The influence of crude terpenoid constituents on mule deer preference for big sagebrush and black sagebrush

by Timothy Lee Personius

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Range Science
Montana State University

Abstract:
Samples of current year's growth of leaves and stems were collected in February, 1983, for four Artemisia taxa (A. tridentata tridentata, A. t. wvomingensis. A. t. vasevana, and A. nova) from a mule deer winter range near Gardiner, Montana. Samples represented both lightly and heavily used plants within each taxon. Epidermal crude terpenoid concentrations were measured by rapid chloroform extraction of fresh whole tissue. This extract was then separated into volatile and non-volatile components by steam distillation. Highly volatile compounds in the tissue, normally lost during solvent extraction or steam distillation, were quantified by gas-liquid chromatography of headspace vapors. Major constituents in each fraction were identified and quantified for comparison with preference ranks between taxa and between form classes within each taxon. The importance of considering complete plant chemistry when investigating preference is stressed.

Highly volatile compounds (methacrolein+ethanol), monoterpene irritants (rho-cymene and alpha-phellandrene), and non-volatile sesquiterpene lactones appear to influence mule deer selection among the sagebrush species studied. Vasevana. the most preferred taxon, was characterized by low levels of methacrolein+ethanol and irritants, and moderate amounts of sesquiterpene lactones, but without any major individual lactones. Novaf the least preferred taxon also had low levels of methacrolein+ethanol, but had the highest amounts of rho-cymene, alpha-phellandrene, and sesquiterpene lactones. Wvomingensis and tridentata were intermediate in preference, wvomingensis being slightly more preferred than tridentata. This could be explained by the low lactone content of wvomingensis (compared to all taxa), and its lower level of methacrolein+ethanol (compared to tridentata). Both taxa had significantly greater quantities of methacrolein+ethanol than vasevana. and significantly less quantities of rho-cymene, alpha-phellandrene, and lactones than nova. The role of several other compounds in determining preference is discussed.

Form classes were less clearly determined by chemical criteria. Nova form classes were chemically indistinguishable. Wvomingensis and tridentata form classes could be chemically separated most of the time, but the role of the identifying compounds in determining preference is questionable. Vasevana form classes appeared to be strongly influenced by the presence or absence of acetone, borneol, and rho-cymene. Plants in the heavy-use form class had the lowest levels of these three compounds.

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ABSTRACT

Samples of current year's growth of leaves and stems were collected in February, 1983, for four Artemisia taxa (A. tridentata tridentata, A. t. wyomingensis, A. t. vaseyana, and A. nova) from a mule deer winter range near Gardiner, Montana. Samples represented both lightly and heavily used plants within each taxon. Epidermal crude terpenoid concentrations were measured by rapid chloroform extraction of fresh whole tissue. This extract was then separated into volatile and non-volatile components by steam distillation. Highly volatile compounds in the tissue, normally lost during solvent extraction or steam distillation, were quantified by gas-liquid chromatography of headspace vapors. Major constituents in each fraction were identified and quantified for comparison with preference ranks between taxa and between form classes within each taxon. The importance of considering complete plant chemistry when investigating preference is stressed.

Highly volatile compounds (methacrolein+ethanol), monoterpene irritants (rho-cymene and alpha-phellandrene), and non-volatile sesquiterpene lactones appear to influence mule deer selection among the sagebrush species studied. Vaseyana, the most preferred taxon, was characterized by low levels of methacrolein+ethanol and irritants, and moderate amounts of sesquiterpene lactones, but without any major individual lactones. Nova, the least preferred taxon also had low levels of methacrolein+ethanol, but had the highest amounts of rho-cymene, alpha-phellandrene, and sesquiterpene lactones. Wyomingensis and tridentata were intermediate in preference, wyomingensis being slightly more preferred than tridentata. This could be explained by the low lactone content of wyomingensis (compared to all taxa), and its lower level of methacrolein+ethanol (compared to tridentata). Both taxa had significantly greater quantities of methacrolein+ethanol than vaseyana, and significantly less quantities of rho-cymene, alpha-phellandrene, and lactones than nova. The role of several other compounds in determining preference is discussed.

Form classes were less clearly determined by chemical criteria. Nova form classes were chemically indistinguishable. Wyomingensis and tridentata form classes could be chemically separated most of the time, but the role of the identifying compounds in determining preference is questionable. Vaseyana form classes appeared to be strongly influenced by the presence or absence of acetone, borneol, and rho-cymene. Plants in the heavy-use form class had the lowest levels of these three compounds.

INTRODUCTION

Knowledge of the motivation behind ungulate forage selection is vital to predicting and manipulating range forage/herbivore interactions. This study examines the relationship between observed mule deer (Odocoileus hemionus hemionus) preference for four sagebrush (Artemisia L. spp.) taxa and certain constituents of sagebrush chemistry; based on plant samples, and shrub utilization data collected from a mule deer winter range near Gardiner, Montana. The four taxa are black sagebrush (A. nova Nels.),basin big sagebrush (A. tridentata Nutt. tridentata), Wyoming big sagebrush (A. t. wyomingensis Beetle and Young), and mountain big sagebrush (A. t. vaseyana (Rydb.) Beetle).

Analysis centers on a broad group of chemicals loosely called crude terpenoids. Crude terpenoids can be divided into two basic fractions: essential oils, including monoterpenes and volatile nonterpenoids; and nonvolatile compounds, principally the sesquiterpene lactones and cuticular waxes (Kelsey et al. 1982).

Crude terpenoids contain a variety of individual constituents (31 were isolated in this study), many of which posess animal feeding deterent properties. These chemicals are largely located in epidermal sac-like glands called trichomes, increasing their potential sensibility to mule deer by both taste and smell (Kelsey et al. 1983).

The purpose of this study was to explain observed differential mule deer preference between and within four sagebrush taxa on the basis of the plants crude terpenoid chemistry. If plant chemistry is

a factor in establishing preference, then more preferred species or individuals should have a significantly different chemical makeup (in kind or quantity) than less preferred species or individuals.

The objectives of the study were to identify and quantify the major constituents of epidermal crude terpenoids in four sagebrush taxa and to compare the results of that analysis to observed mule deer preference for those taxa.

LITERATURE REVIEW

The relative palatability of western sagebrush species to mule deer appears to be low (Smith and Hubbard 1954, Anderson et al. 1972), but widely variable and significant utilization of sagebrush does occur, especially on seasonally occupied mule deer winter ranges (Welch et al. 1981). On the Gardiner winter range in southwestern Montana, big sagebrush and black sagebrush can incur heavy seasonal use (Wambolt 1983). Heavy use in winter may be due to the evergreen nature of the shrub and low alternate forage availability.

