



The influence of selected *Artemisia* compounds on mule deer preference
by Robert Owens Bray

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

Montana State University

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Abstract:

Previous studies on a mule deer (*Odocoileus hemionus hemionus* Rafinesque) winter range near Gardiner, Montana found differences in mule deer preference for 4 taxa of sagebrush growing in close proximity to each other. Mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vasevana* (Rydb.) Beetle) was the most preferred taxon, followed by Wyoming big sagebrush (*A. t.* ssp. *wyomingensis* Beetle and Young), which was slightly preferred to basin big sagebrush (*A. t.* ssp. *tridentata*). Black sagebrush (*A. nova* Nels.) was the least preferred taxon. Differences in preference were attributed to differences in plant chemistry.

The objective of this study was to test, by means of a feeding trial, the effect on preference of compounds previously identified by discriminate analysis to be probable preference determinants. Compounds tested included; p-cymene, 1,8-cineole, methacrolein (tested at 2 concentrations), and the nonvolatile crude terpenoid fraction (NVCTF) from each of the 4 sagebrush taxa. The compounds were tested at concentrations closely approximating those found in nature. The compounds were applied to chopped alfalfa hay and tested against an untreated control in a 2-choice preference test. Eight deer were used as test animals.

All compounds tested, including 1,8-cineole a possible attractant, significantly deterred preference. Compound influence on preference, in order of increasing deterrence, was as follows; 50% methacrolein, *vasevana* NVCTF, methacrolein, *tridentata* NVCTF, p-cymene, *wyomingensis* NVCTF, *nova* NVCTF, and 1,8-cineole. The results indicate that 1,8-cineole is not an attractant, and the sesquiterpene lactones, major constituents in the NVCTFs, may be important preference determinants. The results also support the suggestion that methacrolein may be an important preference determinant among big sagebrush subspecies, and p-cymene between black sagebrush and big sagebrush.

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A thesis submitted in partial fulfillment
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Master of Science

in

Range Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

March 1990

N378
B738

APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Carl Wambolt, my major professor, for his knowledge, support, and patience through all phases of this project. Likewise, I would like to thank the members of my graduate committee, Dr. Rick Kelsey and Dr. John (Jack) Taylor, for their suggestions and helpful comments during the completion of this thesis. I am especially thankful to Rick for sharing his expertise of sagebrush chemistry and extraction procedures.

I would also like to thank the faculty and students, especially Dr. Jack Nelson, Bruce Davitt, and Tom Hodgman, of the Department of Forestry and Range Management at Washington State University. Without the use of their facilities, deer, time, and knowledge this project would not have been possible. Thank you Stan, for your regular visits and help with the day to day care of the deer.

Lastly, but no less important, I would like to thank my family, friends, and fellow students for their help, encouragement and friendship, which made this long ordeal enjoyable.

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ABSTRACT

Previous studies on a mule deer (Odocoileus hemionus hemionus Rafinesque) winter range near Gardiner, Montana found differences in mule deer preference for 4 taxa of sagebrush growing in close proximity to each other. Mountain big sagebrush (Artemisia tridentata Nutt. ssp. vaseyana (Rydb.) Beetle) was the most preferred taxon, followed by Wyoming big sagebrush (A. t. ssp. wyomingensis Beetle and Young), which was slightly preferred to basin big sagebrush (A. t. ssp. tridentata). Black sagebrush (A. nova Nels.) was the least preferred taxon. Differences in preference were attributed to differences in plant chemistry.

The objective of this study was to test, by means of a feeding trial, the effect on preference of compounds previously identified by discriminate analysis to be probable preference determinants. Compounds tested included; p-cymene, 1,8-cineole, methacrolein (tested at 2 concentrations), and the nonvolatile crude terpenoid fraction (NVCTF) from each of the 4 sagebrush taxa. The compounds were tested at concentrations closely approximating those found in nature. The compounds were applied to chopped alfalfa hay and tested against an untreated control in a 2-choice preference test. Eight deer were used as test animals.

All compounds tested, including 1,8-cineole a possible attractant, significantly deterred preference. Compound influence on preference, in order of increasing deterrence, was as follows; 50% methacrolein, vaseyana NVCTF, methacrolein, tridentata NVCTF, p-cymene, wyomingensis NVCTF, nova NVCTF, and 1,8-cineole. The results indicate that 1,8-cineole is not an attractant, and the sesquiterpene lactones, major constituents in the NVCTFs, may be important preference determinants. The results also support the suggestion that methacrolein may be an important preference determinant among big sagebrush subspecies, and p-cymene between black sagebrush and big sagebrush.

INTRODUCTION

This study is one of a series examining the forage relationships between Rocky Mountain mule deer (Odocoileus hemionus hemionus Rafinesque) and sagebrush (Artemisia L.) on an important big game winter range near Gardiner, Montana. Sagebrush taxa examined included basin big sagebrush (Artemisia tridentata Nutt. ssp. tridentata), Wyoming big sagebrush (A. t. ssp. wyomingensis Beetle and Young), mountain big sagebrush (A. t. ssp. vaseyana (Rydb.) Beetle), and black sagebrush (A. nova Nels.). The study site is unique in that all 4 taxa grow in close proximity to each other forming natural "cafeterias". This provides an ideal situation to measure the relative forage qualities of the 4 taxa. Previous studies identified the study site (McNeal 1984), and considered environmental factors influencing the selection of foraging sites (Wambolt and McNeal 1987). Additional studies measured the relative preference of mule deer for the 4 sagebrush taxa (Personius et al. 1987, Wambolt unpublished data). Striby et al. (1987) measured the digestibilities of the 4 taxa and the effect of crude terpenoid content on digestibility. Personius et al. (1987) identified some of the crude terpenoids as potential preference determinants.

Numerous studies (Scholl et al. 1977, Sheehy and Winward 1981, Welch et al. 1981, Welch et al. 1983, Behan and Welch 1985, Welch and McArthur 1986, Personius et al. 1987, Wambolt unpublished data) have

measured the relative palatabilities of various sagebrush taxa. The results of these studies are variable, indicating that palatability cannot be accurately predicted on the basis of species or even subspecies. In order to predict if a given population of sagebrush will be preferred by mule deer a better understanding of what influences preference is needed.

Presumably plant chemistry plays a major role in shaping sagebrush palatability. Which compounds or groups of compounds are important in determining mule deer preference for sagebrush is poorly understood. Many studies (Behan and Welch 1985, Schwartz et al. 1980b, Sheehy 1975, Narjisse 1981, Welch et al. 1983, Personius et al. 1987) have been made which attempt to correlate plant chemistry to palatability. The results have not been consistent. Many of the studies considered "essential oil" or monoterpene contents. However, essential oils are just one of several broad groups of compounds occurring in sagebrush. It may be one of these other groups of compounds, in addition to, or instead of the essential oils that determines palatability, or it may be one, or a few specific compounds within these broad groups of compounds.

With wildlife becoming an increasingly important consideration in resource management decisions it becomes critical to have a thorough understanding of wildlife-forage relationships, and be able to identify and predict which browse sources will be desirable as forage. This is especially important for the sagebrush species which are very widespread and often major components of big game ranges in the western states. If palatable populations of sagebrush could readily be identified it would be useful information to have when making

management decisions such as where sagebrush should be controlled or protected.

With a better understanding of which compounds, and what concentrations of these compounds, determine an animal's preference for a potential forage species, plant breeders can select for or against palatable varieties. More palatable varieties of sagebrush would be desirable for revegetation of big game ranges following large scale disturbances such as fire and mining, or improving ranges that currently have little desirable browse. Selection of varieties with low palatabilities is desirable for ornamental and timber species which may be damaged by browsing. This is not of particular importance with sagebrush, but it certainly is with many conifer species which contain some of the same compounds believed to influence the palatability of sagebrush.

Personius et al. (1987) took forage samples from the 4 sagebrush taxa present on the Gardiner winter range. From these forage samples they isolated 31 chemical constituents and with a stepwise discriminate analysis related compound concentrations with mule deer preference for the 4 taxa. This procedure statistically identified probable preference determinants. However, it did not separate compounds with a real biological effect on deer, from compounds with no significant role in determining deer preference, but were merely correlated positively, or negatively, with the actual preference determinants. The objective of this study was to determine, by means of feeding trials, the effect on mule deer preference of certain compounds found in sagebrush which were previously identified through statistical analysis to be probable

preference determinants (Personius et al. 1987). Thus, the hypothesis tested was: compounds found in sagebrush taxa do not influence mule deer preference.

LITERATURE REVIEW

Sagebrush (Artemisia L.) is a dominant or co-dominant over vast acreages of the western United States. Of the western vegetation types the sagebrush type occurs over the greatest range of longitude and altitude (Branson, 1985). In the western states the various sagebrush taxa occupy 109,369,225 ha (422,275 mi²) (Beetle 1960). Of this big sagebrush (A. tridentata Nutt.) is by far the most dominant taxon, occurring on 58,630,866 ha (226,374 mi²), and black sagebrush (A. nova Nels.) is the third most extensive in distribution occurring on 11,214,959 ha (43,301 mi²) (Beetle 1960). Sagebrush is also a very important taxon in Montana occurring on 14,040,649 ha (54,211 mi²), of this 7,796,159 ha (30,101 mi²) is big sagebrush and 259,000 ha (1000 mi²) is black sagebrush (Beetle 1960).

With such large geographical, and diverse climatic ranges it is reasonable to expect that genetically distinct populations (ecotypes) would evolve within a species in response to local environmental conditions. This appears to be the case. There are 3 widely recognized subspecies of big sagebrush, basin big sagebrush (A. t. Nutt. tridentata), Wyoming big sagebrush (A. t. wyomingensis Beetle and Young), and mountain big sagebrush (A. t. vaseyana (Rydb.) Beetle). Within these subspecies are numerous forms, accessions and even other subspecies that have been described by various authors.

