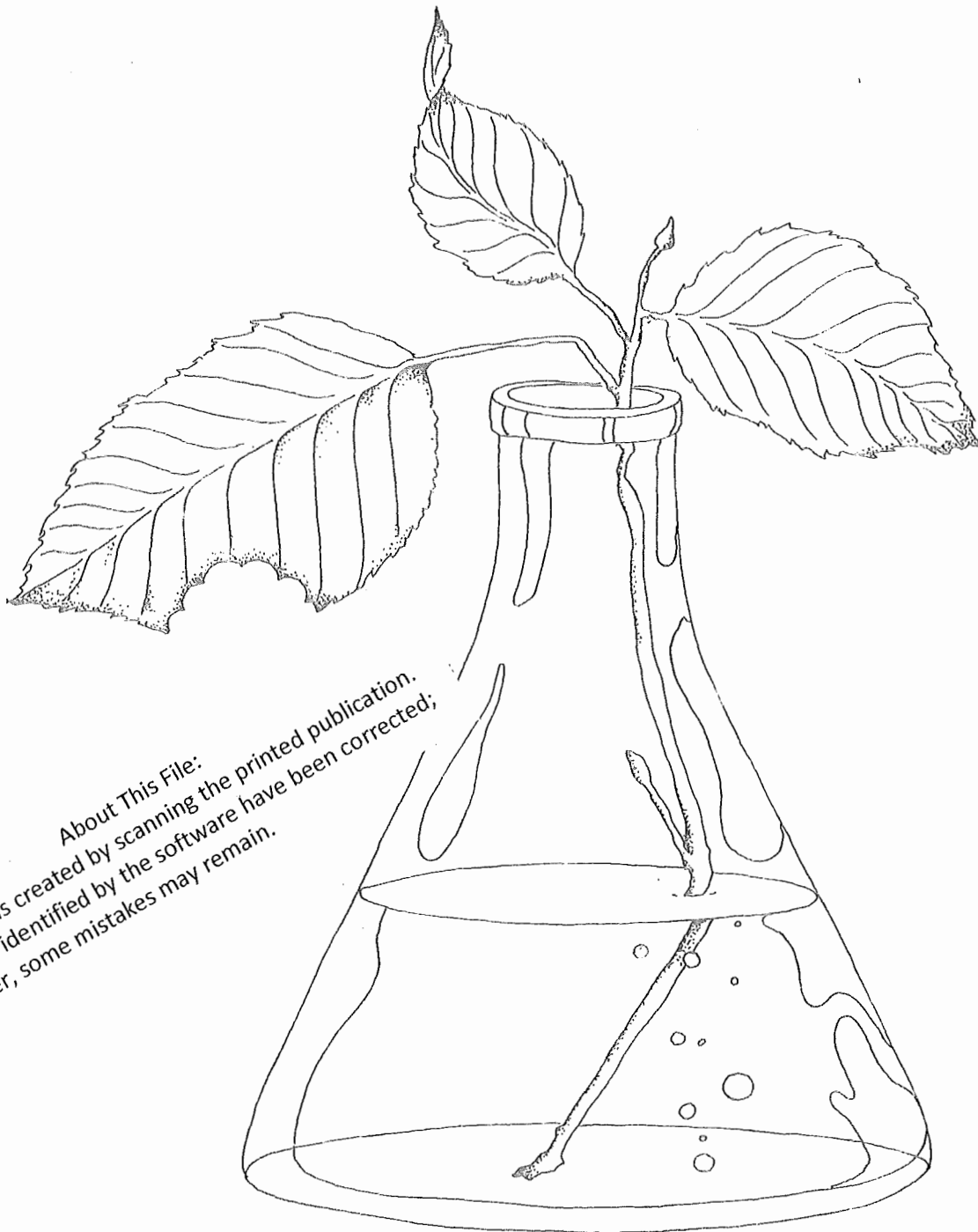




About This File:
This file was created by scanning the printed publication.
Misscans identified by the software have been corrected;
however, some mistakes may remain.

CHEMICAL COMPOSITION AND DEER BROWSING OF RED ALDER FOLIAGE

M. A. Radwan
W. D. Ellis
G. L. Crouch

Research Paper PNW-246
Pacific Northwest Forest and Range Experiment Station
U.S. Department of Agriculture

December 1978
Forest Service
Portland, Oregon

M. A. RADWAN is Principal Plant Physiologist with the Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Olympia, Washington.