While this study is not concerned with those factors that affect the relative preference between sagebrush species and other forages, it is still important to consider how those factors (grazing stimuli) contribute to or control the animal's forage selection process. The process is one of perceiving stimuli and responding to them.

Skiles (1984) stated that animals can show one of three mutually exclusive responses to any grazing stimuli: 1) Positive, meaning that the animal prefers that stimulus; 2) Negative, meaning that the animal will avoid that stimulus; and 3) Neutral, the stimulus has no effect on the animal's preference for a forage possessing that stimulus. A positive or negative stimuli may still not affect forage preference if the stimuli is equally present in all forage choices. This can further limit an animal's perceptual ability to discriminate among forage choices and may force them to consider other criteria.

The Role of the Senses

While our understanding of what motivates preferential selection

of forage by ungulates is limited, it is generally believed that the special senses of touch, sight, taste, and smell, alone, or in combination, are used to differentiate acceptable from less acceptable or unacceptable plants. With regard only to forage selection by mule deer between the three subspecies of big sagebrush and black sagebrush, it would seem that only taste and smell are ultimately important (Longhurst et al. 1968).

Krueger et al. (1974) reported that touch and sight selection by ungulates is related to such specific plant conditions as succulence, coarseness, and growth form. While mule deer may select sagebrush over other forages in winter on the basis of succulence, all sagebrush samples tested in this study showed similiar water contents--equal succulence--and similiar gross morphology.

Cook and Stoddart (1960) showed that repeated herbage removal could induce a hedged appearance in big sagebrush. McNeal (1984) found that subjectively assigned form classes in big sagebrush reflected actual grazing use. Kelsey (1985) observed no visible morphological change in mountain big sagebrush following two years of clipping 50% of annual growth. If sagebrush plants can be trained into recognizable form classes, it is likely that several years of continuous browsing are required to visibly alter plant morphology. Form classes then can be viewed as indicators of historic preference for individual plants.

Since individual sagebrush plants that receive heavy, habitual grazing use may be trained into recognizably different forms, it can be argued that mule deer may use sight to select historically grazed

(and presumably more preferred) plants. However, ocular selection of plants is not considered important on the Gardiner study area for three reasons:

1) Young sagebrush plants must be initially selected by some other means (taste/smell) to ever be trained into a recognizable heavy-use form class.

2) Plants, even if initially selected by sight, are ultimately accepted or rejected on a taste and/or smell basis (Longhurst et al. 1968, Schwartz et al. 1980).

3) Personal field observations revealed widespread use on plants in an obvious, growthy, light-use form class. While that may reflect a situation where heavy grazing pressure forces use on non-sight-preferred plants, those same plants and sight-preferred plants (if such categories exist) are still ultimately accepted or rejected after the animal smells or tastes the plant.

If, as proposed, touch and sight are not important to mule deer in selecting among sagebrush plants, then smell and/or taste must be.

Selection Motivation Based on Taste and Smell

If mule deer select sagebrush by taste and smell, what are they selecting for or against?

It has long been argued that wild ungulates possess some sort of 'nutritional wisdom' that allows them to select the most nutritious forage available (Swift 1948). The supposed correlation between nutritional value and forage selection is not apparent in mule deer/sagebrush interactions.

Nagy et al. (1964) and Oh et al. (1967) found the volatile oil content of big sagebrush to be inversely correlated to its digestibility. Yet Kelsey et al. (1983) found the highest volatile oil content in the most preferred taxon. Welch and Pederson (1981) also reported that the most digestible sagebrush accessions were among the least preferred, while Welch and McArthur (1979) found the same inverse relationship between crude protein and preference rank. Sheehy (1975) found no correlation between digestibility and preference among sagebrush and further reported no significant relationship between preference rank and crude protein, fiber, or lignin content. There is no evidence to date that suggests nutritional wisdom is playing a role in mule deer selection among sagebrush plants.

On the other hand, a possible explanation for the low average preference for sagebrush is the high concentration of secondary metabolic compounds they typically contain. Crude terpenoids constitute the major fraction of secondary compounds; up to 24.8% of dry matter (Kelsey et al. 1982) or more. Also present are lesser amounts of coumarins (Shafizadeh and Melnikoff 1970), flavonoids (Rodriquez et al. 1972), and some highly volatile non-terpenoids such as acetone and methacrolein (Scholl et al. 1977). The properties associated with the individual compounds that make up the crude terpenoids, and the sensitive olfactory and gustatory detection abilities of mule deer, strongly suggest an other than neutral response by mule deer to crude terpenoid stimuli (Longhurst et al. 1968). If crude terpenoids also occur in sensibly different quantities between individuals or species, then certain components of

the crude terpenoids may also determine preference between individual sagebrush plants as well.

Goatcher and Church (1970) studied domestic ruminants and concluded that of the four primary taste sensations (sweet, sour, salty, bitter), bitterness was the most stimulatory taste and was the most important in determining forage preferences. Crude terpenoids contain a number of bitter compounds, particularly the sesquiterpene lactones (Rodriquez et al. 1976). In addition, a number of compounds are listed as skin and eye irritants, and/or are toxic if ingested in sufficient quantities (Tatken and Lewis, 1983). Many of the compounds have also been shown to act as insect feeding deterrents and repellants, phytotoxins, antimicrobial agents, and plant growth inhibitors (Kelsey et al. 1984). And most are located in epidermal sac-like glands that are easily ruptured during mastication; releasing potent quantities of secondary chemicals in the mouth (Kelsey et al. 1983).

Chapman and Blaney (1979) provide a comprehensive overview of how animals detect and perceive secondary metabolites, which include the crude terpenoid group. They noted that the response stimulus of a given chemical or compound can vary between animals and depends on the amount of the chemical in a forage. They further defined the concentration of a compound that is required to produce a sensible response (taste sensation, allergic reaction, irritating reaction) in an animal as a threshold level--a parameter that depends on the sensibility of the animal, the potency of the compound, and the mix of associated compounds that collectively comprise a forage sample.

Apart from realizing that olfaction and gustation are the principal methods ungulates use to detect chemicals in forages, we still do not know the real mechanisms of detection; what specific characteristics make a compound bitter versus sweet, or attractive versus repellant (Harborne, 1982). This makes prediction of untested chemical stimuli uncertain.

Animal Preference and Secondary Compounds

Since sagebrush is so strongly 'flavored' by the presence of secondary chemicals, it is reasonable to assume that these chemicals are important causal factors influencing mule deer selection among sagebrush (Freeland and Janzen 1974). However, recent studies attempting to equate differential preference for sagebrush to certain fractions of the plants secondary metabolites--largely terpene and florescent phenolic compounds--have failed to achieve a concensus on which compounds or groups of compounds are playing a role in determining preference.