Six subspecies, forms and varieties of big sagebrush were recognized in Montana by Morris et al. (1976). Included were Wyoming big sagebrush, basin big sagebrush, high elevation, low elevation and hot springs varieties of mountain big sagebrush as well as the sub alpine form of mountain big sagebrush (A. t. ssp. vaseyana f. spiciformis (Osterhout) Beetle). They were separated by geographical distribution, site characteristics, morphology and chemical properties.

Compared to big sagebrush taxa not as much taxonomic work has been done with black sagebrush in regards to formally separating it into different subspecies. However, at least 2 different forms have been recognized. Stevens and McArthur (1974) separated 2 forms based on the color of foliage-water extracts when viewed under longwave ultraviolet light. This is an indirect measure of the foliage's chemical constituency. Beetle (1960) and Brunner (1972) separated the two forms simply on the basis of differences in leaf color.

Sagebrush Utilization

With such an extensive range, and being such a dominant species over much of this range, it is not surprising that a wide variety of wildlife species, as well as domestic animals, have come to utilize or even depend upon sagebrush for forage and/or cover. Most of the research involving utilization of sagebrush by animals has centered around species which are important to man economically, or for recreational purposes, and relatively conspicuous in the landscape. As these species and especially mule deer, are the most pertinent to this study they will be emphasized in this discussion. However, it must be remembered that many other less economically important, and less

conspicuous species undoubtedly depend upon sagebrush in one way or another.

Due to the habitat preference of sagebrush taxa for relatively dry sites with low to moderate snow accumulations its range frequently coincides with mule deer winter ranges. In addition, due to its erect growth habit it is available when many low stature species are covered with snow.

Tueller (1979) looked at the diets of mule deer throughout the year on several mule deer ranges located in Nevada. In most analyses he lumped the sagebrush species together, however it was primarily big sagebrush. On the winter ranges he found sagebrush to occur in 93.3% to 100% of the mule deer rumens and it made up 11.7% to 68.6% of the vegetative composition of the rumens. Even during summer when sagebrush utilization is lowest it was found in 90.8% of the mule deer rumens on one range and made up 9.4% of the vegetative composition of the rumens on another range. Other studies in Montana (Eustace 1971), British Columbia (Willms et al. 1979), Colorado (Hansen et al. 1977) and California (Leach 1956) show big sagebrush to be an important mule deer forage for at least part of the year, particularly the winter months.

Big sagebrush can also be a very important forage for pronghorn antelope (Antilocapra americana Ord) and tends to be so during a greater portion of the year than it does for mule deer. Olsen and Hansen (1977) found sagebrush, primarily big sagebrush, to make up 78% of pronghorn diets in Wyoming on a year round basis. Ninety five percent of their winter diets and 42% of their summer diets were sagebrush. Pronghorn feeding site analysis in Montana during January

showed big sagebrush to make up 76% and silver sagebrush (Artemisia cana Pursh) 19% of the diet, for a total of 95% sagebrush diet (Bayless 1969).

Black sagebrush has also been shown to be an important forage for pronghorns. Smith and Shandruk (1979) used 4 different methods to determine antelope diets in Utah. Depending on the method used, the diet ranged from 17.4% to 27.5% black sagebrush. Averages of the 4 methods showed antelope diets to be 22.4% black sagebrush.

Work conducted in Utah by Cook and Harris (1968) during the winter showed both big and black sagebrush to occur in domestic sheep diets at 5% and 17%, respectively. Green et al. (1951) also found wintering sheep utilized black sagebrush. In another Utah study Narjisse (1981) found the June diets of sheep to contain up to 57% big sagebrush in pastures considered to have low forage availability and 26% in pastures with high forage availability. Although not generally thought of as a cattle forage, cattle will utilize sagebrush (Johnson 1979).

Sagebrush Nutrient Content

Many authors have looked at the nutrient content of big and/or black sagebrush, alone, or compared to other species available on the same range (Cook et al. 1951, Smith 1957, Cook and Harris 1968, Eustace 1971, Tueller 1979, Welch and McArthur 1979, Kelsey et al. 1982, Remington and Braun 1985, Behan and Welch 1986). Differences in sites, years, season, time of collection, and other environmental conditions, as well as differences in analytical technique make it difficult to make valid comparisons between studies. In general though, sagebrush, and big sagebrush in particular, appear to have a very high nutritional

value relative to other forage species commonly found on winter ranges. In a review of numerous studies Welch (1983) calculated averages for the nutrient contents of big sagebrush and numerous other species commonly encountered on winter ranges. He found big sagebrush to rank second of 16 species in total digestible nutrients (TDN) at 61.3%, third of 13 in digestible protein at 6%, third of 9 in carotene content at 8.2 mg/lb, tied for first of 15 with 0.20% phosphorus, and relatively low in calcium at 0.65%. With the exception of curlleaf mahogany (Cercocarpus ledifolius Nutt. ex Torr. & Gray) no other species was consistently high in all nutrient categories listed. Black sagebrush with a TDN of 47.0, 4.5% digestible protein, 8.0 mg/lb of carotene, 0.17% phosphorus and 0.62% calcium did not have the consistently high nutrient contents that big sagebrush did, but was always near or above the middle of the range. A similar review by Behan and Welch (1986) showed big sagebrush to rank second in crude protein (CP) content out of 25 winter forage species listed, with an average of 11.4% CP and a range of 9.9-14.2% CP. Only green regrowth of crested wheatgrass (Agropyron desertorum (Fisch.) Schult.) was higher in crude protein. Black sagebrush was ranked fifth with 9% CP and ranged from 6.9 to 11.7% CP. Big sagebrush was ranked first of 25, and black sagebrush was third of 25 species reported in dry matter digestibility (in vitro) at 57.4% and 53.7%, respectively.

The nutritional needs of wild mule deer are not well understood. They are probably highly variable in response to the different physiological needs of deer differing in sex, age, stage of production, and local environmental conditions. After reviewing several studies

Dean (1975) concluded that wild ruminants the size of deer "require in excess of 15% CP for maximum growth and 12-15% for reproduction".

From data presented by Behan and Welch (1986) it can be seen that big sagebrush is one of only a few species that can attain these levels during winter. Even among the ranges of CP reported for big sagebrush the lower values do not attain the recommended levels of CP. Dean (1975) concluded that growing deer need 0.45-0.50% calcium and 0.25% phosphorus. Levels of calcium presented by Welch (1983) for big sagebrush (0.65%) and black sagebrush (0.62%) easily meet these requirements. The level of phosphorus is somewhat short at 0.20% for big sagebrush and 0.17% for black sagebrush, but still high relative to other species available. Ullrey (1975), using data from Ammann et al. (1973) to support his position, said that white-tailed deer need to have a diet of at least 50% dry matter digestibility, or gut fill would limit dry matter intake to below maintenance levels. It is very likely this applies to mule deer as well. Dry matter digestibilities of 57.4% for big sagebrush and 53.7% for black sagebrush (Behan and Welch 1986) are among the few species listed exceeding 50% dry matter digestibility.

Due to the lack of complete information on the nutrient requirements of mule deer Welch (1983) assumed that because of digestive tract similarity to domestic sheep, the 2 species would have similar requirements. Welch (1983) compared his composite nutrient values to the nutrient requirements of sheep (National Academy of Sciences 1975) and found that big sagebrush met, or exceeded, the requirements of sheep for total digestible nutrients, digestible

protein, calcium, phosphorus, and carotene. Black sagebrush did not meet the requirements for total digestible nutrients and just missed the minimum requirements of phosphorus, but was adequate in digestible protein, calcium and carotene.

The important point is not whether sagebrush can meet the recommended nutritional requirements of mule deer, but that sagebrush is an important forage species of high nutritional quality relative to the other species available on the winter range. Even if it does not meet the mule deer's recommended nutritional requirements it will minimize deficiencies. On his study site in southeast Montana, Eustace (1971) rated big sagebrush as the best winter browse species due to its "high protein and fat content, low crude fiber and a well balanced calcium-phosphorus ratio". This comment is probably pertinent to most mule deer winter ranges where big sagebrush is present.

Influence Of Secondary Compounds On Nutrition

Evidence supporting the commonly held theory that secondary compounds in sagebrush inhibit rumen microbial growth and hence digestibility, was obtained by Nagy et al. (1964), Nagy and Tengerdy (1967, 1968) and Oh et al. (1968). This was further supported by work with the secondary compounds in juniper (Juniperus L. spp.) (Schwartz et al. 1980a), Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Oh et al. 1967) and several other species (Oh et al. 1968).

Essential oils steam distilled from big and black sagebrush exhibited antibacterial properties to a wide range of aerobic bacteria (Nagy et al. 1964, Nagy and Tengerdy 1967). These essential oils also inhibited the growth of captive and wild mule deer rumen microbes in

culture (Nagy 1964, Nagy and Tengerdy 1968). At low concentrations the essential oils reduced the rate of cellulose digestion by mule deer rumen fluid and stopped it completely at higher concentrations. In addition, the oils reduced gas production and lowered volatile fatty acid concentrations in vitro with mule deer rumen fluid (Nagy 1964). On a daily basis, Nagy et al. (1964) placed 7 lbs. of big sagebrush through a fistula into a steer's rumen. Rumen moisture content, rumen motility and appetite decreased each day. After the third day, rumen contractions and intake of hay had stopped completely, and the feces became bloody.

Oh et al. (1967) and Schwartz et al. (1980a) fractionated the essential oils into 3 major components; monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes. They also identified and tested some of the oxygenated monoterpenes and monoterpene hydrocarbons separately. Schwartz et al. (1980a) found the oxygenated monoterpenoids in juniper inhibited mule deer starch, cellulose, and dry matter digestibility, in vitro, more than did the monoterpene hydrocarbons or sesquiterpenes. The monoterpene hydrocarbons were the least inhibitory. When the fractions were tested at low concentrations, inhibition of digestion by sesquiterpenes was similar to that of oxygenated monoterpenes. When tested at higher concentrations, inhibition of digestion by sesquiterpenes was similar to that of monoterpene hydrocarbons.

