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The Concentration and Composition of Big Sagebrush Essential Oils From Oregon

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Key Word Index – *Artemisia tridentata*; Anthemideae; Asteraceae (Compositae); essential oils; monoterpenes; variation; herbivory.

Abstract – The concentration of essential oils in big sagebrush foliage, *Artemisia tridentata* subsp. *tridentata*, subsp. *vaseyana* and subsp. *wyomingensis* collected from Oregon, differed significantly between individual shrubs with no correlation to geographic site, subspecies genotype, or plant age. Essential oil composition, measured by headspace analysis, was not affected by plant age, location of tissue within the crown, or geographic site. Six of 16 compounds differed quantitatively by subspecies, with *wyomingensis* and *tridentata* having nearly the same composition. *Vaseyana* could be recognized by its significantly lower concentration of acetone and methacrolein, two highly volatile, non-terpenoid, constituents. The implication of these results and those of recent studies are discussed in relation to herbivory.

Introduction

Since the recognition of three subspecies within big sagebrush [*Artemisia tridentata* Nutt. subsp. *tridentata*, subsp. *vaseyana* (Rydb.) Beetle and subsp. *wyomingensis* Beetle and Young] in 1965 [1] evidence has been mounting for the need to separate them for management purposes [2–6].

Although sympatric throughout the semiarid rangelands of the western United States these subspecies have evolved often subtle but distinct morphology [2–4], phenology [3], physiology (from measurable differences in chemical composition) [7–11] and ecology [2–6, 12]. Plants and populations remain difficult to separate or identify at the subspecies level, even by experienced individuals [6], due to great genetic variability, phenotypic plasticity and, to a more limited extent, hybridization and introgression. In recent years there has been an increased intensity of research with these shrubs because most work prior to 1965 was directed toward taxonomy and methods for eradication and control. We now know that early ecological studies were frequently confusing, contradictory and of limited current use because of taxonomic indiscrimination, particularly within big sagebrush, the single most widespread species.

Native herbivores (pygmy rabbits, sage grouse, mule deer, antelope) that have coevolved with these shrubs consume the plants as part of their diet, whereas introduced domestic herbivores (horses, goats, sheep and cattle) in general avoid or limit consumption [13–20]. Economically, dense stands of sagebrush have a negative impact on commercial livestock production and provide incentive for its removal and replacement by grass [21, 22]. Interest has also arisen in utilizing these shrubs as a source of energy and specialty chemicals [23, 24]. Both their presence and their removal have considerable environmental and ecological effect. Management and vegetative manipulation of the sagebrush ranges will be most reliable and successful if based on a thorough biological knowledge of the subspecies.

To further this knowledge a study was designed to measure and correlate soil, plant (shrubs and associated vegetation) and hydrologic characteristics for the habitats of big sagebrush subspecies in eastern Oregon. In this paper we present our results from the analysis of subspecies essential oil

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concentrations and composition, with emphasis on sources of variation at the individual plant and subspecies levels under the combined influence of genetic and natural environmental factors. These results and those of recent studies are discussed in relation to herbivory.

Results

Essential Oil Concentrations

The quantity of steam distillable essential oil within a plant was not related to its age, subspecies genotype, or geographic site (Table 1). Differences in oil concentrations between individual shrubs were significant ($\alpha = 0.05$) in both the 1979 and 1980 growing seasons.

Composition of Headspace Vapors and Steam Distilled Oils

Twenty-one compounds (Table 2) were identified in the headspace vapors and steam distilled oils. Sixteen, including a combined value for santolina triene and α-pinene (because of poor separation), were used in the analyses.

The quantity of a chemical vaporized from plant tissue into the atmosphere of a sealed chamber with restricted volume will not by itself give a true measure of the concentration in the tissue. Less volatile materials will be poorly represented in comparison to the highly volatile substances. If, however, the conditions for preparing the samples are constant and the tissues are chemically similar, then the headspace technique can be used to measure relative differences in volatile constituents.