Most of the investigations have centered on the monoterpene fraction of the crude terpenoids. Narjisse (1981) found that goats discriminated against the taste of monoterpenes in pelleted feeds while sheep selected against monoterpenes by smell only. Schwartz et al. (1980) discovered that tame mule deer in cafeteria feeding trials preferred forages with lower amounts of volatile oils. Scholl et al. (1977) found virtually no correlation between mule deer preference and monoterpenoid content, but did find an inverse relationship between preference and certain highly volatile non-terpenoids. Welch et al.

(1983) also found no significant relationship between monoterpenoid content and mule deer preference among five sagebrush taxa, while Sheehy (1975) reported that the relative concentration of eight monoterpenes could account for 90% of the variation of mule deer use between seven sagebrush taxa.

Other animal and plant interactions have been investigated as well, and with equally variable results. Radwan et al. (1982) found that some individual components of ponderosa pine (Pinus ponderosa) stem and root oils were strongly related to pocket gopher (Thomomys spp.) preference among individuals of that plant. Farentinos et al. (1981) came to a similar conclusion investigating tassel-eared squirrels (Sciurus aberti) feeding preference for ponderosa pine as did White et al. (1982) studying pygmy rabbits (Brachylagus idahoensis) and big sagebrush. None of the three studies found total monoterpenoid content to be significantly related to preference, but did find at least one individual compound of that fraction that was strongly correlated to preference.

Reviewing the literature revealed two major shortcomings of previous work investigating animal preference and plant chemistry. Both center on analysis of the plant's chemistry.

The first problem encountered is the piecemeal focus of individual studies. Most investigations examine only one part (sometimes a minor part) of a plants secondary compounds, such as the monoterpenes. This ignores the fact that other compounds may be involved and must be considered together to infer a complete picture of animal/chemical interaction.

The second problem deals with the method of analyzing the chemical data. While any of several statistical procedures can be used, depending on the experimental design, only individual compounds should be analyzed. Several studies have only analyzed chemically related groups such as total monoterpenes, or total oxygenated compounds, or they have combined individual plant samples into composite samples, ignoring inter-plant chemical and preferential variation. To lump individuals into a group for analysis is to proceed from a false assumption that all samples are chemically or preferentially homogenous, or that the individual chemicals making up the group have the same properties (irritating reactions or taste sensations) and the same threshold levels for those properties (potencies). The latter is the logical equivalent to saying that two apples plus one pineapple (3 fruits) are the same as three oranges (3 fruits). Equivalent measures of gross chemical content (percent monoterpenes or crude terpenoids) among forages do not suggest equivalent stimulatory response in animals consuming that forage unless and only if the gross fractions are identical in measure at the individual compound level (Freeland and Janzen 1974).

SITE DESCRIPTION

Sagebrush samples were collected from a mule deer winter range near Gardiner, Montana, located on U.S. Forest Service administered rangeland, immediately adjacent to Yellowstone National Park.

The area is dominated by big sagebrush and black sagebrush, and sample collection was restricted to plants from so-called "cafeterias"; areas where several sagebrush taxa occur in close proximity.

Cafeterias are characteristically a mosaic of distinct microsites controlled by slope angles, soil properties, and exposure, that in turn result in a plant community most noticeably dominated by a combination of sagebrush taxa not ordinarily found in such close proximity.

Cafeterias studied fall into one of several habitat types described by Mueggler and Stewart (1980) and refined by NcNeal (1984). By either classification, big sagebrush (at least one subspecies) and/or black sagebrush are dominants or co-dominants, with bluebunch wheatgrass (Agropyron spicatum) and/or Idaho fescue (Festuca idahoensis) subdominant.

Elevations range from the Yellowstone River bottom at 1615m (5300 feet) to the rolling benches at the base of the Absoroka Mountains at 1950m (6400 feet). Precipitation along the elevational gradient varies from 30.5cm (12 inches) along the river, to about 40cm (16 inches) in the adjacent foothills (Farnes 1975). About half of this moisture falls as snow.

Winter forage for mule deer is limited to shrub and grass species. Heavy snow cover may confine deer to a shrub-only diet for parts of the winter. McNeal (1984) estimates that 400 or more mule deer seasonally occupy the winter range around Gardiner.

Cafeteria areas are traversed in winter by elk (Cervus elaphus nelsoni) as well as mule deer. However, data from Greer et al. (1970), and personal observations, suggest that elk make relatively light use of sagebrush in winter, at least in the areas sampled in 1983. The cafeterias were located close to travelled roads and elk were hunted in the area until February. Mule deer in contrast were not hunted after November and were observed in late January to be quite tame, grazing at mid-day on the cafeterias and seemingly oblivious to passing traffic. Most of the observed sagebrush utilization on the cafeterias sampled in 1983 can be attributed to the wintering mule deer population. Mule deer were observed consistently on the cafeteria areas, actively browsing sagebrush throughout the winter and into spring (April).

EXPERIMENTAL

Sample Collection

Plant samples were collected in early February from cafeteria areas exhibiting a gradient of light to heavy use measured between individual plants. Collections were timed to coincide with the most intense browsing period, when it is believed that the influence of secondary compounds on preference is established (Scholl et al. 1977).

Ten plants were subjectively selected to represent either a more preferred, or less preferred individual of each of the four taxa, using form classes as the criteria of selection. Five plants representing the light-use form class (open, growthy, relatively unbranched crowns), and five plants representing the heavy-use form class (dense, intricately branched crowns, club-like appearance) made up the ten individuals sampled for each taxon.

Individual plant utilization was not estimated since mule deer were actively grazing plants at the time of collection and were observed to continue heavy use into April. Form classes were used to estimate preference within a taxa and a two year utilization study by Wambolt (1983) of permanently tagged sagebrush plants on the same cafeterias was used to estimate preference between the four taxa. This allowed for within, as well as between taxa comparison of chemical makeup to relative preference.

A sample consisted of 15 grams of current years leaves and stems--closely approximating the actual tissue consumed by mule deer. Plant samples were sealed in individual, air tight plastic bags,

double bagged, placed on dry ice, and transported to a freezer for storage at -20°C until chemically analyzed.

Chemical analysis

Crude terpenoid isolation:

Epidermal crude terpenoids were removed with a rapid chloroform ($CHCl_3$) extraction (Kelsey et al. 1982). Seven to eight grams of frozen leaf and stem tissue were brought to room temperature in a sealed plastic bag, placed in a beaker with 24 ml of chloroform per gram of tissue, and stirred gently for 5 minutes. The extract was then filtered through sharkskin (Schleicher and Schuell) filter paper into a preweighed round bottom flask for concentration. Extracted tissue, including hairs that collected in the filter paper, was dried at 100°C overnight, desiccated, and reweighed to determine sample dry weight.