W. D. ELLIS is a chemist with the Forest Products Laboratory, Madison, Wisconsin.

G. L. CROUCH is Principal Wildlife Biologist with the Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado.

CHEMICAL COMPOSITION AND DEER BROWSING OF RED ALDER FOLIAGE

Reference Abstract

Radwan, M. A., W. D. Ellis, and G. L. Crouch.

1978. Chemical composition and deer browsing of red alder foliage. USDA For. Serv. Res. Pap. PNW-246, 6 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oreg.

Chemical factors suspected of influencing seasonal change in deer preference for red alder leaves were investigated. There were many differences in the leaves between seasons. It is postulated, however, that changes in contents of crude fat and total phenols were the important factors in increasing deer preference for the species from June to September.

KEYWORDS: Browse preference, foliar analysis, phenols, deer (black-tailed), *Odocoileus hemionus columbianus*, *Alnus rubra*.

RESEARCH SUMMARY

Research Paper PNW-246

1978

Selected chemical properties suspected to influence feeding preference of black-tailed deer were compared in red alder leaves obtained from one plant population in western Oregon. Leaves were collected in June when browsing was minimal and in September when utilization had begun. In general, the preferred September foliage had lower levels of moisture, acidity, crude protein, and total phenols, and higher contents of available carbohydrates and crude fat. The leaves also contained both saturated and unsaturated fatty acids commonly found in palatable plants, and fatty acid composition changed during the growing season. Despite the many differences found in leaves between seasons, it is postulated that changes in contents of crude fat and total phenols were the important factors in increasing deer preference from June to September. Because of the extreme complexity of the animal-plant relationships, however, other chemical or nonchemical differences cannot be completely ruled out as factors of preference.

Introduction

Red alder (*Alnus rubra*) is one of the most widely distributed hardwoods in the Douglas-fir region of the Pacific Northwest. Despite its abundance and easy availability in the spring and summer, alder foliage is not much utilized by black-tailed deer (*Odocoileus hemionus columbianus*) in western Washington and Oregon until early fall (Brown 1961, Crouch 1968). Although many factors may be suspected of influencing this seasonal change in deer preference, it is likely that the major deciding factor is the changing chemical composition of the foliage during the growing season. Such changes may influence preference through their effect on important foliage properties, such as nutritive value, taste, odor, and digestibility (Arnold and Hill 1972).

This paper reports chemical analyses of red alder foliage collected from one plant population in western Oregon in June when deer use of the species was minimal and again in September when browsing had begun. The analyses include both nutritional and nonnutritional chemicals suspected of influencing feeding preferences of herbivorous mammals for plants (Heady 1964, Arnold and Hill 1972, Radwan 1974), and results are discussed in relation to differences in deer browsing preference.

Materials and Methods

LEAF SAMPLING

Test leaves were obtained from alder trees growing at the Tillamook Burn in northwest Oregon. Three composite samples, of approximately 500 g each, were taken in June and again in September. Each sample was obtained from about 20 healthy trees selected at random from approximately 10 acres of similar elevation, aspect, soil series, and vegetation composition.

Samples were collected by clipping leaves growing within the reach of deer, and collections were made in early morning when deer commonly feed. Samples were individually placed in plastic bags and brought to the laboratory in a portable cooler.

CHEMICAL ANALYSES

In the laboratory, fresh foliage tissue was chopped into small pieces; and subsamples were taken for determination of dry matter, pH, and total phenols. Remaining tissue was dried to constant weight at 65°C, ground to 40 mesh in a Wiley mill, and stored in closed containers at -15°C until used for other analyses.

Moisture content was determined by drying to constant weight in a forced-air oven at 65°C, and ash in the ground tissue was estimated by heating in platinum crucibles at 500°C-550°C for 4 hours. Hydrogen-ion concentration (pH) was determined with glass electrode on aqueous extracts obtained by homogenizing fresh tissue in recently boiled distilled water. Total phenols, obtained by extraction in Soxhlet apparatus with 80-percent methanol, were estimated by using the Folin-Denis reagent according to the procedure of Swain and Hillis (1959); and tannic acid was used as the reference standard. Total available carbohydrates were extracted and hydrolyzed with 0.2 N H₂SO₄ (Smith et al. 1964), and resulting sugars were determined as glucose by the ceric sulfate method (Hassid 1937).