Quantities for 12 of the 16 compounds in the headspace vapors (from analyses of nine plants, three of each subspecies) were positively correlated with the quantities of their respective steam distilled oils. The lack of correlation for Me_2CO and methacrolein was attributed to their high volatility and loss during steam distillation. Low concentrations and variability of GLC sensitivity were considered the causes of noncorrelation for *p*-cymene and santolina epoxide. Borneol was only detectable in the steam distilled oils and not in the vapors because of low volatility. Since most of the compounds displayed a correlation, this verified the headspace technique as a reliable method for studying the essential oil composition and, in addition, provided the ability to detect the highly volatile constituents.

Analysis of headspace vapors from whole and ground leaves at room temp. confirmed methacrolein and the other more volatile compounds, absent in distilled oils, to be natural products and not artifacts of the analysis preparation process.

Effect of Intraplant Location and Plant Age on Essential Oil Composition

The chemical composition of the essential oils (analysed by headspace vapors) was not affected by the relative age of the plant, or the location of tissue development within the crown (north, south, east, west and top). However, during the analysis of data by crown location, five compounds (artemiseole, methacrolein, santolina epoxide, methyl santolinate and camphor) differed significantly in quantity between the three plants.

Effect of Site and Subspecies Genotype on the Chemical Composition of Essential Oils

About half of the compounds (six) in the headspace vapors of mature plants were quantitatively discernable between subspecies (Table 2), whereas only one compound differed quantitatively between the geographic sites. Plants at Baker had significantly ($\alpha = 0.05$) less cineole

TABLE 1. THE ESSENTIAL OIL CONCENTRATION OF BIG SAGEBRUSH SUBSPECIES AT VARIOUS LOCATIONS IN EASTERN OREGON

Location	Subspecies			1980 sites
	wyomingensis	*tridentata*	*vaseyana*	
	% leaf dry wt*			
Squaw Butte, 1979	1.5 ± 1.1	2.1 ± 0.2	2.9 ± 1.1	–
Baker, 1980	6.2 ± 3.3	2.6 ± 0.7	3.4 ± 2.9	4.1 ± 2.8
Frenchglen, 1980	2.8 ± 0.6	2.8 ± 0.4	2.0 ± 0.3	2.5 ± 0.5
Millican, 1980	3.2 ± 1.1	2.1 ± 0.6	2.7 ± 1.2	2.9 ± 0.9
1980 subspecies	4.1 ± 2.4	2.5 ± 0.6	2.9 ± 1.6	–

*Mean ± s.d. from three plants each representing a distinct population of the subspecies. The 1980 values from the mature plant age group. The 1979 and 1980 data analysed separately by nested classification ANOVA, $\alpha = 0.05$. None of the means was significantly different.

TABLE 2. AVERAGE PERCENT COMPOSITION OF HEADSPACE VAPORS FROM MATURE PLANTS OF BIG SAGEBRUSH SUBSPECIES FROM OREGON

	Subspecies					
	wyomingensis		*tridentata*		*vaseyana*	
Compound*	Mean†	Range†	Mean	Range	Mean	Range
Methylbutene‡	–	–	–	–	–	–
Me_2CO	7.4[a]	4–11	4.6[a]	2–6	2.3[b]	1–4
Methacrolein	29.0[a]	11–48	37.0[a]	18–57	9.1[b]	3–23
EtOH‡	–	–	–	–	–	–
Santolina triene and α-pinene	7.6[a]	2–21	3.3[b]	0–8	15.0[a]	3–43
Camphene	6.7	1–17	9.2	4–19	6.8	1–19
β-Pinene	0.5	0–2	0.5	0–1	1.1	0–3
Artemiseole	13.0	0–27	11.0	2–21	13.0	0–31
1,8-Cineole	7.7	1–17	9.0	2–24	13.0	0–24
p-Cymene	1.1	0–3	1.2	0–2	1.0	0–2
Santolina epoxide	0.7[a]	0–3	0.4[a]	0–2	0.0[b]	0.0
Artemisia ketone	8.3	0–47	0.1	0–1	22.0	0–62
Yomogi alcohol	0.4	0–1	1.0	0–5	0.2	0–1
Artemisia acetate	1.9	0–4	2.9	0–10	2.5	0–5
Methyl santolinate	3.7[a]	2–11	5.7[a]	1–10	1.3[b]	0–5
Thujone	0.0	0.0	0.0	0.0	0.4	0–6
Artemisia alcohol	0.6	0–3	0.8	0–2	1.1	0–3
Camphor	2.8[a, b]	0–8	4.6[b]	2–9	2.7[a]	0–12
Terpinen-4-ol‡	–	–	–	–	–	–
Borneol‡	–	–	–	–	–	–
Total	91	–	91	–	92	–