The round-bottom flask containing the extract was placed on a roto-evaporator under vacuum and the chloroform was evaporated with a 30°C water bath (extract achieved an oil like consistency). Water bath temperature was then increased to 60°C and roto-evaporation continued for one hour to drive off the residual chloroform. The flask was then dried thoroughly, desiccated for 30 minutes, and reweighed to determine crude terpenoid extract weight.

Steam distillation was used to seperate the extract into volatile (essential oils) and non-volatile (sesquiterpene lactone) fractions. The dried extract was redissolved in chloroform and steam was bubbled through the solution for 10 minutes. Volatile compounds were captured

in a cold water (10°C) condenser and collected in a round-bottom flask with chloroform and water. Non-volatile components remained in the distillation flask with condensed water. Resulting volatile and non-volatile fractions were concentrated as above for the crude terpenoid fraction, dried, and weighed.

Essential oils analysis:

The dried essential oil fraction from the crude terpenoid extract was separated into individual compounds by gas-liquid chromatography (GLC). A 0.1 ul sample of extract was injected onto a 4m column of 10% Carbowax (TPA) on Gas Chrom Q, 80/100 - 100/120 mesh, in a Varian Aerograph 1800 gas chromatograph with a flame ionization detector and nitrogen carrier gas. Column temperature was programmed for 50-200°C, with a 10°/min rise and final temperature hold. Chromatograms were printed on a Hewlett Packard 3380A integrator recorder. Integrator conversion factors for quantifying individual oil components were determined by injecting known quantities of methacrolein and camphor as reference compounds. Concentrations were then standardized by adjusting for initial sample dry weights and are expressed as percent of sample dry weight.

Compounds were identified by comparison of retention times to known references, and by GLC/mass spectrometry. Approximately 0.05-0.1 ul of essential oil (neat) was injected onto the GLC column (4m, 10% Carbowax on 80/100 CWHP, He carrier gas) with a programmed temperature of 50-200°C at 10°/min. Mass spectra of the oil components were identified by comparison with known reference samples

and published spectra (Stenhagen et al. 1974, Epstein et al. 1976). Ten of the twenty compounds isolated could not be identified, but together constituted a very minor fraction of the total essential oils.

Sesquiterpene lactone analysis:

Analysis of the non-volatile fraction of the crude terpenoid extract centered on isolation, quatification, and identification of sesquiterpene lactones; one of the major constituents in that fraction. Other constituents present but not studied included cuticular waxes, some flavonoids, and possibly other unknown compounds.

Dried non-volatile extracts from each plant sample were dissolved in chloroform or a mixture of choroform and ethanol, and diluted to a volume of 25.0 ml with the same solvent. A measured volume (between 2 and 6 ul) was injected onto a 1.83m column of 3% OV-17 on Gas Chrom Q, 100-120 mesh, in a Varian Aerograph 1800 GLC. Column temperature was set isothermally at 205°C for the entire analysis. Chromatograms were printed on a Hewlett Packard 3380A integrator recorder. A 2.0 ul standard of the sesquiterpene lactone matricarin was also run to determine a response factor for use in quantifying sesquiterpene lactones that were to be identified later using thin-layer chromatography (TLC), infrared spectroscopy (IR), and GLC.

For TLC analysis, microliter quantities of diluted extracts were spotted on 0.5 mm silica gel G TLC plates and developed in $CHCl_3$:pet ether:EtOH (5:4:1). Spots were visualized by spraying with

concentrated sulfuric acid and heating at 100-110°C. The plates were heated 10-60 minutes, photographed, returned to the oven for complete charring, and then photographed again (Kelsey et al. 1976).

To isolate and identify specific compounds as sesquiterpene lactones, measured aliquots were taken from each extract and combined by taxa. Compounds were separated and purified by preparative TLC using 1.0 mm thick silica gel G plates. Compound locations were visualized with iodine vapor; the silica gel was then scraped from the plate, washed with solvent ($CHCl_3$, $CHCl_3$:EtOH, and EtOH), and filtered. The solvent was evaporated from the combined washes and isolated compounds were compared with reference samples using TLC, GLC, and IR spectroscopy.

For IR quantification of the sesquiterpene lactone concentration, a Nicolet model MX-1 Fourier Transform Infrared Spectrometer was used. The basin big sagebrush extracts were analyzed in a Beckman liquid cell with a 0.025 cm path length between NaCl plates. The other extracts were held in a Research and Industrial Instruments Co. liquid cell with a 0.05 cm path length between NaCl plates. Quantification of the total amount of sesquiterpene lactone in a sample was accomplished by comparing the integrated area of the lactone moiety absorbance band with that of a reference compound at known concentrations. Deacetylmatricarin and cumambrin B were used as references for basin big sagebrush and black sagebrush respectively. Deacetoxymatricarin was the reference used for mountain big sagebrush. The extracts of Wyoming big sagebrush gave no measurable lactone peak; there were either no sesquiterpene lactones present, or only very low

concentrations, below the detection limits of this technique.

Headspace technique:

Rigorous distillation, evaporation, and drying procedures used in crude terpenoid isolation result in a loss of highly volatile compounds from the essential oil fraction. To compensate for this, headspace vapors were analyzed using a modification of the procedure reported by Scholl et al. (1977).

Approximately 1 gram of frozen whole tissue was sealed in a 160 ml vial fitted with an air-tight rubber septum. The sample was then adjusted to room temperature and placed in an oven at 60°C for 15 minutes. An air-tight, locking syringe was used to extract 1.0 ml of vapor from the vial. The syringe was locked (sealed), the vapor compressed to 0.1 ml, and injected onto a column in a GLC as described for essential oil fractionation. The tissue sample was then removed from the vial, dried overnight at 100°C, desiccated, and weighed.

Integrator counts for individual compounds were converted to micrograms after injecting known quantities of camphor and methacrolein as references. All concentrations were standardized to compensate for differences in tissue dry weights. Compounds were identified as described for the essential oil fraction.

Incidental Analysis

Extracted sample dry tissue from the crude terpenoid analysis was also used to determine sample moisture content and leaf:stem ratio for each taxon (Table 9, Appendix A). In addition, percent transmittance at 340n of a five minute water extract of fresh whole tissue was

determined for each sample (Table 9, Appendix A).

Statistical Analysis

The data set consisted of 40 plant samples--grouped into four taxa of 10 samples each, and 2 form classes of 5 samples each within each taxon--with a quantified list of individual compounds for each sample.

To illuminate interactive chemical components--those compounds which, when considered together and on a sample by sample basis, could be used to separate taxa or form class groups on a chemical basis--a stepwise multiple discriminant analysis was used. Only individual compounds were entered in the analysis to let the discriminant function chose the group of compounds best able to distinguish or separate the taxa or form class groups.

For comparison of individual compound means between taxa, a one-way analysis of variance (AOV) using Tukey's Honestly Significant Difference (HSD) Multiple Range Test was computed. A comparison of group means between the two form classes within a taxon was made using a simple t-test.

