so that the surface does not begin to solidify. Once the

solution has cooled to 45°C, add 0.1 g BSA. The protein will denature if it is added to a hot solution, but the agar will solidify if it cools any further, so 45°C is optimum. The BSA dissolves best if it is sprinkled over the surface of the agar solution and left unstirred for about 1 minute, then stirred gently until dissolved. Use a wide-mouth pipet to dispense 10 ml aliquots into standard plastic petri dishes. Make sure that the agar covers the bottom of the plate, and place on a level surface. An unlevel surface will result in an uneven agar thickness, and greatly affect the results. To prevent solidification of agar along the sides of the pipet, preheat the pipet (not above 45°C), and work quickly. Bubbles and an inconsistent agar surface will also affect your results. Allow to cool on a level surface. Seal with parafilm and store at 4°C until use (preferably no more than 3 days).

Tannic acid standard curve: The tannic acid standard makes an excellent, very straight standard curve. A new standard curve should be made with each run, however. While each run is consistent within itself, runs do seem to differ from day to day. Changing conditions, whether it be room humidity or variability in the agar batch, seem to affect the tannin movement and precipitation enough to significantly alter the resulting tannic acid equivalents. Dissolve 0.125 g tannic acid in distilled water and fill to 10 ml in a volumetric flask. In ten test tubes, make a series of dilutions from this stock solution as outlined in the table below: Run the standard at the same time as the samples, following the same procedure.

ml stock solution	ml Distilled water	Final mg T.A. in 8 uL aliquots	Final mg T.A. in 16 uL aliquots
0.1	0.9	0.01	0.02
0.2	0.8	0.02	0.04
0.3	0.7	0.03	0.06
0.4	0.6	0.04	0.08
0.5	0.5	0.05	0.10
0.6	0.4	0.06	0.12
0.7	0.3	0.07	0.14
0.8	0.2	0.08	0.16
0.9	0.1	0.09	0.18
1.0	0.0	0.10	0.20

Procedure:

1. When ready to proceed with the assay, uniformly punch 4 mm diameter holes, spaced approximately 1.5 cm apart, into the agar. I was able to easily fit five holes, with one in the center. Holes that are made too early may fill with moisture and decrease the quantity of extract that can be added to each well.

2. The wells may then be filled with up to 16 uL of extract. Test your samples to find out how much should be added to produce a readable ring, and be consistent with the quantity that you choose. Sometimes more extract is required than will fit in the well. Additional amounts may be added after the first aliquot has partially absorbed into the agar, if the well has not dried completely. This is not advisable, however; the agar can absorb at different rates, creating a

time-consuming waiting and checking game. It would be better to use a more concentrated sample.

3. Reseal with parafilm and incubate at 30°C for 96-120 hours (4-5 days).

4. Measure and record the ring diameters to within a tenth of a millimeter. This may be accomplished with an accurate ruler and good lighting, or with a microscope ruler.

5. Use the diameters from known quatities of tannic acid to create the standard curve, by graphing diameter vs. quantity tannic acid.

6. Compare the sample diameters to the standard curve to determine tannic acid equivalents and record. For final results calculate the amount of tannic acid equivalent in one gram of leaf material.

Appendix C Blue-dye Labelled BSA Protein-binding Test

(Asquith and Butler 1985; Waterman and Mole 1994)

Note: "It is essential that not even traces of acetone contaminate the precipitation reaction." (Hagerman and Robbins 1987; as written in Waterman and Mole pg. 114). The rotoevaporator was used to evaporate all acetone from our solutions, leaving purely an aqueous solution (B.P. of acetone is 56.2 °C).