*Listed in order of increasing R_t on a 3.7 m 10% Carbowax CWHP column. All compounds, except yomogi alcohol, were identified by MS, additional confirmation was obtained by RR_ts [26, 53] and in some instances, co-injection with reference compounds. MS of unknowns were identified by comparison to published spectra [52, 53]. Structures for irregular compounds can be found in ref. [53], where artemiseole is the same as arthole.

† Average percent of total GLC integrator counts. Means without letters or followed by the same letter are not significantly different between species at the 5% level. Ranges rounded to the nearest whole percent.

‡ These compounds not used in the statistical and cluster analysis. Borneol detected in steam distilled oils, but not in headspace vapors.

(3.2 ± 3.6% s.d.) than plants at Squaw Butte (13.4 ± 7.0%) or Millican (15.2 ± 5.8%). Based on the means in Table 2, *tridentata* and *wyomingensis* were more similar to each other than they were to *vaseyana*. The only significant difference between the former pair was in the combination santolina triene plus α-pinene concentration. *Vaseyana* varied from both the other two subspecies with lower quantities of Me_2CO, methacrolien, santolina epoxide and methyl santolinate. It further differed from *tridentata* with lower amounts of camphor, but higher levels of the santolina triene plus α-pinene mixture. Mean concentrations of artemisia ketone were wide ranging, 0.1, 8.3 and 22.0 for *tridentata, wyomingensis* and *vaseyana*, respectively; but were not significantly different because of substantial variations. Most *wyomingensis* contained no artemisia ketone but, when present, it was in large quantities, resulting in a higher average than *tridentata*.

The mean concentrations for the chemical components in the essential oils (Table 2) suggest some subspecies delineation of the compounds, quantitatively. A cluster analysis (for all plants sampled in 1979), with the 16 compounds analysed by the headspace technique, gave a small grouping of *vaseyana* predominantly from a single geographic site (Squaw Butte) and a mixed cluster of both *tridentata* and *wyomingensis* representing all geographic sites. Eliminating compounds with no significant differences improved clustering for *vaseyana*, whereas *tridentata* and *wyomingensis* remained a mixed group.

The extensive variation, both within and between subspecies, documented by the overlapping and widely divergent ranges (Table 2), limited the taxonomic utility of the essential oils constituents. The identity of *vaseyana* plants could be reliably determined by their lower concentrations of Me_2CO (≤3.5%) and methacrolein

(≤15%). Artemisia ketone, when present in amounts of 5–60%, was a good marker for separating *vaseyana* from *tridentata*. In combination with low levels of Me_2CO and methacrolein it provided an additional character for distinguishing *vaseyana* from *wyomingensis*. There were no diagnostic compounds to discriminate between *tridentata* and *wyomingensis*. The constituents in a single plant or subspecies were predictable only in the most general terms.

Discussion

Plant (size and vigor) and environmental characteristics (soil texture and chemistry) have been associated with significant differences in essential oil concentrations between populations of big sagebrush in Colorado [25]. Genetic influences by subspecies were not investigated. The effects of subspecies genotype on oil concentration were documented after eliminating environmental variation by transplanting shrubs into a uniform garden [9]. Subspecies *vaseyana* had a higher oil content than either *wyomingensis* or *tridentata*, with no difference between the latter two.

Chemical constituents in the oils from garden plants varied significantly between and within subspecies for populations of different geographic origin [9]. Variation in oil composition was greatest at the plant and population level, within rather than between taxonomic groups, in Scholl's [26] garden study. He concluded these compounds were a poor index of genetic differences for otherwise, morphologically, physiologically and ecologically, distinct taxa. Narjisse [20] also commented on the substantial chemical variation in oils between individual plants.