All statistical analysis was generated by SPSS (release 9.1) software (Nie et al. 1975), adapted for use on a Honeywell CP-6 computer.

RESULTS AND DISCUSSION

Chemical Constituents

Thirty-one compounds were isolated and quantified from the crude terpenoid extract; seventeen compounds were identified (Tables 1 and 2). No individual compounds were quantified from the sesquiterpene lactone fraction, but several were identified and total lactone concentration was determined (Table 3). Black sagebrush had the highest average lactone concentration, consisting of two major components, cumambrin A and B, and several other unidentified compounds. Wyoming big sagebrush had the lowest lactone concentration and no individuals were identified. Basin big sagebrush and mountain big sagebrush both contained significant quantities of lactones but differed in the chemical complexity of their respective fractions. Basin big sagebrush lactones were dominated by three compounds: matricarin, deacetylmatricarin, and deacetoxymatricarin. Mountain big sagebrush lactones, comprising many minor compounds, had no dominant or major constituents. No individual lactones of mountain big sagebrush could be identified, but TLC analysis indicated that these plants were high elevation chemotypes (Kelsey et al. 1973), and should have produced artevasin and dehydroleucodin (Kelsey and Shafizadeh 1979).

In the headspace fraction, methacrolein and ethanol could not be consistently separated, and were considered as one compound. However, in the samples that were separable, methacrolein constituted some 80-90% of the total of the two compounds.

Table 1. Mean concentrations[1] by taxa[2] of *Artemisia* headspace constituents.

Chemical	ATV	ATW	ATT	AN
methyl butene	6.64^{b3}	8.21^{b}	6.14^{b}	1.44^{a}
acetone	0.84^{a}	5.67^{b}	5.67^{b}	0.00^{a}
UHN-1*	0.00^{a}	0.00^{a}	0.00^{a}	0.36^{b}
methacrolein+ethanol	0.45^{a}	15.96^{b}	30.06^{c}	0.43^{a}
UHV-1*	0.38^{a}	0.00^{a}	0.00^{a}	0.00^{a}
UHV-2*	0.15^{b}	0.00^{a}	0.00^{a}	0.00^{a}
santolina triene	0.00^{a}	3.63^{bc}	1.89^{b}	7.45^{c}
alpha-pinene	4.07^{b}	0.00^{a}	0.00^{a}	3.12^{b}
camphene	5.92^{b}	2.25^{a}	1.90^{a}	2.09^{a}
beta-pinene	1.15^{b}	0.14^{a}	0.17^{a}	0.22^{a}
artemiseole	0.16^{a}	1.96^{b}	1.32^{b}	0.00^{a}
1,8, cineole	5.33^{c}	0.70^{b}	0.79^{b}	0.00^{a}
rho-cymene	0.24^{a}	0.13^{a}	0.13^{a}	0.70^{b}
artemisia ketone	0.32^{a}	0.00^{a}	0.00^{a}	0.00^{a}
thujone	0.84^{a}	0.00^{a}	0.00^{a}	0.00^{a}
camphor	1.49^{b}	0.54^{ab}	0.25^{a}	0.15^{a}
alpha-phellandrene	0.00^{a}	0.00^{a}	0.00^{a}	0.20^{b}

[1] ug/gram of dry tissue.
[2] ATV: *A. t. vaseyana*, ATW: *A. t. wyomingensis*, ATT: *A. t. tridentata*, AN: *A. nova*.
[3] Individual compound means sharing a subscript, were not significantly different ($p<.05$) using Tukey's HSD multiple range test.
* Unidentified compounds

Table 2. Mean concentration[1] by taxa[2] of *Artemisia* essential oils.

Chemical	ATV	ATW	ATT	AN
artemiseole	0.02[a3]	0.53[b]	0.41[b]	0.00[a]
1,8, cineole	1.10[b]	0.30[a]	0.32[a]	0.04[a]
rho-cymene	0.06[a]	0.04[a]	0.06[a]	0.24[b]
santolina epoxide	0.00[a]	0.18[b]	0.43[c]	0.00[a]
methyl santolinate	0.00[a]	0.84[b]	1.07[b]	0.00[a]
methacrolein	0.00[a]	0.12[b]	0.24[c]	0.00[a]
thujone	0.73[a]	0.00[a]	0.00[a]	0.00[a]
UETW-1*	0.00[a]	0.22[b]	0.34[c]	0.00[a]
UEV-1*	0.14[b]	0.00[a]	0.00[a]	0.00[a]
camphor	1.82[ab]	2.11[b]	1.70[ab]	0.96[a]
borneol	0.23[b]	0.00[a]	0.00[a]	0.19[b]
UETW-2*	0.00[a]	0.56[b]	0.37[b]	0.00[a]
UETW-3*	0.00[a]	0.40[b]	0.31[b]	0.00[a]
UETW-4*	0.00[a]	0.40[b]	0.46[b]	0.00[a]
UEV-2*	0.32[b]	0.00[a]	0.00[a]	0.00[a]
UEV-3*	0.18[b]	0.00[a]	0.00[a]	0.00[a]
UEV-4*	0.50[b]	0.00[a]	0.00[a]	0.00[a]
UEV-5*	0.17[a]	0.00[a]	0.00[a]	0.00[a]
UEV-6*	0.06[a]	0.00[a]	0.00[a]	0.00[a]
UETW-5*	0.00[a]	0.22[b]	0.31[b]	0.00[a]

[1] % extracted-tissue dry weight.
[2] ATV: *A. t. vaseyana*, ATW: *A. t. wyomingensis*, ATT: *A. t. tridentata*, AN: *A. nova*.
[3] Individual compound means sharing a subscript were not significantly different ($p<.05$) using Tukey's HSD multiple range test.
* Unidentified compounds.

Table 3. Mean concentration[1] by taxa[2] of the major fractions of *Artemisia* secondary compounds.

Fraction	ATV	ATW	ATT	AN
crude terpenoids	19.58^{a3}	22.36^{ab}	24.59^{b}	21.94^{ab}
essential oils	5.71^{b}	6.81^{bc}	7.61^{c}	1.82^{a}
headspace	28.84^{ab}	39.94^{bc}	49.25^{c}	16.44^{a}
sesquiterpene lactones	2.58^{bc}	0.10^{a}	2.37^{b}	3.05^{c}

[1] Headspace: ug/gram dry tissue. All others are % extracted-tissue dry weight.

[2] ATV: *A. t. vaseyana*, ATW: *A. t. wyomingensis*, ATT: *A. t. tridentata*, AN: *A. nova*.

[3] Individual fraction means sharing a subscript, were not significantly different ($p<.05$) using Tukey's HSD multiple range test.