Materials:

distilled water
Remazol brilliant blue R (150 mg)
sodium bicarbonate (0.4 g)
acetic acid
sodium chloride (10 g)
BSA (2 g)
(fraction V, fatty acid free)
SDS (1 g)
triethanolamine (5ml)
isopropanol (20 ml)
1N sodium hydroxide solution
parafilm
weighing papers
scoop

100 ml volumetric cylinder
10 ml pipette and bulb
250 ml beaker
400-600 ml beaker
1000 ml beaker
2 1-liter volumetric flasks
18 inches of dialysis tubing
(45 mm x 50 ft 12,000-14,000)
stir bars
centrifuge
centrifuge test-tubes
cuvettes
spectrophotometer
analytical balance

Preparation:

Sodium hydroxide solution (NaOH): Prepare 2 N NaOH by adding 8.24 g NaOH (MW=40.00; 97% pure) to 90 ml distilled water (dH_2O) and filling to a final volume of 100 ml.

Acetate buffer: Prepare a 0.2 M acetate buffer, pH 4.9, containing 0.17 M sodium chloride (the NaCl adds ionic strength to the reaction medium). Add 11.28 ml/L acetic acid and 9.93 g NaCl to 800 ml dH_2O. Add 2 N NaOH until the pH is 4.9. Then fill to 1 liter with dH_2O.

Sodium bicarbonate solution ($NaHCO_3$): Dissolve 0.4 g $NaHCO_3$ in 40 ml dH_2O to make a 1% bicarbonate solution.

Protein solution: Dissolve 2 g BSA (Fraction V, fatty acid free) protein in 40 ml of 1% sodium bicarbonate solution, pH 8.2. Add 150 mg of Remazol brilliant blue R. Stir mixture for 30 minutes at room temperature. Dialyse the blue-dye BSA against the buffer solution at 4°C overnight to replace the reaction buffer with a buffer more suitable for the protein-binding assay. After dialysis, pour the BSA solution into a 1 liter volumetric flask and fill to 1 liter with the buffer solution to make a 2.0 mg BSA/ml solution. This solution is used in the precipitation reaction. The blue-dye is covalently attached to the protein and will act as a marker, allowing the spectrophotometer to measure the amount of BSA protein precipitated by the tannin.

SDS solution: Mix 5 ml triethanolamine, 20 ml isopropanol, 50 ml distilled water and 1 g SDS in a 100 ml volumetric flask. Fill to 100 ml with distilled water.

Procedure: (Make sure everything is well mixed and at room temperature)
Note: For best results the reaction must be run at the equivalence point. This is the tannin to protein ratio which precipitates the most BSA per extract concentration. Changes in the extract concentration, or total tannin concentration, can greatly alter the results. If too much tannin is present, BSA precipitation will be maximized before the protein-binding potential of the tannin

is exhausted. On the other hand, a threshold of tannin must be present for tannin-protein complexes to precipitate. With too little tannin, only soluble tannin-protein complexes form, which are not detectable by this method. Since the tannin concentration of an extract is unknown, it is best to run several concentrations to determine the range that contains the equivalence point. Once this range is determined, I recommend continuing to run the samples with three concentrations. The equivalence point can change from one sample to the next, even with the same plant species, so determining the equivalence point of one sample does not guarantee that the equivalence point will be the same for other samples of the same species. Keep in mind also, that the equivalence point may be more sensitive and difficult to determine for some species than for others.

1. Add 2 ml of the blue-dyed protein solution to 1 ml of the tannin extract in centrifugable test tubes (Corex). In an additional test tube mix 1ml of distilled water and 2 ml of blue-dyed BSA; this will be the blank. Mix and allow to stand at room temperature for 15 minutes.

2. Centrifuge at 5000 G for 15 minutes, then carefully discard the supernatant with a pastuer pipette. The remaining precipitate will be redissolved in the next step.

3. Add 4 ml of the SDS solution to the precipitate in the test tube and mix to resuspend the tannin-protein mixture.

4. Zero the spectrophotometer on the blank solution. This is necessary, because a small amount of BSA is precipitated even when tannin is not present; zeroing the machine on a blank will subtract out the non-tannin precipitants.