In our study, the concentration and composition of the essential oils were analysed for individual plants under the combined genetic and environmental influence of their natural populations. The concentration of steam distillable oils in the leaves of big sagebrush from Oregon varied significantly between individual shrubs, overshadowing any correlation with plant age, subspecies genotype or general environmental conditions. It is possible that some of the environmental effect may have been diminished by the lengthy sampling time between geographic sites in the fall of 1980 (9–10 September, Millican; 16–17 September, Baker; 4–5 October, Frenchglen). Concentration changes have been reported during this season [24, 27, 28]. Subspecies and age group sampling occurred within a 30 h period over two consecutive days at each site and should have minimized temporal variation.

Chemical composition of the oils was extremely variable and individual constituents were not affected by intraplant location of the leaves, plant age, or geographic site. In analysing for effects of intraplant location, five compounds differed significantly between the three plants examined. Quantitatively, six of 16 compounds were affected by subspecies genotype with most differences occurring between *vaseyana* and the other two subspecies. Low concentrations of Me_2CO and methacrolein allowed recognition of *vaseyana*, whereas *wyomingensis* and *tridentata* were inseparable by chemical characters.

These results demonstrate that the quantities of the individual chemicals in the essential oil vary between plants and between subspecies. This limits their taxonomic value, but possibly enhances their ecological significance as a defense adaptation to herbivores [29]. One must suspect chemical defense to be quite important to sagebrush, because of its apparency (in both time and space) in rangeland communities and its relative successful resistance to herbivory.

Much of the current interest in sagebrush phytochemistry has been directed toward the effects of the monoterpenes on ruminant digestion and forage selection [30]. Sheep use smell, and goats use taste to discriminate against feed contaminated by monoterpenes [20]. Ungulates will preferentially select individual plants, populations and subspecies of sagebrush for consumption [7, 31–34]. Within big sagebrush, *vaseyana* is more heavily utilized than *tridentata* or *wyomingensis* by both mule deer and sheep [32–34], when all three subspecies are available. The role of monoterpenes in these selections has been investigated, but their importance under natural feeding conditions has not been resolved [30].

Although monoterpenes are quantitatively the major class of secondary metabolic products in sagebrush tissues they are not the only compounds that could influence herbivores. Scholl *et al.* [32] examined the relative concentrations of the headspace volatiles and found that methacrolein (a highly volatile nonterpenoid) appeared to be inversely correlated with mule deer utilization. In our study, *vaseyana*, the most preferred subspecies by

ungulates, had the lowest mean concentration of Me_2CO and methacrolein. These nonterpenoids were major constituents in the headspace vapors of whole and ground leaves at room temp., so they may be the most volatilized component from whole plants and readily released during mastication. Methacrolein is a mucus tissue irritant [35] with an oxygen functionality. Oxygenated monoterpenes in a forage can reduce animal preference and also inhibit the metabolism of ruman microbes, possibly interfering with normal digestion [36–39].

Synergism between the volatile essential oils and other secondary metabolic products, sesquiterpene lactones and phenolics (coumarins and flavonoids), may provide a simultaneous stimulus to the olfactory and gustatory sensors of herbivores. The terpenoids and, probably, the phenolics are stored in glandular trichomes on the tissue epidermis [24, 40]. These structures at maturation are easily ruptured releasing their fluid contents; the liquid essential oil portion acting as a solvent for the sesquiterpene lactones and other nonliquid components. The volatiles released may initially interact with taste sensors while simultaneously vaporizing into the olfactory organs of animals. The nonvolatiles in the liquid would be restricted to stimulation of the gustatory organs. Sesquiterpene lactones are major sagebrush constituents [40] and, because of their wide ranging biological effects [41–43], including mammalian toxins, feeding deterrents, and insect feeding deterrents, must be suspect as part of a chemical defense mechanism.

The occurrence of these compounds in the epidermal trichomes enhances their concentrations relative to being diluted by distribution throughout the tissue. Trichome protrusion from the epidermis insures their rupture by minimal mechanical disturbance without irrepairable tissue damage or loss; possibly an important factor with insect herbivores.