Some compounds were identified in both the essential oil and headspace fractions. In general, the means of nearly every compound differed significantly ($p< .05$) between at least two taxa.

In contrast, the means of individual compounds rarely differed ($p< .1$) between the two form classes of the same taxon. Table 4 lists only those compounds found to be significantly different between the two form classes of at least one taxa. Black sagebrush form classes were chemically inseparable; no compound differed significantly between the light and heavy use form classes. Wyoming and basin big sagebrush had three and two significantly differing compounds between their respective form classes. Mountain big sagebrush had ten.

Table 4. Compounds differing significantly ($p < .1$) between the two form classes of a taxon.

Taxon[1]	Compound	Source[2]	Quantity[3] Light-use	Quantity[3] Heavy-use	p-value
ATV					
	acetone	HD	1.67	0.00	.01
	UHV-2*	HD	0.31	0.00	.01
	camphene	HD	2.62	9.22	.04
	camphor	HD	0.40	2.58	.05
	UEV-1*	ES	0.27	0.01	.05
	camphor	ES	0.84	2.79	.02
	UEV-2*	ES	0.43	0.20	.08
	UEV-4*	ES	0.98	0.01	.04
	UEV-5*	ES	0.33	0.00	.06
	UEV-6*	ES	0.12	0.00	.07
ATW					
	camphene	HD	3.20	1.30	.07
	beta-pinene	HD	0.20	0.09	.05
	UETW-2*	ES	0.35	0.77	.09
ATT					
	acetone	HD	4.57	6.76	.07
	camphene	HD	1.39	2.41	.06
AN					
	-- none significant --				

[1] ATV: *A. t. vaseyana*, ATW: *A. t. wyomingensis*, ATT: *A. t. tridentata*, AN: *A. nova*.
[2] HD: headspace fraction, ES: essential oils.
[3] HD: ug/gram dry tissue, ES: % extracted-tissue dry weight.
* Unidentified compounds.

Utilization and Preference

Table 5 lists mule deer preference ranks for big sagebrush and black sagebrush, based on the 1982-1983 winter utilization of 1134 permanently tagged leaders of 124 plants within a cafeteria on the Gardiner study site.

Table 5. Preference ranks of four Artemisia taxa.

Species	% Use[1]	Preference Rank
A.t. vaseyana	52	1
A.t. wyomingensis	24	2
A.t. tridentata	19	3
A. nova	8	4

[1] Percent of leaders browsed; from Wambolt 1983, unpublished data.

Mountain big sagebrush was by far the most preferred taxon, Wyoming and basin big sagebrush were intermediate, and black sagebrush was the least preferred. Preliminary data of 1983-1984 winter utilization, indicate that use of all taxa increased, but preference rank remained unchanged.

Discriminant Analysis

Table 5 indicates that all four sagebrush taxa had at least some plants that were utilized. It is therefore more useful to classify sagebrush taxa as more preferred or less preferred.

Discriminant analysis seems to most closely approximate the conscious or unconscious process of the mule deer in grouping forage choices into preferred or less preferred groups. Discriminant analysis selects the subset of chemical variables (stimuli) that best categorize or most accurately assign plants into their respective taxa (less preferred or more preferred groups). The stepwise procedure identifies the most important (significant) variables first and the least important last (Nie et al. 1975).

This analysis is somewhat artificial in that the groups are taxanomic units and not strictly preference determined. This is a result of not having individual plant utilizations for each sample collected. As stated earlier, it was not believed tenable to assume that early February utilization data was an accurate indicator of true preference for individuals sampled.

Among the subset of variables (compounds) chosen by the discriminant function to best separate the samples into their respective groups, both positive and negative correlations to preference were observed for different variables. While those compounds negatively correlated to preference (highest amounts in the least preferred taxa) are suspected of being the most important in actually determining preference, it is possible that mule deer may be selecting for, instead of against, certain compounds.

Chapman and Blaney (1979) suggest that a sort of mimicry can occur between plants, based on an association (by the animal) of a toxic or irritating principle with a recognizable chemical characteristic of the plant that is not itself harmful or irritating.

Thus, animals may key in on these so-called chemical markers as indicators of low concentrations of irritating or unpleasant compounds, even though the response stimulus of the marker is neutral.

Goatcher and Church (1970) described another situation where positive correlation of a chemical (higher quantities in the most preferred taxa) to preference might occur. In general, they showed that some compounds can have an ameliorating or deactivating effect on other, associated chemicals. The presence of such a compound in sagebrush could, for instance, reduce the potency or effect of irritating compounds.

Both the situations where positive correlation to preference may occur are speculative, and not likely involved in mule deer preference for sagebrush, given the general nature of the compounds involved. They are possible occurrences however, and for that reason all compounds were entered into the analysis, regardless of apparent correlation.

Table 6 lists the compounds in order identified by the discriminant function as being the group of compounds best able to predict group membership of individual samples. All samples were correctly placed into their respective groups (taxa) using these chemical criteria. Mean separations (Tables 1, 2, and 3) show that all compounds listed in Table 6 differed significantly between at least two taxa. Significance values for individual compounds change at each step of the stepwise procedure, and may increase or decrease, depending on the nature of the chemical interaction.

Table 6. Compounds selected by stepwise discriminant analysis for four *Artemisia* taxa[3]. P (to enter)=.01.

Compound	Source[1]	Quantity[2] ATV(1)	ATW(2)	ATT(3)	AN(4)
1. UETW-4#	ES	0.00	0.38	0.46	0.00
2. Lactones	--	2.58	<0.10	2.37	3.05
3. 1,8,cineole	ES	1.10	0.30	0.32	0.04
4. Methacrolein	HD	0.45	15.96	30.06	0.43
5. Methyl butene	HD	6.64	8.21	6.14	1.44
6. Rho-cymene	ES	0.06	0.04	0.06	0.24
7. Santolina triene	HD	0.00	3.63	1.88	7.45

1 ES: essential oil fraction, HD: headspace fraction.
2 ES and lactones: % extracted-tissue dry weight, HD: ug/g dry tissue.
3 ATV: *A.t. vaseyana*, ATW: ssp. *wyomingensis*, ATT: ssp. *tridentata*, AN: *A. nova*; numbers in () are preference ranks.
Unknown compound.

All samples were correctly placed into their respective groups (taxa) using these chemical criteria.

Table 7 lists the compounds that best assign the samples into their respective form classes within a taxon. Because of the reduced sample size (5 per form class), the significance level of the test was also lowered.

Chemical differences between the two form classes of a taxon were less pronounced than the differences between taxa. All 10 samples were assigned the correct form class for mountain big sagebrush, using 5 compounds. Eight of 10, and 9 of 10 samples were assigned the

correct form class for Wyoming and basin big sagebrush respectively, using only one compound each. Black sagebrush samples could not be assigned to form classes using chemical criteria.