Oh et al. (1967) working with Douglas fir essential oils found that monoterpene hydrocarbons had no effect, or slightly increased mule deer rumen microbial activity as measured by gas production. Sesquiterpenes

increased microbial activity. Oxygenated monoterpenes, as a group, were strongly inhibitory to rumen microbial activity, although some of the individual oxygenated monoterpenes had little, or a slight positive effect on gas production. Oh et al. (1967, 1968) proposed that since some of the compounds inhibit rumen microbial activity, and others promote microbial activity, it is not the absolute concentrations of the individual fractions that determine the effects of essential oils on digestion, but the concentration ratios of the compounds.

Although experimental evidence clearly indicates essential oils can have an antibacterial action and reduce in vitro digestibility, the influence of these essential oils on digestibility of sagebrush in the natural system is not as clear. Critics point out that preparation for the in vitro technique leads to the loss of many volatile compounds, which would artificially increase digestibility. Welch and Pederson (1981) showed the monoterpenoid content of sagebrush samples to be reduced by 78% with freeze drying and 100% with oven drying at 100°C. Improved preparation techniques such as grinding fresh frozen tissue under liquid nitrogen to keep the essential oil concentration near natural levels can be used. Even with improved techniques in vitro digestibilities remain high (Welch and Pederson 1981, Pederson and Welch 1982, Striby et al. 1987).

Striby et al. (1987) looked at the digestibility of 3 subspecies of big sagebrush and black sagebrush, intact, and with the crude terpenoids removed by solvent extraction. This procedure extracts nonvolatile compounds such as sesquiterpene lactones and cuticular waxes, as well as the essential oils. They examined the in vitro

organic matter digestibility (IVOMD) of 4 intact and extracted taxa, from 3 collection dates, and with 3 sources of rumen inoculum, including mule deer, steer and sheep. Extraction of the crude terpenoids increased IVOMD by an average of 12.3%. Digestibility was high before the extraction procedure, ranging from 37.6% to 63.4% IVOMD, depending upon the taxon, collection date, and inoculum source. IVOMD was consistently lower for black sagebrush and higher for basin big sagebrush. Within each taxon digestibility tended to decrease with increasing crude terpenoid concentration. This was not the case among the taxa. Basin big sagebrush, which had the highest concentration of crude terpenoids was the most digestible. This lends support to the theory of Oh et al. (1967) which postulates it is not just the absolute concentrations of terpenoids that effect bacteria (digestion), but the concentration ratios of the various fractions. Other studies confirm there is no relationship between digestibility of sagebrush and its total essential oil content (Welch and Pederson 1981).

It is not fully understood why sagebrush essential oils do not reduce digestibility in nature to the degree their antimicrobial properties would suggest. Pederson and Welch (1982) believed Nagy et al. (1964) tested the antimicrobial and digestibility reducing properties of big sagebrush essential oils at higher concentrations than found in nature. In addition Pederson and Welch (1982) thought that placing the extracted essential oils directly into the culture exposes the microbes to the full concentration of essential oils. While in nature some of the essential oils are trapped in sagebrush tissues and unavailable to affect rumen microbes until released through further

processing of the tissue by rumination. Thus, the concentration of essential oils available to affect the microbes would be lower than the actual concentration. In addition, it would give the essential oils initially released time to dissipate from the rumen before the remaining essential oils were released. Pederson and Welch (1982) also thought the closed system Nagy et al. (1964) used would trap the monoterpenoids and keep them at an artificially high concentration when compared to a rumen, which allows them to escape. Cluff et al. (1982) found the concentration of monoterpenoids in rumen ingesta of mule deer was 80% less than expected from the forage ingested. They attributed this loss "to one or more of the following: mastication, rumination, eruction, absorption through the rumen wall into the cardiovascular system and excreted in the urine or metabolized in the liver." A similar study by White et al. (1982b) showed a 77% reduction in the monoterpenoid content of pygmy rabbit (Brachylagus idahoensis) stomach ingesta. Volatilization during mastication and ingestion accounted for some of this loss. While the ingestion process of mule deer may not be the same as that of pygmy rabbits, it can be speculated that sagebrush ingested by a mule deer would certainly lose some of its monoterpenoid content during the initial mastication and rumination of the sagebrush. Welch and Pederson (1981) found that the body temperature of mule deer was high enough to volatilize some monoterpenoids and drive them from the digestion solution during an in vitro digestion trial. They could then leave the rumen through eructation as proposed by Cluff et al. (1982).

Losses of essential oils due to volatilization reduces not only the absolute concentration of essential oils in the rumen, but also changes the concentration ratios, which are believed to be a factor influencing digestibility by Oh et al. (1967, 1968). The ratios would tend to become skewed toward the low volatility sesquiterpenes, and oxygenated monoterpenes, which Oh et al. (1967, 1968) found to stimulate and depress digestion, respectively.

Whether or not essential oils influence forage digestibility has generally been tested with artificial models, which may or may not approximate what is occurring in nature. The hypotheses that arise from these experiments are theoretical in nature and irrelevant to the deer. The point is that sagebrush is an important mule deer forage and can make up a large portion of their diet, especially during the winter. Their nutritional plane may or may not be optimal theoretically, but mule deer have been living and reproducing on sagebrush ranges for eons, so the nutritional plane is at least adequate.

Preference For Sagebrush

In recent years there have been numerous studies measuring the preference of mule deer for various sagebrush taxa, in particular big and black sagebrush (Scholl et al. 1977, Sheehy and Winward 1981, Welch et al. 1981, Welch et al. 1983, Behan and Welch 1985, Welch and McArthur 1986, Personius et al. 1987, Wambolt unpublished data). Additional studies have been conducted to determine the preference of sheep (Sheehy and Winward 1981, Welch et al. 1987), pygmy rabbits (White et al. 1982a), and sage grouse (Remington and Braun 1985) for different big sagebrush taxa.

From data presented by Scholl et al. (1977) it appeared that mountain big sagebrush was preferred to basin big sagebrush, which was preferred to Wyoming big sagebrush. Palatability of the 2 accessions of black sagebrush examined was very dissimilar with 1 accession being highly preferred with 61.0% utilization and 1 much less preferred with 28.1% utilization. Behan and Welch (1985) also showed preferences of free roaming mule deer for different accessions of black sagebrush to be highly variable, ranging from 0 to 82.7% utilization in a transplanted garden.

Sheehy and Winward (1981) looked at the preferences of captive mule deer for 7 sagebrush taxa in a transplanted garden. Based on a relative preference index of the percentage of a species utilized divided by the percent available they measured winter mule deer preferences for the 7 sagebrush taxa. Of the taxa common to this study they found mountain big sagebrush to be the most preferred taxon followed by Wyoming big sagebrush then basin big sagebrush and lastly black sagebrush. The difference in preference between Wyoming and basin big sagebrush was not significant, the others were significantly different ($P < 0.05$).

Welch et al. (1981) looked at the preferences of free roaming mule deer for different accessions of big sagebrush and black sagebrush. Mountain big sagebrush was clearly preferred to basin and Wyoming big sagebrush. Preference for the 1 accession of Wyoming big sagebrush tested was intermediate to the 4 accessions of basin big sagebrush tested. Preference for black sagebrush could not be compared directly to the big sagebrushes as its utilization was estimated ocularly rather than by measurements of pre and post browsing leader lengths as was

done for the big sagebrush accessions. However, it was clear that the deer showed a great deal of variation in their preferences for the black sagebrush accessions, with utilization ranging from 0 to 60%.

Welch et al. (1983) looked at several accessions of different sagebrush taxa and found that mountain big sagebrush was clearly preferred by mule deer to basin big sagebrush and black sagebrush. However, it is not clear whether black sagebrush or basin big sagebrush is most preferred. Wyoming big sagebrush was not included for comparison.

Welch and McArthur (1986) used 3 different transplanted gardens in Utah to measure preferences of mule deer for the 3 subspecies of big sagebrush. When all the accessions within a subspecies were lumped together and averaged across all 3 gardens and over 3 winters the current years growth utilized was 44.1% for mountain big sagebrush, 35.3% for Wyoming big sagebrush and 32.6% for basin big sagebrush. All means were significantly different ($P < 0.05$) (Welch and McArthur 1986). However, preference for different accessions within a subspecies varied widely. The more preferred accessions of a less preferred subspecies were sometimes more preferred than the less preferred accessions of a more preferred subspecies.

Data presented by Personius et al. (1987) from a natural sagebrush cafeteria located in close proximity to the sagebrush foliage collection sites used for this study showed the percentage of tagged leaders browsed by wild mule deer over the period of one winter to be 52% for mountain big sagebrush, 24% for Wyoming big sagebrush, 19% for basin big sagebrush, and 8% for black sagebrush. Utilization averaged over 4 winters on a similar cafeteria, also located near the foliage

collection sites for this study, showed the percentage of tagged leaders browsed by wild mule deer to be 63% for mountain big sagebrush, 47% for Wyoming big sagebrush, 40% for basin big sagebrush, and 12% for black sagebrush (Wambolt unpublished data).

From these papers it can be generalized that wintering mule deer preferences for the 3 big sagebrush subspecies and black sagebrush are as follows; most to least preferred, mountain big sagebrush > Wyoming big sagebrush \geq basin big sagebrush > black sagebrush. This generalization may be particularly inappropriate for black sagebrush which can be highly preferred, or greatly discriminated against, depending upon the accession. Selection takes place at the accession, or more correctly the individual plant and ultimately the bite level, and not at the species or subspecies level.

Sheep like mule deer show clear preferences for different species and accessions of sagebrush (Sheehy and Winward 1981, Welch et al. 1987). In similarly designed trials, in the same transplant gardens used for mule deer, sheep showed preferences similar to, but not identical to, those of mule deer (Sheehy and Winward 1981, Welch and McArthur 1986).

Remington and Braun (1985) showed that wild sage grouse preferred Wyoming big sagebrush to mountain big sagebrush. However, this preference seemed to be largely determined by feeding site selection and species availability rather than a true measure of preference.

