Use of UV absorption for identifying subspecies of *Artemisia tridentata*

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Abstract

Use of UV absorption spectra for identifying subspecies of *Artemisia tridentata* Nutt. was investigated by analyzing the relative optical densities of alcohol extracts from herbarium and fresh plant material at 240 nm, 250 nm, and 265 nm. In all but 1 comparison, mean relative optical densities were significantly different (p=0.95) between subspecies, but intraplant and intrasubspecies variation and overlap was found to be too large to permit use of UV absorbance alone for identifying individual specimens. These results held whether dry or fresh leaves were extracted, or whether methanol or ethanol was used as the extracting solvent.

Key Words: big sagebrush, phenotypic expression, chemotaxonomy

Recognition of major evolutionary trends (formally established as taxonomic subspecies) within *Artemisia tridentata* Nutt. has led to a better understanding of the ecology of big sagebrush (Gifford et al. 1979, Winward 1980, Tisdale and Hironaka 1981, Hironaka et al. 1983), but subspecific identification based on morphological features is not always easy (Winward and Tisdale 1977, Tisdale and Hironaka 1981, Hironaka et al. 1983). Several workers have reported, however, that UV fluorescence or absorption characteristics of tissues or extracts may be valuable in identifying subspecies of *A. tridentata* (Tisdale and Hironaka 1981, Shumar et al. 1982, Hironaka et al. 1983). Subjective evaluations of UV properties is a major limitation in their use, but Shumar et al. (1982) recently published some quantified spectral descriptions of alcohol leaf extracts. Their results indicated that spectra between 220 nm and 270 nm are consistent and unique for each subspecies and might be used as the sole criterion for identification. Their results were the same for fresh or dried material, although their main findings were based upon extracts from fresh leaves.

Following this report, we attempted to use similar methods for identifying various specimens of big sagebrush, but in preliminary trials, we were not consistently able to obtain distinctive subspecific spectra. Subsequent investigations suggest that UV spectra may not always be suitable as the sole criterion for specimen identification. In these investigations we evaluated intraplant variation as well as interplant differences within subspecies. In contrast to Shumar et al.'s use of ethanol (EtOH) and fresh leaves, we primarily used methanol (MeOH) as an extracting solvent and dry material from herbarium specimens, but additional studies indicate that these differences in techniques do not account for the differences in the results.

Materials and Methods

Subspecific identifications were made using the morphological criteria presented in Winward (1980). Specimens from the University of Idaho Herbarium (ID) were used as the source of dried leaves (Table 1); 23 specimens of *Artemisia tridentata* Nutt. subsp. *tridentata*, 31 of *A.t.* subsp. *vaseyana* (Rydb.) Beetle, and 8 of *A.t.* subsp. *wyomingensis* Beetle & Young. Twelve duplicates of the above taxa plus 2 specimens of uncertain identity were used for investigating intraplant variation and for comparing the 2 methods of extraction (MeOH vs. EtOH). In the first case, 3 subsamples from each species were used, while in comparing solvents, material from 2 leaves was divided between the 2 solvent systems for each paired sample. In all cases a single leaf was found to provide sufficient material.

Limited studies were also done with fresh material of subsp. *tridentata* for intraplant and intrataxon comparisons. In this case, 6 single-leaf subsamples were obtained and extracted from 5 individuals from each of 2 populations near Lewiston, Idaho (Table 1).

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Table 1. Source of leaf material. All dried material from University of Idaho Herbarium (ID). Specimens are listed by location, collector and number. Numbers appearing in () are ID accession numbers in lieu of a collector's number.

Dried Material

A. tridentata subsp. *tridentata*

USA. **California:** Riverside Co., Hendrickson 4257. **Idaho:** Ada Co., Keithly 19; Bingham Co., Lyman s.n.(8028); Cassia Co., Biggers s.n.(8826), Wellner 3007; Gooding Co., Wellner 2911; Idaho Co., Johnson 77227; Lewis Co., Sattler 13; Oneida Co., Richardson 47; Owyhee Co., Henderson 5571, Tisdale s.n.(65312); Valley Co., Henderson 5239. **Montana:** Carbon Co., Booth 54574; Park Co., Suksdorf 993.

Nevada: Elko Co., Kinnaman s.n.(30846). **Oregon:** Deschutes Co., Steward 6849; Malheur Co., Tisdale s.n.(69162). **Wyoming:** Natrona Co., Dueholm 8500.

CANADA. **British Columbia:** Anonymous (63051).

A. tridentata subsp. *vaseyana*

USA. **Colorado:** Routt Co., Christ 1420. **Idaho:** Adams Co., Bingham 245, Wellner 1678, 2066; Bear Lake Co., Wellner 2407; Butte Co., Zink s.n.(34392); Caribou Co., Wellner 3329; Clark Co., Moseley 463, Wellner 2365, 2385; Custer Co., Henderson s.n.(74140), Wellner 1563, 1956; Franklin Co., Wellner 3336; Fremont Co., Rust 728; Idaho Co., Bingham 169; Lemhi Co., Baker 14666, Henderson 5038; Valley Co., Wellner 1447, 1890A, 2688; Teton Co., Brunsfeld 2341. **Montana:** Big Horn Co., Booth 55781; Gallatin Co., Booth s.n.(38945); Madison Co., Ellison 58Q; Powell Co., Booth 55677. **Nevada:** Humboldt Co., Rosentreter 3491. **Oregon:** Klamath Co., Coombs 157; Wallowa Co., Mohan 28. **Wyoming:** Teton Co., Lowrie s.n.(24865).