For fatty acid analyses, lipids were extracted with petroleum ether (b.p. 30°C-60°C) in Soxhlet, saponified with methanolic NaOH, and methylated with BF₃ in methanol (Metcalf et al. 1966, Horwitz 1970). Resulting fatty acid methyl esters were taken up in heptane and analyzed with a gas chromatograph equipped with flame ionization detector and a 3-m x 3-mm (ID) stainless steel column packed

with 10-percent diethylene glycol succinate (DEGS) on 80- to 100-mesh Chromosorb W (AW-DMCS). Operating conditions were: injection port, 225° C; detector, 225° C; column, isothermal at 190° C; and H₂, N₂, and air flows of 25, 30, and 250 ml/minute, respectively. Heptadecanoic acid (17:0), added to samples prior to esterification, was used as an internal standard. Resolved peaks were identified by comparing unknowns' relative retention times with those of known compounds and by peak enrichment; and compounds were quantified by measuring peak areas with electronic integrator.

Analyses of other tissue components were made as follows: total nitrogen by the micro-Kjeldahl technique, and crude fat from loss in tissue weight after extraction with ether in Soxhlet (Horwitz 1970); nitrate by the phenoldisulfonic acid method (Johnson and Ulrich 1950); and

acid-detergent fiber and acid-detergent lignin according to Van Soest (1963).

All analyses were made in duplicate on each of the three replicate samples, and mean values for the two leaf collections were compared.

Results

The average chemical characteristics of the leaf tissues of red alder collected at two different times during the year are summarized in table 1. Relative acidities are shown in pH units, and concentrations of the different chemical constituents are expressed on dry weight basis.

Differences among leaves of the two collections were apparent in most properties studied, but the magnitude of the differences and the direction of change varied with the property in question. Brief consideration of the individual properties follows.

Table 1--*Chemical properties of red alder foliage collected at two different times during the year^{1/}*

Chemical property	Unit of measure	Collection date of foliage	
		June 1974	September 1974
Moisture	percent	67.69	58.67
Ash	percent	2.82	3.49
H-ion concentration	pH	4.60	5.55
Acid-detergent fiber	percent	21.80	20.69
Acid-detergent lignin	percent	12.48	10.86
Total available carbohydrates	percent	10.43	12.31
Total nitrogen	percent	2.38	1.83
Nitrate nitrogen	ppm	68.00	62.00
Total phenols	percent	10.22	5.13
Crude fat	percent	3.52	7.86

^{1/}Values are averages of three composite samples each. Total phenols are expressed as tannic acid equivalents.

Moisture.--Moisture was the highest of all constituents determined; the average moisture content for all leaves was approximately 63 percent. There was a decrease in moisture content as dry matter accumulated during leaf maturation. Consequently, leaves were less succulent in September than in June.

Minerals.--Mineral elements, as shown by the ash content, averaged about 3 percent for the two collections. This indicates that an average of approximately 97 percent of the dry matter was organic in nature. The ash content increased from June to September reflecting increased absorption of minerals as the season progressed. Difference between the two collections, however, was small.

Acidity.--The pH of the leaves increased during the growing season; it ranged from 4.60 in June to 5.55 in September. Leaves, therefore, were less acidic during the period of deer browsing.

Fiber and lignin.--The acid-detergent fiber, representing the acid insoluble cell-wall materials (Van Soest 1965), was, as expected, the highest of the measured organic constituents of the leaves. The fiber decreased slightly from June (21.80 percent) to September (20.69 percent).

Trends of acid-detergent lignin were similar to those of the fiber. Lignin content for both collections averaged 11.67 percent of dry matter.

Available carbohydrates.--Total available carbohydrates averaged over 11 percent in the leaf tissues. Clearly, available carbohydrates constituted a major nutritional component of the leaves' organic matter. Also, as expected, carbohydrate levels were higher in September (12.31 percent) than in June (10.43 percent).

Nitrogen.--Total nitrogen ranged from a high of 2.38 percent in June to a low of 1.83 percent in September. For comparison with other data in the literature, these nitrogen levels are equivalent to approximately 14.9 and 11.4 percent crude protein, respectively. Red alder leaves, therefore, are characterized by high levels of protein. In both the June and September leaves, crude protein was much higher than the minimum requirement for black-tailed deer as proposed by Einarsen (1946).

Nitrates were low, averaging 65 ppm for the two collections. Also, both the June and September leaves contained similar amounts of nitrates.

Phenols.--Total phenols ranged from 10.22 percent in June to 5.13 percent in September. This decrease by approximately 50 percent was the greatest noted among the leaf components which declined during the growing season. The reduced phenolic content also coincided with the period of increased deer browsing.