White et al. (1982a) looked at the preference of captive pygmy rabbits for several accessions of mountain and basin big sagebrush and

concluded that there was no difference in preference for the 2 subspecies. However preference was shown at the accession level.

Diet Selection And The Senses

There are 2 widely held, though sometimes contradictory theories as to why animals select the diets they do from the array of forages available to them. The first is; within the constraints of availability they select forages that are most pleasurable, or least deleterious, to their senses. If the diets they select are higher in quality than what is available, it is coincidental. If an animal's senses direct the animal to a high quality diet it will increase the chance that a particular animal will contribute to the gene pool. Thus, over time, diet selection will become an evolved trait, but the individual animal will not know why it selects the diet it does, it is simply the most pleasurable to the senses.

The alternative is through "nutritional wisdom" an animal can detect a potential forages nutritional properties and select the diet that best meets its nutritional needs. In a variation of nutritional wisdom proposed by Freeland and Janzen (1974) plant tissues contain potentially toxic compounds, which are used as a defense mechanism against herbivory. Rather than trying to maximize nutrient content the herbivore selects a diet which minimizes the quantity of these compounds it must ingest. There is little evidence to support nutritional wisdom in ruminants, although it appears to occur with the monogastric rat (Rozin 1969).

Mule deer do not appear to have nutritional wisdom with regards to sagebrush. When offered a choice of several species, subspecies or

accessions they do not select the taxon with the highest nutritional value, nor the lowest content of supposedly toxic compounds (Welch and McArthur 1979, Striby et al. 1987).

Regardless of the ultimate factors driving forage selection it is apparent that the actual selection of forage is determined in response to the individual animals sensory perception of the potential forages. Arnold (1966) tested the influence of sight on forage selection using sheep fitted with "blinkers", which allowed the sheep to see at a distance, but prevented them from seeing in close proximity. He concluded that sheep used sight to orientate themselves and select grazing sites, but sight did not influence preference ranking within a grazing site. Work by Krueger et al. (1974) with totally blindfolded sheep showed similar results. However, both authors noted that highly preferred species, which were easily recognized by sight at a distance, were selected by sight.

Krueger et al. (1974) concluded that in sheep the sense of touch was the least influential of the senses for selecting forage, and no plants were significantly discriminated against with the sense of touch. However, he looked only at grasses and forbs, browse species may be different.

Personius (1985) pointed out that the sagebrush taxa growing in the cafeterias used for his (and this) study had equal succulence and similar gross morphology, which reduces the chance that sight, and or touch, influence selection of a particular taxon. He further concluded that although individual, apparently preferred plants, may become "hedged" and possibly visually (or tactually) recognizable to mule deer

after several years of heavier browsing, the mule deer must initially select the preferred plants by taste and/or smell before they can be preferentially browsed and take on the recognizable hedged appearance.

Influence of Secondary Compounds on Diet Selection

It is poorly understood which compound, or combination of compounds, in sagebrush affect a mule deer's senses of taste and smell, to influence preference for a particular taxon. Studies have found mule deer preference for terpenoid containing taxons to be negatively correlated with total monoterpenoid concentrations (Schwartz et al. 1980b, Sheehy 1975, Narjisse 1981). These correlations are not always strong (Sheehy 1975), and those of Narjisse (1981) were confounded with season. Other researchers have found no correlation (Behan and Welch 1985, Personius et al. 1987, Welch et al. 1983). This inconsistency indicates it is not just the total concentrations of secondary compounds, or the concentrations of broad groups of secondary compounds, such as total monoterpenoids, that determine palatability. This leaves the concentrations of specific compounds, or narrow groups of compounds, and the interactions of these compounds, to be probable preference determinants.

Schwartz et al. (1980b) found that when the terpenoids of juniper species were fractionated into oxygenated monoterpenes, monoterpene hydrocarbons and sesquiterpenes and applied to feed pellets there were significant differences ($P < 0.05$) in intake by mule deer for the treated pellets. Greatest intake was of the sesquiterpene treated pellets and least intake was of the oxygenated monoterpene treated pellets. Schwartz et al. (1980a) and Oh et al. (1967) also found the oxygenated

monoterpenoids to be the most inhibitory to rumen microbes. Schwartz et al. (1980b) found that mule deer preferred alligator juniper (Juniperus deppeana) to the other juniper species tested. They believed the reason for this was the low total monoterpene concentration. However, it also had the lowest oxygenated monoterpene concentration, which was the most deterrent group of compounds. This may be the reason it is the most preferred, and not that it had the lowest total monoterpene concentration.

Welch et al. (1983) found no significant relationships between the total monoterpene concentration or the concentration of 3 individual monoterpenoids, and preference for 21 accessions of sagebrush. Behan and Welch (1985) found no significant relationships between mule deer preference for 7 accessions of black sagebrush and total monoterpene concentration, or the concentration of 8 individual monoterpenes, including 1,8-cineole which is common to this study.

While looking at the coumarin content of sagebrush taxa as a taxonomic indicator, Stevens and McArthur (1974) found coumarin content seemed to be positively correlated to mule deer preference for the sagebrush taxa studied. Welch and McArthur (1986) looked at coumarin contents and mule deer preferences for the 3 subspecies of big sagebrush and found that on the average the more palatable subspecies did have higher coumarin contents. However, coumarin content could not predict mule deer preference at the accession level.

Scholl et al. (1977) reported only low levels of correlation between concentrations of 8 dominant volatile compounds found in 8 taxa of sagebrush and utilization of the sagebrush by free ranging mule

deer. Three of these compounds, methacrolein, 1,8-cineole, and p-cymene are common to this study. However, they did conclude that in general the concentration of oxygenated compounds, especially methacrolein, showed a stronger (negative) correlation to utilization by mule deer than did hydrocarbon monoterpenes.

Other studies have shown the concentrations of specific compounds to accurately predict mule deer preference for sagebrush. Sheehy (1975) found the concentrations of 8 volatile oils to account for 90% of the variation in utilization, by mule deer, for 7 taxa of sagebrush. Personius et al. (1987) identified 7 compounds that predict mule deer preference for the 4 taxa of sagebrush examined in this study.

MATERIALS AND METHODS

Site Description

Sagebrush foliage used to extract certain compounds was collected on U.S. Forest Service land immediately north of Yellowstone National Park, near Gardiner Montana. This area has been previously described in detail (McNeal 1984, Personius et al. 1987, Striby et al. 1987, Wambolt and McNeal 1987). It consists of steep south and west facing slopes interrupted by nearly flat to rolling benches. Elevations range from 1615 m to 2200 m and precipitation from 300 mm to 460 mm (USDA 1988), with increasing precipitation positively correlated with higher elevations. The vegetation is predominantly sagebrush-grassland with one or more big sagebrush subspecies; basin, mountain, Wyoming, and/or black sagebrush as the overstory. Bluebunch wheatgrass (Agropyron spicatum (Pursh) Scribn.) and/or Idaho fescue (Festuca idahoensis Elmer) are the most common understory dominants. The area is considered to be an important winter range receiving heavy winter utilization by both Rocky Mountain mule deer and Rocky Mountain elk (Cervus elaphus nelsoni Bailey) (Wambolt and McNeal 1987). The "cafeterias" and sample collection sites described by Personius et al. (1987) and Striby et al. (1987), at approximately 1950 m elevation, are near the middle of the range. Natural sagebrush cafeterias are relatively limited communities with high interspersions of several sagebrush taxa. They are formed by

variations in microclimatic conditions along the ecotone between 2 or more sagebrush-dominated habitat types.

Sample Collection

Since large quantities of plant material were needed for extraction of compounds, the cafeteria areas could not provide enough material without being destroyed for current and future research. In addition, personnel used in collecting the material for extraction could not always detect the subtle morphological differences among the taxa. Thus, large single taxon stands of sagebrush located up to 2.2 km from the cafeterias were selected for collection. There were continuous stands of sagebrush between the cafeterias and the collection areas so they were assumed to contain genetically similar plants. Collection sites ranged from 1615 m for basin and Wyoming big sagebrush to 2200 m for mountain big sagebrush. A shallow-limy site near the cafeteria (Personius et al. 1987, Striby et al. 1987), was selected for collection of black sagebrush.

For big sagebrush subspecies the current year's vegetative leaders were clipped in a manner similar to Personius et al. (1987) and Striby et al. (1987), which closely approximates the foliage utilized by browsing mule deer. Due to the extremely small annual leader growth of black sagebrush, and time constraints during collection, some older growth and reproductive structures were collected with the current year's material. Clippings were placed in airtight plastic bags on ice in a cooler and transported to the laboratory each evening for storage at -23°C until extraction. Big sagebrush subspecies were collected August 13-15 and 18-19, and black sagebrush September 17-19, 1986.

Extraction Procedure

Nonvolatile crude terpenoid fractions (NVCTFs) were extracted from sagebrush tissue with chloroform using a procedure similar to Kelsey et al. (1982) and Personius et al. (1987). Modifications, described below, were made to accommodate larger quantities of material. Since none of the volatile fractions were needed, procedures necessary to recover them were omitted.

Two hundred and fifty grams of sagebrush clippings was placed in a 4000 ml beaker, covered with 3000 ml of chloroform, and gently stirred for 5 minutes. The extract was first filtered through a large funnel with a brass screen to remove coarse debris, then through a small funnel with Whatman #4 filter paper to remove fine debris such as epidermal hairs and dust.

Chloroform and the more volatile components were removed from the extract with a roto-evaporator under vacuum and a 50-55°C water bath. When the chloroform was nearly gone the water bath temperature was increased to 60-65°C. This was continued for 10 minutes after any liquid was observed to drip from the condenser in the evaporator.