Table 7. Compounds selected by stepwise discriminant analysis of *Artemisia* form classes. P (to enter)= .10.

Taxon[1]	Compound	Source[2]	Quantity[3] Light-use	Quantity[3] Heavy-use	% ID[4]
ATV					100
	1. Acetone	HD	1.67	0.00	
	2. Borneol	ES	0.21	0.25	
	3. Rho-cymene	ES	0.08	0.05	
	4. UEV-3*	ES	0.25	0.12	
	5. UEV-2*	ES	0.43	0.20	
ATW					80
	1. Beta-pinene	HD	0.20	0.09	
ATT					90
	1. Camphene	HD	1.38	2.41	
AN					0
	--none significant--				

[1] ATV: *A.t. vaseyana*, ATW: *A.t. wyomingensis*, ATT: *A.t. tridentata*, AN: *A. nova*.
[2] ES: essential oil fraction, HD: headspace fraction.
[3] ES: % extracted-tissue dry weight, HD: ug/g dry tissue.
[4] % of samples within a taxa assigned to their respective form class.
* Unknown compounds.

The stepwise function uses statistical significance to rank the compounds, but actual importance in determining (and not just indicating) preference is also a function of compound characteristics

and potencies.

Chemical Properties

Physiological effects of crude terpenoid constituents ingested by mule deer, are largely unknown. Nagy et al. (1964) suggested that monoterpenes may inhibit mule deer rumen microbes and lower digestibility, while Welch and Pederson (1981) reviewed the literature and refuted that claim. Cluff et al. (1982) found that an unexplained loss of monoterpenoids, from 1.64% dry weight in the forage to 0.3% dry weight in the rumen ingesta of mule deer, may explain the discrepencies between in vitro and in vivo digestibilities.

Schwartz et al. (1980) offered confined mule deer a choice of 3 pelleted feeds treated with identical quantities of either monoterpenes, oxygenated monoterpenes, or sesquiterpenes. While all treated feeds were less preferred than the untreated control, pellets containing the oxygenated compounds were the least preferred of the three treated feeds, suggesting that oxygenated compounds are more potent feeding deterents than other monoterpenes or sesquiterpenes.

Sesquiterpene lactones are bitter tasting and were found to negatively influence white-tailed deer (Odocoileus virginianus) preference for three Veronia species. White-tailed deer chose the two species containing no sesquiterpene lactones, avoiding the species with a measurable lactone content and avoided the preferred species when lactones were applied artificially (Burnett et al. 1977). The effect is probably dependant on the individual compounds involved, and certainly depends on the amount of those compounds present. For

instance, Mitchell and Dupuis (1971) tested the matricarins and cumambrins identified in basin big sagebrush and black sagebrush respectively. They found the cumambrins to be the more reactive compounds, causing contact dermititus in some patients, while the matricarins did not.

Most of the compounds identified in the crude terpenoid fractions have been tested for some types of biological activity. Test animals were generally mice, rats, rabbits, or humans. A summary of the results of such tests is presented in Table 8. Application of these tests to mule deer can only be inferred, but the results are useful indicators of potential animal responses to specific chemical stimuli.

Table 8. Biological activity in certain mammals, of compounds isolated from sagebrush crude terpenoids.[1]

Irritants[2]	Toxins[3]	Untested
acetone*	camphor*	santolina epoxide*
methacrolein*	1,8, cineole*	santolina triene
ethanol*	borneol*	methyl santolinate*
alpha-phellandrene	thujone*	artemisia ketone*
rho-cymene	camphene	artemiseole*
alpha-pinene	methyl butene	
beta-pinene		

1 From Tatken and Lewis 1983.
2 Skin (causing contact dermititus) or mucous tissue irritants.
3 Also causing reproductive problems, tumors, or mutations.
* Oxygenated compounds.

Every compound tested was either toxic or irritating, or both, when applied or ingested in sufficient quantities. However, the threshold level for mule deer (the concentration at which a compound

becomes irritating or toxic, or a negative or positive feeding stimulus) cannot be implied with certainty. Feeding trials (beyond the scope of this study) are the only way to establish such thresholds. In their feeding trials, Schwartz et al. (1980) found that mule deer were able to differentiate between pelleted feed containing 0.7% volatile oil, and untreated (oil-free) pellets. Only gross fractions were used, no individual compounds were identified, and no finer detection levels were tested.

Little can be inferred about the activity of the unknown compounds isolated in this study. All are volatile to varying degrees, most are likely terpenes or hydrocarbons, and some are probably toxic or irritating. There is, however, no way to discern which specific compounds possess any of these or other properties.

Chemical Determinants of Preference

Compounds identified by discriminant analysis and listed in Tables 6 and 7 are indicators of relative preference between and within the four sagebrush taxa studied. In order for the compounds to actually influence or determine preference, they must meet additional criteria. Preference determining compounds must effect a positive or negative feeding stimulus, occur in sensible quantities and in sensibly different quantities between forage choices, and they must correlate in some way to the observed preferences for different forages.

Discriminant analysis does not produce correlation coefficients, and because individual values for the dependant variable (percent

utilization) were not obtained, regression analysis would produce unrealistic correlation coefficients. However, the discriminant function surpasses the need for correlation coefficients by considering the importance of a compound on a sample by sample basis, and in relation to other compounds.

For instance, in the ATV form class analysis presented in table 7, the second compound entered, borneol, does not differ significantly between the two form classes. At step 0 in the stepwise analysis, the significance of the F statistic for borneol was .80. But at step 1, after acetone was entered into the analysis, the F value for borneol dropped to .004, indicating that borneol was important in discriminating against some, but not all samples. Examining the raw data illuminates that point. Four of five samples in the light-use form class of mountain big sagebrush contain acetone, an irritant, while none of the heavy-use plants do. Borneol occurs in low concentrations in all the heavy-use plants, but in only two samples of the light-use group. Significantly, borneol occurs in the one light-use sample that contains no acetone, and at a concentration twice that of any other sample.

Borneol is a toxic, oxygenated monoterpene, and occurs in the heavy-use plants of the most preferred taxon. If it is an actual feeding deterent, as its chemistry suggests, then it must not be present in the heavy use plants at concentrations above its threshold level, but does occur in one light-use sample at a sensible level. This example suggests that correlation to preference needs to be considered on a sample by sample basis to account for the complexity

of sagebrush chemistry. Discriminant analysis appears to be the most appropriate technique for dealing with the chemical variability encountered.

The ATV example also illustrates the need for establishing compound threshold levels. Unfortunately, no experimental evidence directly applicable to this study exists. Threshold levels can only be inferred from chemical characteristics (irritants or oxygenated compounds are probably more potent than simple toxins or non-oxygenated compounds), statistical significance of chemical differences, and the magnitude of those differences (as above, where the borneol concentration of one sample was twice that of any other).