Artificial ingestion of sagebrush can cause digestive disruption or death to livestock [44, 45] and the steam distilled oils inhibit rumen microbial growth and metabolism *in vitro* [27, 44, 46]. The implications are that the ingestion of sufficient quantities of sagebrush could disrupt normal digestion and, subsequently, influence plant selection. Despite these toxic and antibiotic effects, *in vitro* and *in vivo* digestibility [20, 28, 31, 47] remains high relative to other forage plants. Welch and Pederson [47] found no relationship between monoterpene content and *in vitro* digestibility. The rumen ingesta of wild mule deer and the stomach contents of pygmy rabbits contained only 20% and 23%, respectively, of the monoterpenes present in their diet. It was hypothesized that these losses were associated with one or more of the following: mastication, rumination, eructation, absorption and excretion [48, 49]. Ingestion of 3 g of monoterpenes daily for 4 days did not affect the rumen microbial activity of sheep and goats and 4 h after infusion no monoterpenes were detectable in the ruminal fluid [20]. Although sagebrush tissues and chemicals can disrupt digestion, the plants are relatively digestible, and monoterpenes under natural conditions do not seem to cause digestive problems. The influence of the non-volatiles, sesquiterpene lactones and phenolics, has yet to be examined. Sagebrush digestibility is probably a more important factor in quantitative diet selection of herbivores (total amount of sagebrush consumed) than in qualitative selection (individual shrub preference).

The concentration and chemical composition of big sagebrush essential oil varies substantially from plant to plant when influenced by it's natural environment. This puts some restrictions on the usefulness of the oils as a taxonomic character at the subspecies level, but may provide chemical heterogeneity needed to maintain an effective chemical defense. The monoterpenes in these oils do have an effect on herbivore selection of sagebrush [20], but the extent of their influence alone is difficult to evaluate and may be of limited value since they always occur in combination with other chemicals that could, by themselves or synergistically (with the monoterpenes), influence palatability. Furthermore, each class of chemicals may be differentially significant to the type of herbivore, i.e. insect vs. vertebrate. Evaluation of sagebrush chemistry as a defense against herbivory must await more thorough investigations.

Experimental

Study sites. Four study sites ranging from 946 to 2135 m elevation were selected in eastern Oregon: Millican, Baker, Frenchglen and Squaw Butte (see Fig. 1). At all sites, three distinct populations of each big sagebrush subspecies (*Artemisia tridentata* Nutt. subsp. *tridentata,* subsp. *vaseyana* (Rydb.) Beetle, and subsp. *wyomingensis* Beetle and Young) were identified and permanently marked.

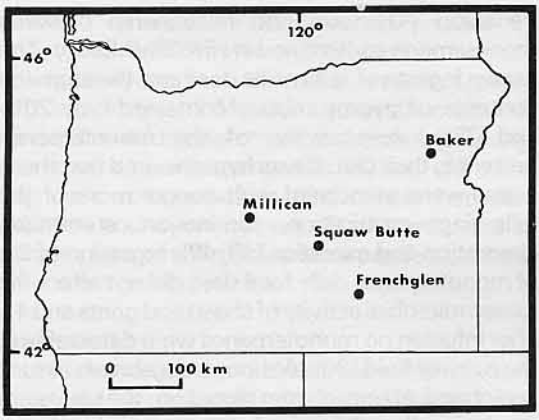

FIG. 1. LOCATION OF STUDY SITES IN OREGON.

Associated plant–soil studies in some of these populations have been described elsewhere [50, 51].

Samples for headspace analysis of essential oils. To measure the effect of subspecies genotype and geographic site on the essential oil composition, tissue samples were collected from one randomly selected mature plant in each study population (36 total) during the fall of 1979 (1–14 November inclusive).

Variation in the oil composition, due to plant age, or intraplant location was checked with additional samples from Squaw Butte. One young and one old plant were sampled in each of the three populations of *vaseyana*. Intracrown sampling (north, south, east, west and top) was repeated for three plants within one population of this subspecies. Approximately 10–50 g of leaf and twig material was collected per sample, loosely packed in an air-tight plastic container and maintained below 0° until analysed by the headspace procedure.

Samples for steam distilled essential oil concentrations. To quantify the essential oil concentrations by steam distillation approximately 100 g of leaf and twig tissues was clipped from one randomly selected mature plant in each population of the three subspecies at Squaw Butte in 1979 (21 November to 11 December inclusive). These were not the same plants sampled for the headspace analysis above, but the essential oil composition of these plants was analysed by the headspace technique for correlation with the chemical composition of the steam distilled oils.