Next, the volatile steam-distillable compounds were driven off. Approximately 250 ml of extract was warmed in a 50°C water bath to facilitate handling and mixing. The extract was redissolved with approximately 250-300 ml. of chloroform to get it into solution. Steam was passed through the solution until 1000 ml of water had evaporated from the boiling flask generating the steam. This drove off the steam-distillable compounds as well as the chloroform used to redissolve the extract. This procedure left a suspension of nonvolatile compounds and

water. Upon cooling, most of the nonvolatile compounds settled out of suspension. The water was poured off the extract and saved. It had a milky color, indicating there were still some nonvolatile compounds in suspension. This water was washed 3 times with 100 ml of chloroform. This "dirty" chloroform was saved and used to redissolve the next batch of roto-evaporated extract, from the same taxon, to be steam-distilled. Water from the last distillation of each taxon was discarded.

The nonvolatile crude extract was dried by spreading a 5-7 mm layer in a glass dish and placing it into a drying oven at 60°C for 72 hours. The mountain big sagebrush nonvolatile extracts were solid; the other taxon's were tar-like (Appendix A). Mountain big sagebrush nonvolatile extracts were ground with a mortar and pestle to a fine powder to facilitate weighing and handling. All material extracted within a taxon was placed in a single container and mixed to ensure uniformity. Volatile compounds tested in this study were not extracted as they were available commercially and purchased from the Aldrich Chemical Company, Milwaukee Wisconsin.

Compound Application

Compounds selected for testing were among those identified by Personius et al. (1987) as probable preference determinants. Those tested included: 1,8-cineole, methacrolein, p-cymene, and each taxon's nonvolatile crude terpenoid fraction, which contains the sesquiterpene lactones.

Volatile terpenoids (1,8-cineole, methacrolein and p-cymene) were tested at the same concentrations Personius et al. (1987) found in the taxon having the greatest quantity (% extracted dry weight). The

highest concentration of 1,8-cineole (1.10%), a possible attractant (Personius et al. 1987), was found in mountain big sagebrush. The greatest concentrations of methacrolein (0.24%), and p-cymene (0.24%), both possible preference deterrents (Personius et al. 1987), were found in basin big sagebrush and black sagebrush, respectively.

To get an indication of the influence of compound concentration on preference, methacrolein was also tested at 0.12% concentration, or one half its greatest concentration. The lower concentration of methacrolein (50% methacrolein) tested was also the same concentration Personius et al. (1987) found in Wyoming big sagebrush.

Nonvolatile extracts were applied to the feed to achieve a 7% concentration. Seventy five grams of extract was dissolved in approximately 1000 ml of chloroform. This was poured over 1000 g of chopped alfalfa hay. After thoroughly mixing by hand it was spread under a fume hood to dry for 90 minutes at 22°C, with stirring at 30 minute intervals. The hay was then spread out and air dried at 22°C. After 12 hours it was placed in plastic bags and stored until needed.

Volatile compounds were applied to the feed with a water carrier. To minimize concentration changes due to evaporation, volatile compounds were applied to the feed in the feed bunks immediately prior to the start of the preference tests. Twenty ml of a water-compound suspension, calculated to deliver the desired concentration (Appendix B), was sprayed onto 250g of feed. The suspension was sprayed with an adjustable-nozzle finger pump spray bottle. The finger pump's intake tube, which normally goes to the bottom of the bottle, was removed so the pump would work well when the bottle was turned upside down. When

pumped until no more suspension came out, regardless of the bottle angle, 5 ml of suspension remained, so to apply 20 ml of suspension, 25 ml was placed in the bottle. Each compound had its own bottle, which was used throughout the trial to prevent contamination.

To speed treatment and minimize concentration changes, the water was placed in each spray bottle in the laboratory, and the proper amount of each compound added immediately before application. The bottle was agitated vigorously during application to keep the compound in suspension. To ensure even application the hay was mixed by hand as the compound was being applied. Spray bottles were held 10-15 cm from the feed being treated and the nozzle was adjusted to produce a coarse mist so the solution was evenly applied. A fine mist was avoided to reduce losses of the compound due to spray drift and increased evaporation area of the fine droplets. Twenty ml of untreated water were similarly applied to the control feed so preference would not be biased by water content.

Deer Biographies

Eight deer were used in the preference tests, including 6 Rocky Mountain mule deer, 1 Columbian black-tailed deer (Odocoileus hemionus columbianus Richardson) and one believed to be a hybrid between the 2 subspecies. Five were females and 3 were castrated males. They ranged from 1.5 to 6.5 years of age. The deer came from a wide geographic area including the states of Montana, Washington and Oregon (Table 1).

Table 1. Deer biographical information.¹

Deer	<u>ssp.</u> ²	sex	age (yr.)	origin (state)
BIM2	BT	M	2.5	OR
BTF6	MDxBT	F	6.5	WA
MDM1	MD	M	1.5	WA
MDM2	MD	M	2.5	WA
MDF1A	MD	F	1.5	MT
MDF1B	MD	F	1.5	WA
MDF1C	MD	F	1.5	WA
MDF1D	MD	F	1.5	WA

¹ Deer biographical information is based on personal communication with Washington State University personnel, primarily Bruce Davitt and Dr. Jack Nelson, and material on file at the range wildlife habitat lab Washington State University.

² MD: mule deer, BT: black tail

All the deer were captured as young fawns, bottle raised at Washington State University and treated essentially the same from shortly after birth. After weaning, the deer were normally kept in pastures adjacent to the metabolism trial building. They were maintained on free choice diets of 1 or more of the following: alfalfa hay, alfalfa-grain concentrate pellets, and grass growing in the pastures. They also had free access to water and trace mineralized salt blocks. The 2.5 and 6.5 year old deer had been exposed to a variety of grass, forb, and browse diets, including terpenoid-containing conifers, but no sagebrush in previous feeding trials. The 1.5 year olds had no

such previous experience. All deer were accustomed to being fed in the metabolism cages and were totally comfortable in them prior to the start of the adaption period proceeding the preference tests.

Test Facilities

The preference tests were conducted during November, December and January of 1986-87 at the E. H. Steffen Center, Department of Forestry and Range Management, Washington State University, Pullman Washington.

During the preference tests and the pre-test adjustment period the deer were kept in individual wire mesh pens 190 cm wide, 445 cm long and at least 183 cm in height. One of the narrow sides of each pen opened into an adjacent wire mesh metabolism cage which measured 193 cm long, 122 cm wide and 152 cm in height. Each cage and 107 cm of each pen were under the roof of an unheated open sided shed, the rest of each pen was uncovered (Figures 1 and 2).

In each cage, on the end opposite the pen, was a feed and water bunk (Figures 3 and 4). The top of each bunk is 56 cm from the cage floor. The bunks were approximately 23 cm deep, 29 cm wide at the top, 20 cm wide at the bottom and 102 cm long. Each bunk was divided into thirds with plywood dividers. A 2 gallon rubber water bucket was placed in one end. The remaining 2 sections were used for the treated and control feeds as well as the normal daily ration. The feed bunks had lids so the treated and control sides of the bunk could be presented to the deer simultaneously (Figure 3). A trace mineralized salt block was located on the floor of each cage. A maximum-minimum thermometer was located in the open sided building containing the metabolism cages.

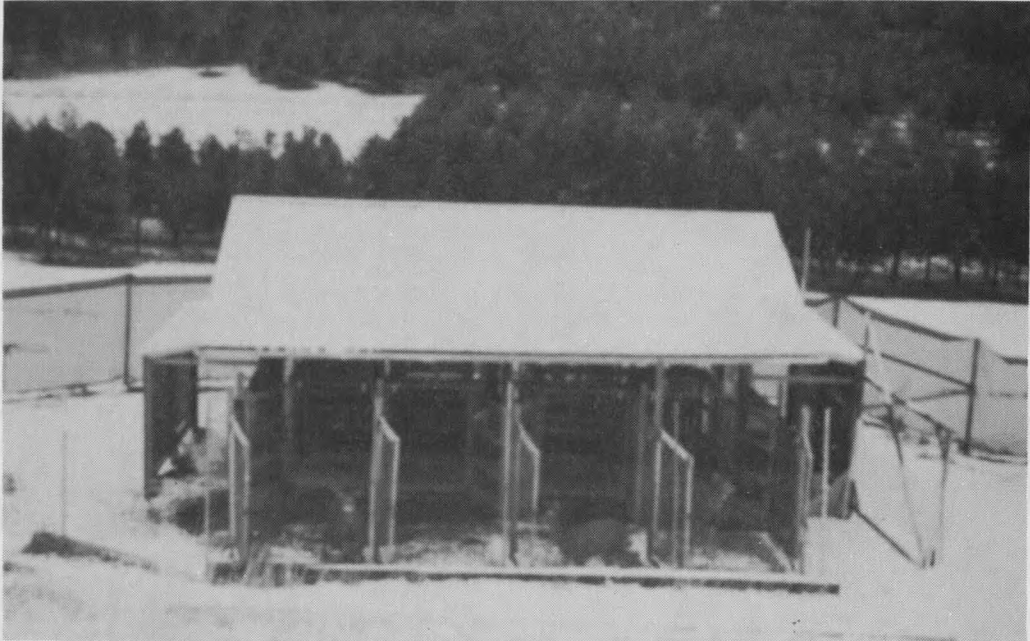


Figure 1. Metabolism trial building and adjacent deer pens.



Figure 2. View from pen, looking into metabolism cage.



Figure 3. Feed bunk with access panel open.



Figure 4. Interior of metabolism trial building, showing metabolism cages and feed bunks.

Experimental Design and Statistical Analysis

An 8 by 8 Latin square design was used to test deer preference for 7 sagebrush compounds. One of the compounds (methacrolein) was tested at 2 concentrations. Eight deer were used over 8 test periods. During each test period a 2-choice preference test (Bell 1959, Goatcher and Church 1970) was conducted each day for 5 days. This was followed by a 2 day rest during which the deer were fed in the same manner as in the preceding days, except no treated feed was offered. The rest period reduced any possible carryover effect of a compound into the next test period.