Expected feeding response (positive or negative stimulus) must likewise be inferred from known chemical characteristics, but also depends on chemical potency. It is however, reasonable to assume that if a chemical with suspected repellant characteristics is found to be positively correlated to preference, then it is not present in sensible quantities, and the correlation is coincidental.

Between taxa:

Of the seven compounds identified in table 6 as distinquishing between the four sagebrush taxa, some are more probable preference determinants than others.

Methacrolein+ethanol is the largest single constituent of the headspace fraction, and correlates negatively and consistently to preference for the three big sagebrush taxa (Table 1). Both constituents are known irritants. Black sagebrush, the least

preferred taxon, has the lowest amounts of methacrolein+ethanol, but does have significantly (4 times) greater quantities of rho-cymene (another irritant) than any other taxon. Black sagebrush also has the largest amount of sesquiterpene lactones (suspected feeding deterents), but mountain big sagebrush also has a high concentration (Table 3) and is much more preferred. It is interesting to note however, that both black sagebrush and basin big sagebrush lactones were dominated by a few major constituents (page 20), while mountain big sagebrush lactones consisted of many, but no major constituents. Also, the lactones of all three taxa contain significantly different individuals, and the cumambrins of black sagebrush appear to be the most reactive (page 31). Wyoming big sagebrush was virtually lactone free, perhaps contributing to its increased preference over basin big sagebrush and black sagebrush.

The role of the other compounds in determining preference is less clear (Table 6). 1,8, cineole, an oxygenated monoterpene, is positively correlated to preference and a major constituent of mountain big sagebrush essential oils (Table 2). Methyl butene, an extemely volatile compound also exhibits a slight positive correlation to preference (Table 1). Neither compound exhibits a consistant relationship to preference, but both appear to be important taxonomic markers.

Santolina triene is somewhat negatively correlated to preference, particularly between black and mountain big sagebrush (Table 1). However, little is known about this compound and inferences about its charcteristics must be confined.

The last compound (but appearing first in the stepwise procedure) is the unknown UETW-4 (Table 2). It is negatively correlated to preference among the three big sagebrush taxa, but is also absent from black sagebrush. No inference about its possible characteristics or feeding stimulus can be made.

Discriminant analysis maximizes the difference between groups by finding the combination of variables whose values are as close as possible within groups, and as far apart as possible between groups (Lebart et al. 1984). When discriminating between more than two groups, this kind of analysis may overlook or ignore some compounds, if their significance among groups was limited to only a few cases. For instance, alpha-phellandrene (Table 1), an irritant, only occurs in black sagebrush (the least preferred taxon), and may be influencing preference for that species, but it was not identified as such by the discriminant function. Alpha-phellandrene, being negatively correlated to preference and an irritant, appears to be the only obvious omission from the discriminant list.

Between form classes:

No compound or group of compounds differed significantly between the two form classes of black sagebrush (Table 4).

One compound, camphene, correctly assigned 9 of 10 basin big sagebrush samples into their correct form classes. The differences between form classes however, was not great, and the heavy-use samples had the higher concentrations. Camphene is not a strong suspect in explaining form classes in basin big sagebrush, unless it elicits a

positive feeding response.

Wyoming big sagebrush samples were correctly assigned the proper form class in 8 of 10 cases (Table 7), using beta-pinene, an irritant (Table 8). Light-use plants contained about twice as much beta-pinene as heavy-use plants. However, considering that 2 cases were misclassified and considering that the most preferred taxa, mountain big sagebrush, has roughly 7 times the concentration of beta-pinene that Wyoming big sagebrush has, it is unclear whether beta-pinene is actually playing a role in determining preference in Wyoming big sagebrush.

Five compounds correctly assigned all mountain big sagebrush samples into their proper form classes. Two of the compounds, acetone and rho-cymene are irritants and were consistently found in higher quantities in the light-use plants, although the difference for rho-cymene was not statistically significant. Borneol, as described earlier, seemed to have an important negative influence on preference in one or two samples only. The two unknown compounds were both negatively correlated to preference but quantitative differences were not great. Their role in mountain big sagebrush preference is not clear.

Conclusion

Of the seven compounds identified as preference indicators, three (methacrolein+ethanol, rho-cymene, and sesquiterpene lactones) may be preference determinants as well.

The most preferred taxon, mountain big sagebrush, was

characterized by low levels of methacrolein+ethanol and rho-cymene, and moderately high levels of lactones, but without any major individual lactones.

Black sagebrush, the least preferred taxon, also contained low concentrations of methacrolein+ethanol, but had the highest amounts of rho-cymene and lactones, including several major constituents (cumambrin A and B) in the latter.

Wyoming and basin big sagebrush were intermediate in preference, Wyoming being slightly more preferred than basin. This could be explained by the low lactone content of Wyoming big sagebrush (compared to all taxa) and its lower level of methacrolein+ethanol (compared to basin big sagebrush). Both taxa had significantly (>30 times) greater quantities of methacrolein+ethanol than mountain big sagebrush, and significantly less quantities of lactones and rho-cymene than black sagebrush.

Strong inference could not be made about the preference determining role of the other four compounds identified as preference indicators between taxa (UETW-4, 1,8, cineole, methyl butene, santolina triene), but their importance is not dismissed, only questioned. Alpha-phellandrene may also contribute to the low preference rank of black sagebrush.

Form classes were less clearly defined by chemical criteria. Black sagebrush form classes are chemically indistinguishable using the compounds examined in this study. Wyoming and basin big sagebrush form classes could be chemically identified most of the time, but the role of the identifying compounds in determining preference is

questionable. Mountain big sagebrush form classes appear to be strongly influenced by the presence or absence of three compounds (acetone, borneol, rho-cymene).

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APPENDIX

Table 9. Incidental data for *Artemisia tridentata* and *A. nova*.

	ATV[4]	ATW	ATT	AN
leaf:stem ratio[1]	3.91^{b}	1.85^{a}	2.12^{a}	2.38^{a}
% H_2O[2]	39.51^{a}	42.6^{a}	46.3^{b}	40.0^{a}
% transmittance[3]	3.59^{a}	56.06^{d}	42.37^{c}	18.40^{b}

1 Extracted-tissue dry weight (leaf ÷ stem).
2 Weight of H_2O ÷ fresh whole tissue weight (x 100).
3 Five minute water extract of fresh whole tissue at λ=340n.
4 ATV: *A. t. vaseyana*, ATW: *A. t. wyomingensis*, ATT: *A. t. tridentata*, AN: *A. nova*.
5 Individual means in a row, sharing a subscript, were not significantly different ($p<.05$) using Tukey's HSD multiple range test.