5. Measure and record sample absorbances at 590 nm. Measuring at this wavelength restricts color detection to the blue-dye BSA only.

6. Make a 100% solution by mixing 2 ml of the blue-dye BSA solution used in the precipitation reaction, and 2 ml of the SDS solution used to resuspend the precipitate. In another test tube, make a blank containing 2 ml acetate buffer and 2ml SDS. Mix both well and pour each into a cuvette. Zero the spectrophotometer with the blank, and measure the absorbance of the 100% solution at 590 nm. This absorbance reading represents a solution that contains 1 mg BSA/1 ml solution (4mg total), and it will be used to calculate the amount of BSA precipitated by the leaf extracts. *[At this point I made an error; I used the blank solution from step (4) to zero the machine before reading the 100% solution. This lowers the absorbance reading of the 100% solution and results in slightly larger final tannin protein-binding values (by approximately 5%). Because this was done consistently for all samples, comparisons are still valid.]*

7. Calculate the final results in mg BSA/mg leaf: Divide the sample absorbance by the 100% solution aborbance and multiply by 4 mg BSA. This will give the amount of BSA precipitated by the sample. Calculate the amount of leaf (mg) in 1 ml of extract using the known concentration in the original extract and the concentration prepared for this assay. For example, an extract containing 1 g leaf/25 ml solution, with an ideal reaction concentration of 0.1 ml extract/1 ml solution will be calculated: (1000 mg leaf/25 ml final solution)(0.1 ml extract/1 ml solution) = 4mg leaf/ml. Divide mg BSA by mg leaf in 1 ml.

Appendix D Vanillin Procedure for Condensed Tannin Estimation

(Broadhurst and Jones 1978; with modifications from Swain and Hillis 1959)

Materials:

distilled water	aluminum foil	ice water bath
sulfuric acid	permanent marker	thermometer
vanillin	5 ml glass vials w/ screw caps	analytical balance
catechin	pipets, 0.1 ml, 1 ml	vortex
	25 ml volumetric flask	spectrophotometer
	100 ml volumetric flask	
	250 ml volumetric flask	
	cuvettes for spectrophotometer	

Preparation:

70% sulfuric acid: Add about 65 ml of distilled water to a 250 ml volumetric flask sitting in an ice-water bath. Slowly and carefully add 175 ml of concentrated sulfuric acid to the water. The ice bath will help dissipate the heat generated from the reaction. When the solution has cooled, remove from the ice bath and allow to stand at room temperature. Once the solution has reached room temperature, bring to a final volume of 250 ml with distilled water. Because the volume changes with temperature, it is important that the solution reach a stable temperature before bringing it to volume. This will be used in making the vanillin reagent and blank solutions.

Vanillin reagent: Make fresh every three days. Measure 1 g vanillin into a 100 ml volumetric flask and fill to volume with the 70% concentrated sulfuric acid.

Catechin standards: The standards must be made in a concentration range that will include the same absorbance readings as the samples. I used catechin solutions in the range 0.0-1.0 mg/ml. To do this, make a 1 mg/ml stock solution by measuring 25 mg of catechin into a 25 ml volumetric flask and filling to volume with distilled water. Because catechin does not dissolve quickly in distilled water, the stock should be made the night before, allowing the catechin to dissolve overnight. Run duplicates of each standard level to give you confidence in your results. On the following page is an example of a table used to prepare the standards. Use an adjustable pipetter to measure quantities of stock and distilled water directly into the appropriately labeled vial. For the very small amounts (e.g. 0.05 mls), you may need to use a glass Mohr or TD pipet. Each standard will be run in 0.50 ml aliquots (same as sample volumes).