Due to the volatile nature of some compounds tested the daily preference tests were kept short, 20 minutes in length, to minimize any possible concentration changes. One group of 4 deer began at 1:00 P.M., the second group at 1:30 P.M.. The tests followed a 5 hour fast. The fast was initiated after a pre-trial test with domestic sheep showed a fast was necessary to stimulate all of the animals to begin eating immediately upon presentation of the feed. This fast was kept short because it was not desirable for the animals to be so hungry that selectivity would be reduced. Preference was expressed as:

$$\text{Preference} = \frac{\text{intake treated feed (g)}}{\text{intake treated feed (g)} + \text{intake control feed (g)}} \times 100$$

A 12 day adaption period was initiated prior to the start of the preference tests. The deer were placed in their individual pens and taken care of in the same manner as they would be throughout the preference tests, including the fast period. The deer had free access

to water and trace mineralized salt, and were fed the same high quality chopped alfalfa hay they received during the preference tests.

During each preference test each animal was given the choice of 270g of either treated or control feed. Positions of treated and control feeds in the bunk were switched daily to minimize the effect of any position preference. Immediately following each preference test the deer were given their normal daily ration, which consisted of the previous day's normal daily intake, plus 15 percent. The deer had free access to this until 8:00 or 8:30 A.M. the following day, or 5 hours prior to the next day's test. At this time the orts were weighed. The normal daily intake (feed offered minus orts remaining) was added to the treated and control feed intakes from the previous day's preference test to get total daily intake. All intakes were based on the air dried weights of the chopped alfalfa hay.

Immediately following the second test period the deer were confined to the metabolism cages for 8 days. During this time a digestion trial was conducted. The deer were given the same ration they received during the preference tests, however, they did not have the 5 hour fast. Following the digestion trial the deer were again allowed access to their pens and the 5 hour fast was re-initiated. Following 3 days of this treatment preference test 3 began. The remaining preference tests continued uninterrupted.

The blacktail male was brought into the experiment during the digestion trial prior to preference test 3 to replace a mule deer that became sick and had to be removed. This animal had an 11 day adaptation period, but was not tested with 2 compounds. This caused 2 of the 64

cells in the Latin square to be lost. The NVCTF of mountain big sagebrush and the 50% methacrolein were tested by only 7 of the 8 deer.

During preference tests the deer were observed as closely as possible without disturbing them. Construction of the feed bunks made it easy to see whether the deer was on the treated or control side of the bunk. However, except for the deer immediately adjacent to the observer it was difficult to see if the deer was smelling, licking and mouthing the feed, or actually eating it.

"Feeding" was considered to be any time an animal appeared to be feeding (had its head deep in the feed bunk) for a time period of 1 minute or longer. If the deer was seen feeding for less than 1 minute or had its head in the feed bunk, but not deep enough to be feeding, it was considered to be sampling the feed. Length of feeding time and when in the test period an animal switched from control to treated feed, or visa versa, was recorded for all deer during each test period. More subtle aspects of selection behavior, (smelling, licking, coughing etc.) were also recorded when possible. In addition, the maximum, minimum and 1:00 P.M. temperatures were recorded daily, and body weight was monitored on a weekly basis.

Analysis of variance on the Latin square to measure differences in preference among compounds, animals, and test periods was computed using the General Linear Model of SAS (Statistical Analysis Systems 1985). Where significant differences were found least squares mean separation was at ($P < 0.05$). Intakes of treated and control feed for an entire 5 day test period were added together for the preference ratios analyzed, rather than testing the preference ratios of individual days.

The preference ratio derived from the square of the sums, rather than the sum of the squares was used to avoid giving too much weight to days with abnormal intake. This method also handles days when neither treated nor control feed was eaten (this happened on 2 of 310 possible deer test days). On days when neither was eaten the sum of squares method gave a 0/0 preference ratio which could not be defined. Preference ratios were determined to be different from the theoretical no preference ratio of 50 by means of a T-test. Analysis of variance for preference by day and day x compound was also computed with the General Linear Model of SAS (Statistical Analysis Systems 1985), in this case the preference ratios sum of squares was tested. Where differences were found least squares mean separation was at ($P \leq 0.1$).

Time of initiation of feeding on the treated feed was analyzed by constructing a contingency table and placing the time of initiation of treated feed intake into categories of; ≤ 1 , $>1-5$, $>5-10$, $>10-15$, $>15-20$ minutes, and conducting a Chi-square analysis with MSUSTAT (1986). MSUSTAT (1986) was also used to compute regressions between temperature and preference, as well as temperature and total daily intake.

RESULTS AND DISCUSSION

Preference

Analysis of variance (Appendix C) showed mule deer preference, as expressed by the ratio of treated feed intake to total feed intake, was significantly different ($P < 0.05$) for the compounds tested. There were no differences ($P < 0.05$) in preference among the test periods, nor among the test animals.

Table 2 lists the means of the preference ratios for all of the compounds tested. The smaller the mean the greater the compound was discriminated against. The larger the mean, or the closer the mean was to the "no preference" ratio of 50, the less it was discriminated against. If any of the compounds had been selected for, their means would have exceeded 50. All of the means were significantly different from the no preference ratio of 50. All of the compounds except methacrolein, 50% methacrolein, and the NVCTF of mountain big sagebrush were below Bell's (1959) "rejection threshold" of 20% of intake, and fall within Goatcher and Church's (1970) "strong rejection zone". The 3 previously mentioned compounds fall within Goatcher and Church's (1970) "moderate rejection zone". From these results it is apparent that all of the compounds tested are "probable" preference deterrents. It is less apparent which compound(s) are actual preference determinants.

Table 2. Mean mule deer preference¹ for chopped alfalfa hay treated with 8 compounds found in 4 taxa² of sagebrush.

Compound	Preference
1,8-cineole	1.60 ^{A3}
Arno NVCTF	11.06 ^B
Artrwy NVCTF	13.21 ^B
p-cymene	15.09 ^B
Artrtr NVCTF	15.83 ^B
methacrolein	23.00 ^C
Artrva NVCTF	24.85 ^{CD}
50% methacrolein	29.87 ^D

¹ Preference = intake treated feed (g) / intake treated feed (g) + intake control feed (g) x 100.

² Arno: A. nova, Artrwy: A. t. wyomingensis, Artrtr: A. t. tridentata, Artrva: A. t. vaseyana.

³ Means sharing a superscript were not significantly different (P<0.05).

The data in Table 2 indicate that volatility has little influence on preference for the compounds, at the tested concentrations. Volatile and nonvolatile compounds are distributed throughout the list.

1,8-cineole stood alone (P<0.05) as the strongest preference deterrent. Of the 4 volatile compounds tested, 1,8-cineole was the most abundant in sagebrush tissue, and was applied to the test feed at the highest concentration. It is also less volatile than methacrolein, so its concentration on the treated feed should have remained higher for a longer period, although its vapor concentration immediately above the feed might have been lower. 1,8-cineole (Eucalyptol) was still easily

detected by human olfactory senses at the end of the 20 minute test period. Interestingly, several humans (5 of 5 graduate students) agreed it was the most pleasant smelling compound tested. Stepwise discriminant analysis (Personius et al. 1987) had shown it to be a possible preference "attractant", as its concentration among taxa increased with increasing preference by mule deer for the taxa. Data from Sheehy (1975) also indicates 1,8-cineole may be an attractant. My results challenge those observations. However, one cannot say conclusively it is a deciding preference determinant either. Any effects it may have on preference may be masked by 1 or more of the other compounds found in sagebrush.

While preference for p-cymene treated feed was not different from some of the nonvolatile compounds, it was different from, and ranked between other volatile compounds, as a preference deterrent. It was applied at the same concentration as methacrolein, yet it had a greater deterrent effect, so it appears to be a stronger deterrent than methacrolein. Methacrolein came out of the stepwise discriminant analysis before p-cymene (Personius, 1985), indicating it was a better predictor of preference than p-cymene. The preceding statements are not as contradictory as they appear. A stronger deterrent is not necessarily a better predictor of preference. From the data in Personius (1985) it appears that p-cymene was most important as a preference indicator between black sagebrush and big sagebrush. Although the results of my study do not prove p-cymene to be "the" preference determinate between black and big sagebrush, they indicate it could be an important preference determinate between the species.

Methacrolein may be more important as a preference indicator among the 3 big sagebrush subspecies (Personius et al. 1987). This makes a direct comparison of these 2 compounds, as preference determinants, difficult. An additional factor to consider is the extreme volatility of methacrolein. By the end of a preference test the concentration of methacrolein could have been considerably less than its initial concentration, and the concentration of p-cymene. This would reduce its effectiveness as a preference deterrent.

In addition to being extremely volatile, methacrolein is a severe mucosal tissue irritant. When deer stuck their heads into a feed bunk containing fresh methacrolein treated feed they nearly always coughed. In spite of this, methacrolein and 50% methacrolein were the least deterring of all volatile compounds tested, and one deer (BTF6) preferred methacrolein to the control feed on some days and would eat it while coughing. Although there was some tendency to wait until later in the test period to try methacrolein-treated feed, when compared to nonvolatile-treated feed, the delay time was not as long as for other volatile compounds.

Methacrolein was significantly ($P < 0.05$) more of a deterrent than was 50% methacrolein. This indicates that compound concentration does indeed have an effect on preference. This also indicates that methacrolein could be an important preference determinant among the big sagebrush taxa. The highest concentration tested was similar to the concentration found in basin big sagebrush, the least preferred taxon. The 50% methacrolein was a concentration similar to that found in

Wyoming big sagebrush, which is intermediately preferred. Mountain big sagebrush, the most preferred taxon, contains very little methacrolein.

The NVCTF is a mixture of many compounds including; sesquiterpene lactones, cuticular waxes, coumarins and flavonoids (Kelsey et al. 1982, Personius et al. 1987). The NVCTF varies in concentration, and compound composition among the sagebrush taxa. For this reason extracts from all taxa were tested. To avoid confounding the effects of compound composition on preference, with the effects of concentration, the NVCTFs from all taxa were tested at the same concentration. Thus, only the effect of compound composition on preference was measured.