Fat.--The average crude fat content of leaves substantially increased during the growing season; the September value (7.86 percent) was more than twice that of the June concentration (3.52 percent). Such an increase must have greatly enhanced the caloric content of the September leaves since crude fat is a high energy source.

Fats were studied further by hydrolysis and analysis of their component fatty acids by gas-liquid chromatography.

Fatty acids.--The main fatty acids of red alder leaves were lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic (table 2). In both the June and September leaves, saturated acids predominated over unsaturated compounds. Also, in all leaves, palmitic was the most abundant (over 47 percent), followed by linolenic (22.0, 30.8 percent); lauric had

Table 2--Percentage composition of foliar fatty acids of red alder^{1/}

Fatty acid		Collection date of foliage	
		June 1974	September 1974
Lauric	12:0	0.2	0.6
Myristic	14:0	3.4	5.4
Palmitic	16:0	47.7	47.4
Stearic	18:0	7.3	3.8
Oleic	18:1	4.9	3.8
Linoleic	18:2	14.6	8.4
Linolenic	18:3	22.0	30.8

^{1/}Numbers following fatty acids indicate number of carbon and number of double bonds, respectively. Percents are averages of three composite samples each.

the lowest concentrations (0.2, 0.6 percent). During the growing season, levels of lauric, myristic, and linolenic increased, whereas those of stearic, oleic, and linoleic decreased; palmitic concentrations remained unchanged.

Discussion and Conclusions

Results show that in June when utilization by deer was minimal, red alder leaves contained adequate levels of minerals, available carbohydrates, protein, and fats to satisfy the nutritional needs of herbivores, including deer (Maynard 1951). The leaves were also succulent, moderately acidic, had enough roughage as shown by the contents of fiber and lignin, and contained nontoxic levels of nitrates as well as fatty acids which are usually found in palatable plants. The total phenols in the leaves, however, were quite high; they amounted to over 10 percent of the dry matter and greatly exceeded levels found in

plants browsed by black-tailed deer in winter (Radwan and Crouch 1974).

Comparison of the June and September leaves indicated many variations in most chemical constituents. Thus, as leaves grew older and became more preferred by deer in September, moisture, acidity, protein, and phenols had decreased, while available carbohydrates and fats were increased. Fatty acids also changed in their composition during the same period of time. Obviously, it is impossible to state which change or combination of changes were actually responsible for the increased preference or the mechanism by which any of these factors were operating. One may speculate, however, that changes in fats and phenols were the important factors. That changes in these two leaf constituents were much greater than those shown by remaining constituents tends to support this speculation. Crude fat also is recognized as a high energy food source for animals, and associations of high fat contents in forages with high preference by some animals have been

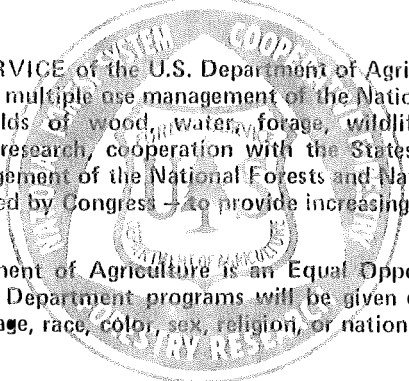
reported (Hardison et al. 1954, Low et al. 1967). Phenols, on the other hand, have been generally considered as defense compounds which protect plants from their natural enemies, including herbivores (Levin 1971). Furthermore, tannins, which are estimated here as total phenols, have been shown to reduce palatability and digestibility of some plants in ruminants (Wilkins et al. 1953, Donnelly and Anthony 1973); and their great decline in September could very well account for the increased utilization of the leaves in the fall.

Factors affecting animal preferences for plants are extremely varied and complex. Accordingly, we recognize that chemical constituents other than those reported here or nonchemical factors such as differences in availability of preferred food may have influenced deer preference.