vial #	Concentration of std in mg/ml	Total catechin mg per aliquot	mls stock	mls dH_2O	spec reading
1	0-sulf acid+van		0	0	
2	0-sulf acid+van		0	0	
3	0-dH_2O+van		0	0.5	
4	0-dH_2O+van		0	0.5	
5	0.1	0.05	0.05	0.45	
6	0.1	0.05	0.05	0.45	
7	0.2	0.1	0.1	0.4	
8	0.2	0.1	0.1	0.4	
9	0.3	0.15	0.15	0.35	
10	0.3	0.15	0.15	0.35	
11	0.4	0.2	0.2	0.3	
12	0.4	0.2	0.2	0.3	
13	0.5	0.25	0.25	0.25	
14	0.5	0.25	0.25	0.25	
15	0.6	0.3	0.3	0.2	
16	0.6	0.3	0.3	0.2	
17	0.7	0.35	0.35	0.15	
18	0.7	0.35	0.35	0.15	
19	0.8	0.4	0.4	0.1	
20	0.8	0.4	0.4	0.1	
21	0.9	0.45	0.45	0.05	
22	0.9	0.45	0.45	0.05	
23	1.0	0.5	0.5	0	
24	1.0	0.5	0.5	0	
25	0-reagent blank		0	0	
26	0-sulf.acid blank		0	0	

Sulf+van=0.5 ml 70% sulfuric acid and 3 ml vanillin rgt *reagent blank*=3.5 ml of vanillin rgt

dH_2O+van=0.5 ml distilled water and 3 ml vanillin rgt *sulf.acid blank*=3.5 ml of 70% sulfuric acid

Procedure:

1. This assay should be conducted at 20± 2° C. Room temperature may be sufficient, but check first. Our lab temperature fluctuates and can be as warm as 24° C. This is also a good time to turn on the spectrophotometer, allowing it to warm up for 30 minutes prior to use.

2. Use small 5 ml glass vials with screw caps. Thoroughly wrap clean vials in aluminum foil to prevent light exposure during the reaction. Label the vials on the foil with a permanent marker.

3. Pipette 0.5 ml of sample into the vials. In the case of standards you will already have made the appropriate dilutions to a final volume of 0.5 mls. Run duplicates of each sample. Record the samples that are in each vial with a sheet like the one above.

4. Add 3 ml of vanillin reagent to each sample and mix thoroughly with a vortex for 10-15 seconds.

5. Immediately place on ice for 5 minutes; the reaction produces heat, and you will need to bring the solution back to around 20° C. After icing, place vials in a rack of room temperature water so they will quickly come to room temp. Allow reaction to stand for 15 minutes at 20 ± 2° C.

6. Read samples and blanks against distilled water (by zeroing the spectrophotometer with water) at 500 nm wavelength, and record this on the data sheet. In our lab see Shimadzu UV-265FW instruction manual: instructions for operation pg. 13, sect. 5.

Diluting samples with high absorbance:

Check the range at which your spectrophotometer can accurately read absorbance. When samples approach the upper limit (4.0 nm for some spectrophotometers), they should be read as diluted solutions. Dilutions can be performed as you are running the samples. If dilutions higher than 1:5 are required, you should consider starting over and diluting the original sample with distilled water before mixing the 0.05 aliquot with the vanillin reagent; if the original sample is too concentrated, the assay will not detect the full condensed tannin content. The diluted absorbance reading may be corrected by simply multiplying the absorbance by the dilution rate.

1. Mix the sample in the vial. Pipet out the volume of sample needed, and add directly to the cuvette. Use a clean pipet for each sample to avoid contamination between samples. (A 1:5 dilution will require 0.7 ml of sample and 2.8 ml of 70% sulfuric acid, for a final volume of 3.5 mls.)

2. Pipet out the volume of 70% sulfuric acid needed and add directly to the sample in the cuvette. Mix the diluted sample in the cuvette.

3. Read the absorbance as usual, and record on the data sheet. Note the dilution so that you may later calculate the absorbance of an undiluted sample. (For a 1:5 dilution, the recorded absorbance will be multiplied by 5 to obtain the final absorbance.)