Of all the NVCTFs tested only that from mountain big sagebrush was significantly different ($P < 0.05$) from the other taxa. As expected it was the least deterrent NVCTF tested. This is especially significant when one considers they were all tested at the same concentration. The NVCTF of black sagebrush naturally occurs at higher concentrations (20.12% extracted-tissue dry weight (ETDW)) than does that of mountain big sagebrush (13.87% ETDW, Personius et al. 1987). This should lower the preference for black sagebrush. Preference for the NVCTF of basin big sagebrush was not significantly different ($P < 0.05$) from the NVCTF of Wyoming big sagebrush. This was not surprising since the 2 taxa are preferred to nearly the same degree, and have about the same quantity of NVCTF (16.98 and 15.55% ETDW, respectively) in nature (Personius et al. 1987). Although the NVCTFs from basin and Wyoming big sagebrush appear to be preferred to the NVCTF from black sagebrush, the difference was not significant ($P < 0.05$). Here again concentration could have an effect. If the black sagebrush NVCTF had been tested at the

higher concentrations found in nature, the difference may have been significant. In addition, plant tissue black sagebrush NVCTF was extracted from had more stems and woody material than did the other taxa. The epidermal extract of the stems and branches contain fewer terpenes, than extracts of the leaves (Kelsey 1986). Consequently, the more woody tissue extracted, the lower the sesquiterpene lactone concentration in the final NVCTF. If sesquiterpene lactones are preference determinants then this woody tissue could reduce the deterrent properties of the extract, which could in part explain why the preference for feed treated with the NVCTF of black sagebrush was not significantly lower ($P < 0.05$) than feed treated with the NVCTF of basin or Wyoming big sagebrush. It is still quite possible that the NVCTF of black sagebrush is what separates it from big sagebrush in terms of palatability.

It appears that the NVCTFs are important preference determinants. The results of this study confer with the findings of Personius et al. (1987), whose stepwise discriminant analysis showed sesquiterpene lactones found in the NVCTF to be good indicators of preference. Burnett et al. (1977) also found sesquiterpene lactones deterred herbivory by eastern cottontail rabbits (Sylvilagus floridanus Allen) and whitetail deer (Odocoileus virginianus Zimmermann).

Preference tests have some problems that are difficult to overcome. Although care was taken to approximate the concentrations found naturally in the taxa studied, there is still significant artificiality in the way the compounds were applied. All sesquiterpene lactones, and probably most of the monoterpenes in sagebrush occur in glandular

trichomes on the epidermal surface (Kelsey and Shafizadeh 1980, Kelsey et al. 1984). Volatile components may be released slowly through the trichome membrane and surrounding cuticular waxes of undisturbed or undamaged leaves, whereas rapid release can occur when the trichomes are burst. Undoubtly, volatile compounds sprayed onto the surface of chopped alfalfa are released more rapidly and in higher concentrations, relative to trichomes, until the supply is exhausted. Thus, the NVCTFs were probably more accurately simulated than were the volatile compounds.

In most cases the preference tests indicated that compounds selected by stepwise discriminant analysis as preference indicators (Personius et al. 1987) may also be preference determinants. There were exceptions. The preference tests showed 1,8-cineole to be a strong preference deterrent, whereas it appeared to be an "attractant" in the earlier stepwise discriminate analysis. Compounds selected by stepwise discriminate analysis predict preference, but are not necessarily the actual preference determinants. This does not reduce the value of the stepwise discriminate analysis procedure. It is still an important tool to identify possible preference determinants. Conducting preference tests on all of the compounds found in sagebrush without the choices having been narrowed by the stepwise discriminant analysis would have been impractical.

To find one, or a few compounds, to be "the" preference determinants for sagebrush is probably not realistic. Sagebrush has too many secondary compounds that are possible preference determinants. These compounds can interact with each other in an endless number of

combinations, cumulatively, synergistically and antagonistically. Although it is not likely that one deciding preference determinant exists for sagebrush, especially among the taxa, a better understanding of preference, and what shapes it, is still needed. To give the most complete picture of the relationship between plant secondary compounds and animal preference future studies should include statistical procedures such as the stepwise discriminant analysis as well as feeding trials.

Adaptation

A separate model (Appendix C) with day of test period included was analyzed to determine if preference changed with repeated exposures to the treated feeds. Preference was found to differ ($P=0.06$) with day of test period. Table 3 lists the means of the preference ratios for each of the five days, from all of the test periods. The smaller the mean the more the compound was discriminated against on that day of the tests.

If adaptation to a compound occurred one would expect preference toward the treated feed to increase progressively from day 1 to day 5 of each test period, or from day 1 to a maximum level of acceptance. This maximum level of acceptance may occur before or after the fifth day of a test period. It appears that the deer did show some adaptation to the compounds after the first day of exposure to the treated feeds. Preference ratios of days 2-5 are similar to each other, and all were higher than day 1. However, the compounds are still strongly discriminated against when compared to the theoretical "no preference"

ratio of 50. There may have been even less discrimination (greater adaptation) if the animals had been exposed to the compounds for longer periods of time each day, or for more days.

Table 3. Mean mule deer preference¹ for treated vs. untreated feeds over 5 days of exposure.

day	preference
1	11.90 ^{A2}
2	18.75 ^B
3	17.92 ^B
4	16.26 ^B
5	18.87 ^B

¹ Preference = intake treated feed (g) / intake treated feed (g) + intake control feed(g) x 100.

² Means sharing a superscript were not significantly different (P≤0.1).

In a two-choice preference test Arnold et al. (1980) showed that over a 72 hour period, sheep completely adapted to the odors of cedarwood oil (a terpene), tannic acid, propionic acid, and amyl alcohol, all of which were initially discriminated against. In the same period of time sheep came to prefer butyric acid treated hay over the control hay. A study by Narjisse (1981) implied that sheep adapted to sagebrush monoterpene odor after 2 days of a 4 day two-choice preference test. In both cases only adaption to odor was tested as the compounds were not applied directly to the feed, but rather to absorbent pads placed in the feed bunks.

In this experiment it appears that adaption to a compound occurs rapidly and preference toward a compound will not change greatly after the second day. However, the intake of treated feed was only a small portion of the deer's daily intake. If the treated feed was a larger part of the diet, adaption could take longer as the deer might have to make additional physiological changes, or allow time for changes to occur in the rumens microbial populations.

Feeding Behavior

Choosing 1 minute as the minimum observation to define feeding behavior was somewhat arbitrary. However, it did give a good indication of what was occurring in regard to intake. Some feed intake was occurring during sampling periods. However, the quantities ingested were very small. Even if the animal was observed sampling one side of a feed bunk several times during a 20 minute test period, its intake from that side would be very low unless it was observed to be feeding from that side for more than 1 minute, at least once. The number of times a compound was observed to be fed upon for more than 1 minute, agrees well with that compound's ranking in the preference test results. 1,8-cineole, the least preferred compound, was observed to be fed upon for greater than 1 minute during only 3 of the 40, 20 minute test periods, although some intake was recorded on more than 3 days. Fifty percent methacrolein, the least discriminated against compound was observed to be fed upon for more than 1 minute on 27 out of 35, test periods. Likewise, how often a deer was observed to be feeding on treated feed, for more than 1 minute, agrees well with that animal's degree of selectivity. The least discriminating deer (MDF1D) was observed to feed

upon the treated feed 27 out of 40, 20 minute test periods. The most discriminating deer (BTM2 and MDF1C) were observed feeding on treated feed 8 of 30 and 8 of 40, test periods, respectively.

Time of initiation of treated feed intake was placed into categories of ≤ 1 , $>1-5$, $>5-10$, $>10-15$, and $>15-20$ minutes. A Chi-square analysis of a 2 way contingency table showed that time of initiation of treated feed intake was significantly different among the compounds tested (Table 4). Feeding on hay treated with volatile compounds was initiated later in the test period than hay treated with nonvolatile compounds. This becomes apparent when the compounds in the contingency table are grouped into volatile and nonvolatile categories (Table 5).

The most likely reason for late initiation of feeding on hay treated with volatile compounds is the reduction of their concentrations over time. Actual concentration of the compound on the feed may not have been greatly reduced, instead, vapors in the air above the feed, remnants of the treatment process, may have dissipated. Another explanation is the deer's olfactory organs become adapted to, or desensitized to, the compounds (Arnold et al. 1980).

Less apparent is why the nonvolatile compounds were not selected against more strongly in the first minute of each test period, relative to the rest of the time periods. The deer usually, but not always, made their decision to initiate feeding behavior on the treatment, or control, within the first minute of the test. Random chance would predict a 50/50 selection. The preference data indicate the control feed was greatly preferred to the treated. Yet, in 42 out of 75 times (56% of the time) nonvolatile compounds were selected for during the

Table 4. Time of initiation of treated feed intake.

Compound	Minutes					Total
	≤1	>1-5	>5-10	>10-15	>15-20	
p-cymene	1 * (4.2)	1 (1.3)	1 (1.8)	6 (2.6)	2 (1.1)	11
1,8-cineole	1 (1.2)	0 (0.4)	0 (0.5)	0 (0.7)	2 (0.3)	3
methacrolein	3 (7.7)	3 (2.4)	8 (3.3)	4 (4.7)	2 (2.0)	20
50% methacrolein	4 (10.4)	3 (3.2)	7 (4.5)	10 (6.3)	3 (2.6)	27
AN NVCTF	12 (6.9)	2 (2.2)	1 (3.0)	2 (4.2)	1 (1.8)	18
ATV NVCTF	14 (10.4)	5 (3.2)	3 (4.5)	3 (6.3)	2 (2.6)	27
AIT NVCTF	9 (5.8)	2 (1.8)	0 (2.5)	3 (3.5)	1 (1.5)	15
AIW NVCTF	7 (4.6)	0 (1.4)	2 (2.0)	3 (2.8)	0 (1.2)	12
Total	51	16	22	31	13	133

Chi-square (28 d.f.)=56.73, P=0.00

* Actual and (expected) counts.

first minute of a feeding period. In their discussion of chemical constituents Personius et al. (1987) speculated that the NVCTF was detectable mainly by taste. This is a plausible explanation for the above data. The nonvolatile compounds were not readily detected by smell and the deer had to "taste-sample" for more than 1 minute before preference could be determined. Also, deer are very curious by nature

and may have been attracted to the novel food momentarily before eating enough to determine preference.