In the fall of 1980 (9 September to 7 October inclusive) 100 g of leaves and twigs was collected from the mature plants (tagged and sampled for headspace analysis in 1979) at Baker, Frenchglen and Millican. In addition, one young and one old plant were sampled from each of the three populations of *vaseyana* at Baker, three populations of *wyomingensis* at Frenchglen and three populations of *tridentata* at Millican. The oils in these specimens were quantified by steam distillation.

Steam distillation. Frozen tissues were placed in a beaker, sprinkled with liquid N_2 and gently stirred to dislodge the leaves. Woody tissues were removed, the leaves sealed in air-tight plastic bags and returned to the freezer. Just prior to distillation they were brought to room temp. and divided (triplicate analysis for Squaw Butte, 1979; duplicate analysis for Baker, Frenchglen and Millican, 1980). A small sample was removed for a duplicate moisture determination (100° to a constant wt). The leaves were weighed and then steam distilled for 2 h, with the essential oils being trapped over a H_2O column in an ice-cooled water condenser. Excess H_2O was drained from the trap and the oils transferred to a pre-weighed vial with screw cap. The last drops of H_2O in the oil were removed with a syringe and the final traces eliminated by placing the open vial into a $CaCl_2$ desiccator for 15 h. The vial was sealed, weighed and the yields calculated as percent dry wt. The triplicate oil samples from Squaw Butte, 1979, were combined after weighing, flushed with N_2 and stored in the freezer until analysed by GLC.

Headspace analysis. Our procedure was a modification from Scholl *et al.* [32]. Leaves, frozen with liquid N_2, were dislodged from the stems, separated from the woody tissue and ground to a fine powder. Approximately 1.0 g was placed into a previously weighed 50 ml hypovial, reweighed, sealed with a rubber septum and stored in the freezer at least overnight (each sample analysed in triplicate). Hypovials were transferred from the freezer to a 100° ± 4° oven for 30 min. A gas-tight syringe with the plunger vol. set at 1.0 ml was inserted through the septum into the gaseous atmosphere of the vial for the last 5 min before GLC injection. The tip of the needle was sealed (a feature of the syringe), the plunger compressed to 0.8 ml and the gas injected into the GLC. A 1.8 m × 3.2 mm stainless steel column packed with 10% Carbowax 20M coated on 80/100 mesh CWHP, 30 ml/min N_2 carrier, 50–200° at 10°/min, FID, digital integration. After injection the hypovial was opened and returned to the oven for 24 h, cooled in a desiccator and weighed. The peak areas were recorded as integrator counts, then normalized for variation in GLC detector response (measured daily by averaging triplicate 0.5 μl injections of EtOAc), leaf wt and hypovial vol. These were averaged for triplicate analysis of each plant to give final peak areas in normalized integrator count/unit vol. of vapor. Individual compounds were identified by R_t, co-injection of standard references and GLC-MS. Spectra were identified by comparison to published spectra [52, 53] and those obtained from standard reference samples.

GLC of steam distilled oils. The steam distilled essential oils from the Squaw Butte plants collected in 1979 were examined by GLC (triplicate 0.25 μl injections, same conditions as in the headspace analysis above). The quantities were normalized for variation in detector response (EtOAc standard) and averaged giving peak areas in integrator counts/vol. For statistical analyses these were converted to counts/g dry wt by multiplying each compound with the inverse of its density and the wt% of the total oil in the plant.

Statistical and cluster analysis. A nested classification analysis of variance (ANOVA) was used to compare concentrations of steam distilled essential oils between subspecies at Squaw Butte in 1979, and between subspecies, geographic sites, and age groups for plants collected in 1980 at Baker, Frenchglen and Millican. Correlation between the chemical composition of the steam distilled oils and the headspace vapors was made with straight line regression analysis. The nonparametric Kruskal–Wallis one-way analysis was used to compare the composition of headspace vapors for subspecies, geographic sites, age groups and intraplant location. The significantly different means were recognized using

Dunn's multiple comparisons test with an experiment-wise controlled error rate, $\alpha = 0.05$.

The computer program used for cluster analysis was BMDP 2M–Cluster Analysis of Cases [54]. It was initially performed with the 16 headspace constituents from all plants (54 total) sampled in 1979. It was rerun using only the six compounds that were significantly different between subspecies.

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