Literature Cited

- Arnold, G. W., and J. L. Hill.
1972. Chemical factors affecting selection of food plants by ruminants. *In* Phytochemical ecology. Proc. Phytochem. Soc. Symp. No. 8, p. 71-101. Academic Press Co., New York.
- Brown, E. R.
1961. The black-tailed deer of western Washington. Wash. Dep. Game, Biol. Bull. 13, 124 p. Olympia, Wash.
- Crouch, G. L.
1968. Forage availability in relation to browsing of Douglas-fir seedlings by black-tailed deer. *J. Wildl. Manage.* 32:542-553.
- Donnelly, E. D., and W. B. Anthony.
1973. Relationship of *Sericea lespedeza* leaf and stem tannic to forage quality. *Agron. J.* 65:993-994.
- Einarsen, A. S.
1946. Crude protein determinations of deer food as an applied management technique. *Trans. N. Am. Wildl. Conf.* 11:309-312.
- Hardison, W. A., J. T. Reid, C. M. Martin, and P. G. Woolfalk.
1954. Degree of herbage selection by grazing cattle. *J. Dairy Sci.* 37:89-102.
- Hassid, W. Z.
1937. Determination of sugars in plants by oxidation with ferri-cyanide and ceric sulfate titration. *Ind. Eng. Chem. (Anal. Ed.)* 9:228-229.
- Heady, H. F.
1964. Palatability of herbage and animal preference. *J. Range Manage.* 17:76-82.
- Horwitz, W., ed.
1970. Official methods of analysis. Assoc. Off. Anal. Chem., Ed. 11, 1,015 p. Washington, D.C.
- Johnson, C. M., and A. Ulrich.
1950. Determination of nitrate in plant material. *Anal. Chem.* 22:1526-1529.
- Levin, D. A.
1971. Plant phenolics: an ecological perspective. *The Am. Nat.* 105:157-181.
- Low, G. N., C. W. P. Steenkamp, and E. L. Steenkamp.
1967. Relationship between the ether extract content of Karoo shrubs and their palatability to sheep. *South Afr. J. Agric. Sci.* 10:867-873.
- Maynard, L. A.
1951. Animal nutrition. 474 p. McGraw-Hill Inc., New York.
- Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka.
1966. Rapid determination of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* 38:514-515.
- Radwan, M. A.
1974. Natural resistance of plants

- to animals. *In* Wildl. and For. Manage. in the Pac. Northwest Symp. Proc., p. 85-94. Oreg. State Univ. Press, Corvallis.
- Radwan, M. A., and G. L. Crouch.
1974. Plant characteristics and deer feeding preference. *J. Wildl. Manage.* 38:32-41.
- Smith, D., G. M. Paulson, and C. A. Raguse.
1964. Extraction of total available carbohydrates from grass and legume tissue. *Plant Physiol.* 39:960-962.
- Swain, T., and W. E. Hillis.
1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Soc. Food Agric.* 10:63-68.
- Van Soest, P. J.
1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Chem.* 46:829-832.
- Van Soest, P. J.
1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. *J. Anim. Sci.* 24:834-843.
- Wilkins, H. L., R. P. Bates, P. R. Henson, I. L. Lindahl, and R. E. Davis.
1953. Tannin and palatability in *Sericea lespedeza* L. Cuneata. *Agron. J.* 45:335-336.

The seal of the U.S. Forest Service is a circular emblem. It features a central shield with a stylized tree and a mountain peak. Above the shield, the words "FOREST SERVICE" are written in an arc. Below the shield, the words "DEPARTMENT OF AGRICULTURE" are written in an arc. The outer ring of the seal contains the words "WATER COOPERATION" at the top and "FOREST RESOURCES" at the bottom.

The FOREST SERVICE of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives — as directed by Congress — to provide increasingly greater service to a growing Nation.

The U.S. Department of Agriculture is an Equal Opportunity Employer. Applicants for all Department programs will be given equal consideration without regard to age, race, color, sex, religion, or national origin.

The mission of the PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION is to provide the knowledge, technology, and alternatives for present and future protection, management, and use of forest, range, and related environments.

Within this overall mission, the Station conducts and stimulates research to facilitate and to accelerate progress toward the following goals:

1. Providing safe and efficient technology for inventory, protection, and use of resources.
2. Developing and evaluating alternative methods and levels of resource management.
3. Achieving optimum sustained resource productivity consistent with maintaining a high quality forest environment.

The area of research encompasses Oregon, Washington, Alaska, and, in some cases, California, Hawaii, the Western States, and the Nation. Results of the research are made available promptly. Project headquarters are at:

Anchorage, Alaska
Fairbanks, Alaska
Juneau, Alaska
Bend, Oregon
Corvallis, Oregon

La Grande, Oregon
Portland, Oregon
Olympia, Washington
Seattle, Washington
Wenatchee, Washington

*Mailing address: Pacific Northwest Forest and Range
Experiment Station
P.O. Box 3141
Portland, Oregon 97208*