Table 5. Time of initiation of treated feed intake, grouped as volatile and nonvolatile compounds.

Compounds	Minutes					Total
	≤1	>1-5	>5-10	>10-15	>15-20	
Volatile	9 *	7	16	20	9	61
	(23.4)	(7.3)	(10.1)	(14.2)	(6.0)	
Nonvolatile	42	9	6	11	4	72
	(27.6)	(8.7)	(11.9)	(16.8)	(7.0)	
Total	51	16	22	31	13	133

Chi-square (4 d.f.)=29.98, P=0.00

* Actual and (expected) counts.

Chi-square analysis of contingency tables was also used to look at the time of initiation of feeding on treated feeds among the test periods and among the days of the test periods, as well as among the individual animals, and between the animals grouped according to sex and age-experience (young=inexperienced, old=experienced). None of these came out significant ($P < 0.1$). However, due to the small sample size, and the disproportionate sizes of the sex and age classes within the study, this analysis is not very strong. The possible effect of sex or age-experience on the time of initiation of feeding on treated feeds (or preference) cannot be dismissed. Rice and Church (1974) found preference differences between male and female black-tailed deer for browse extracts, and organic acids. Several studies with other mammals

have shown age to affect taste sensitivity (Cooper et al. 1959, Cicala and McMichael 1964, Glanville et al. 1964).

Temperature

Temperatures in the open sided shed on preference test days ranged from 9.4°C to -18.3°C. Mean daily maximum, minimum and time of preference test (1:00 P.M.) temperatures were 2.8°C, -2.1°C, and 1.4°C, respectively.

Temperature can affect the sense of taste (Bekesy 1964, Leibetseder 1980). Regression analysis showed no significant ($P < 0.1$) relationships between daily maximum, minimum or 1:00 P.M. temperatures and preference. Lack of a significant relationship between temperature and preference indicates that temperature had no effect on preference. During this experiment there were relatively constant outside temperatures, and the open sided shed had a moderating effect on extreme, or rapid, temperature fluctuation. As a result there may not have been enough variation in temperature to affect deer preference.

Rose and Bradley (1980) included temperature in a list of factors that could affect an animal's smell and taste sensitivity. Fluctuations in temperature could affect an animal's preference toward a particular compound in at least 2 different ways. First, stress (extreme temperature change) could trigger a physiological change in the animal's taste or olfactory organs. This would alter the animal's taste and/or smell detection and acceptance thresholds and ultimately the animal's preferences toward a particular compound. Secondly, changes in temperature would change the volatility (and solubility) of the compounds being tested. A decrease in temperature would cause a

decrease in volatility. With fewer of the compounds molecules in the air immediately surrounding the treated feed the animal would receive reduced olfactory stimulation. Thus, for a given concentration of a compound, olfactory detection and acceptance thresholds would be increased. Conversely, with lowered volatility the compounds would remain on the treated feed, at a high concentration, longer than they would at a higher temperature. For a compound normally discriminated against this slightly higher concentration could possibly be detected and discriminated against by the animal's sense of taste. The above factors may alter the animal's perceived preferences for a given concentration of compound applied to the treated feed, but not the animal's actual preferences.

The effect of temperature on daily intake was estimated on the 2 day rest periods between preference tests, as well as during the 5 day preference tests, but not while the deer were confined to their metabolism cages during the digestion trial. Temperatures ranged from 10°C to -20°C. Mean daily maximum and minimum temperatures were 2.6°C and -2.6°C, respectively.

Regression analysis showed the following relationships:

$$\text{Intake (g)} = 1309 + (-10.60) \times \text{Max. Temp. } ^\circ\text{C}, R^2=.0214, P=.003$$

$$\text{Intake (g)} = 1263 + (-6.141) \times \text{Min. Temp. } ^\circ\text{C}, R^2=.0136, P=.018$$

Although significant, the regression equations have low coefficients of determination, which limits their ability to predict intake. As with preference, there may not have been enough variation in temperature to greatly affect intake. Had there been a wider range of

temperatures, regression analysis may have been better able to predict intake.

CONCLUSION

One nonvolatile extract from each of four sagebrush taxa, and three volatile compounds known to occur in these taxa were tested, in two-choice preference tests, for their deterrent effects on mule deer feeding. Fifty percent methacrolein was the least deterring compound tested, followed by the NVCTF of mountain big sagebrush and methacrolein. Methacrolein was significantly ($P < 0.05$) more of a deterrent than was 50% methacrolein. The NVCTF of mountain big sagebrush was intermediate to, but not significantly different ($P < 0.05$) from, either. The next most deterring group included, in order of decreasing preference: The NVCTF of basin big sagebrush, *p*-cymene, the NVCTF of Wyoming big sagebrush and the NVCTF of black sagebrush. Differences in preference among these compounds were not significant ($P < 0.05$). Data of Personius et al. (1987) indicated 1,8-cineol may have been an attractant. Surprisingly, it was the strongest preference deterrent tested, and the only compound significantly different ($P < 0.05$) from all of the other compounds tested. It is unlikely that 1,8-cineole is a preference attractant.

The deer showed some adaptation to the compounds over time. However, they did not adapt to the compounds completely, as they showed discrimination against the treated feeds on all test days. The deer were more discriminating ($P \leq 0.1$) the first day of the 5 day preference testing periods. There was little change in preference the remaining 4

days. This indicates that adaptation to a new food takes place rapidly, at least for the olfactory and taste senses. Additional exposure to the treated feeds may lead to further adaptation. Temperature did not appear to affect preference for the compounds tested. Over a wider range of temperatures there is still the possibility that temperature could have an affect on preference.

All of the sagebrush compounds and extracts tested were found to be mule deer feeding deterrents. With the exception of 1,8-cineole, compounds selected to be preference predictors by discriminant analysis (Personius et al. 1987), appeared to similar real effects in determining preference. p-cymene may be an important preference determinant between black sagebrush and big sagebrush. Likewise, methacrolein may be an important mediator of deer selection among big sagebrush subspecies. The NVCTFs, containing sesquiterpene lactones, also have an effect on mule deer preference for sagebrush taxa, as predicted. The hypothesis tested: compounds found in sagebrush taxa do not influence mule deer preference, was rejected.

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APPENDICES

APPENDIX A

Results of Nonvolatile Crude Terpenoid Fraction Extraction Procedure

Concentrations of the nonvolatile crude terpenoid fractions in this study were similar to those obtained by Personius et al. (1987). In this study the concentrations were expressed as a percentage of unextracted foliage wet weight. Personius et al. (1987) expressed them as a percentage of extracted dry weight. Using foliage water content data from Personius (1985) it is possible to approximate his values as a percentage of foliage wet weight. This was done in Table 6. Differences between the NVCTF concentrations found in this study, and those of Personius et al. (1987) are not large, for the 3 big sagebrush taxa. If significant differences exist they could probably be explained, at least in part, by seasonal and year to year variation in the concentrations of the nonvolatile crude terpenoid fractions. Kelsey et al. (1982) showed seasonal variations in big sagebrush crude terpenoid concentrations, of which the NVCTFs are a major constituent. The difference between the black sagebrush NVCTF concentration in this study and that of Personius et al. (1987), is larger than the difference between the big sagebrush subspecies. This is probably a result of the greater quantity of stem and reproductive tissue collected in this study. This material has a lower concentration of crude terpenoids (Kelsey, 1986) and subsequently nonvolatile crude terpenoids than the leaves, and therefore lowered the overall yield of these compounds.

Seasonal and yearly variations in concentration could also account for some of the differences.

Table 6. Comparisons of nonvolatile crude terpenoid fractions.

Taxon	Material extracted this study (g)	Color/ Texture	NVCTF this study (% wet wt.)	NVCTF Personius ¹ (% wet wt.)
ATV	12,042	green-brown/hard*	6.73	9.04
ATW	10,630	brown/taffy*	8.08	9.83
ATT	10,372	caramel-brown/taffy*	7.48	10.41
AN	10,400	dark brown/tar-like	7.23	12.57

¹Personius (1985), Personius et al. (1987).

* White waxy film on upper surface.

APPENDIX B

Calculations of Compound Quantities Required to Treat Alfalfa

Example: A 0.24% concentration of p-cymene is desired in the treated feed. p-cymene has a density of 0.860 g/ml.

250 g untreated feed + 20 ml suspension = 270 g treated feed (assume 1 ml = 1 g).

$270 \text{ g} \times 0.0024 = 0.648 \text{ g}$ of compound in treated feed.

$0.648 \times 25 \text{ ml total suspension} / 20 \text{ ml applied suspension} = 0.81 \text{ g compound} / 25 \text{ ml suspension}$.

$0.81 \text{ g} / 0.860 \text{ g / ml} = 0.94 \text{ ml of compound} / 25 \text{ ml water}$.

Table 7. Volatile compound application rate calculations.

Compound	desired concentration ¹ (% dry weight)	compound /25 ml solution (g)	density (g/ml)	compound /25 ml solution (ml)
p-cymene	0.24	0.81	0.860	0.94
1,8-cineole	1.11	3.75	0.921	4.10
methacrolein	0.24	0.81	0.847	0.96
50% methacrolein	0.12	0.41	0.847	0.48

¹ Personius et al. 1987

APPENDIX C

Analysis of Variance

Table 8. Analysis of variance, testing the effects of animal, test period and compound on mule deer preference.

Source	D.F.	Mean Square	F
Animal	7	182.32	1.62
Test Period	7	183.76	1.64
Compound	7	632.52	5.63*
Error	40	112.37	

*Significant at $P < 0.05$

Table 9. Analysis of variance, testing the effects of day and compound by day on mule deer preference.

Source	D.F.	Mean Square	F
Compound	7	3003.02	13.43*
Day	4	513.20	2.30*
Compound x Day	28	246.04	1.10
Error	268	223.61	

*Significant at $P = 0.06$

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