A. tridentata subsp. *wyomingensis*

USA. **Idaho:** Bingham Co., Baker 9985; Butte Co., Andersen 89, Stafford s.n.(84503); Cassia Co., Baker 8784; Lemhi Co., Wellner 2000; Twin Falls Co., Wellner 2900.

A. tridentata (subspecies uncertain)

USA. **Idaho:** Franklin Co., Wellner 3350. **New Mexico:** Hardesty 22.

Fresh Material

A. tridentata subsp. *tridentata*

USA. **Idaho: Nez Perce Co., top of Lewiston Grade along hwy US 95, ca. .5 km N of Lewiston, elev. ca. 820 m; bottom of Lewiston Grade, N edge of Lewiston along hwy US 95, elev. ca. 230 m.**

Whether herbarium or fresh tissue was used, no obviously damaged or discolored leaves were included.

Extraction were made by first crushing the tissues in the bottoms of centrifuge tubes. Five millilitres of solvent (80% v/v aqueous MeOH, except in the comparison of solvents where 70% v/v aqueous EtOH was also used) were then added, and the contents were shaken vigorously for about 15 s. After this material was centrifuged at top speed in a clinical type centrifuge for 5 min., the supernatent was decanted and stored at 4° C until spectral measurements were made. Preliminary studies showed that such storage for several days altered readings by less than 1%.

Optical densities (OD) in all cases were measured against a blank containing the respective solvent in a (doublebeam) Beckman Spectronic 2000. Optical density was measured at 220 nm, 250 nm, and 265 nm in each case. As in Shumar et al. (1982), relative OD was obtained for 250 nm and 265 nm by dividing the respective OD readings at these wavelengths by the OD at 220 nm for that sample. The spectral slope between 250 nm and 265 nm, where Shumar's group found the most significant differences in their spectra, were evaluated by dividing 15 nm into the differences between the 250 nm and 265 nm relative OD values.

Means and standard deviations were computed for the individual specimens that were subsampled. Means from the subsampled herbarium material were then combined with single sample specimen's values for given subspecies to compute the means, standard deviations, and 95% confidence intervals for that subspecies. All differences were tested for significance using a standard *t*-test for unpaired data. The correlation between MeOH and EtOH extractions was assessed using linear regression. A *T*-test (Guenther 1965) was then used to ascertain if the regression slopes were significantly different from 1.00.

Subsequent to the preceding, we became aware that 250 nm might not be an appropriate wavelength for separating subspp. *vaseyana* and *wyomingensis* based upon Shumar et al. (1982). Therefore 5 leaf samples each from 4 herbarium specimens of subspp. *vaseyana* and *tridentata,* and 3 of subsp. *wyomingensis* were extracted with McOH. Optical density was then measured at 220 nm and 240 nm, and analyzed as before.

Results

Spectra obtained in these studies were similar to those of Shumar et al. (1982) with the highest OD's at 220 nm and the lowest at 265 nm. Values at 250 nm were intermediate but closer to those for 265 nm than to 220 nm.

When intraplant samples were compared, considerable variation was observed. The average plant relative OD at 250 nm was 0.817 for the 12 herbarium specimens and the mean standard deviation (SD) was ± 0.081, while the relative OD mean and SD at 265 nm was 0.685 and ± 0.078. Thus the mean SD was 10% or more of sample plant means. The relative OD values at 250 nm differed by 0.001 to 0.321 in individual specimens, and by 0.003 to 0.299 at 265 nm. Similar intraplant variation was found with all three taxa. Fresh material of subsp. *tridentata* exhibited as much variation with a mean of 0.777 and 0.701 for 250 nm and 265 nm, respectively, and mean SD's of ±0.073 and ±0.092.

Intrataxon values were also found to vary widely (Fig. 1), but SD values were generally less in comparison to intraplant SD. There was considerable overlap in values among the subspecies, but mean relative OD values proved to be significantly different ($p = 0.95$) in all cases except for subspp. *tridentata* and *vaseyana* at 250 nm and all 3 subspp. at 240 nm (Table 2). Mean spectral slopes between 250 nm and 265 nm were significantly different between all subspecies. As a whole, fresh tissue extracts of subsp. *tridentata* were not significantly different from herbarium tissue extracts (Fig. 2), but the mean relative ODs for the population at the top of the Snake River Canyon were significantly different ($p = 0.95$) from that of the population at the bottom.

Table 2. Comparisons of relative OD at 240 nm of extracts from 3 subspp. of *A. tridentata.*

subspp.	mean	range	± SD	±95% C.I.
tridentata	0.589	0.520-0.627	0.047	0.086
vaseyana	0.588	0.520-0.714	0.087	0.160
wyomingensis	0.610	0.523-0.664	0.231	0.231

Correlation between the MeOH and EtOH extractions of similar tissues were poor to moderate (Table 3), but this may reflect intraplant variation. More importantly, intraspecific variation was as large among the EtOH extracts as it was among the MeOH extracts, e.g., EtOH extracts from 4 subsp. *tridentata* specimens yielded a mean relative OD of 0.691 and a SD of ± 0.192 compared to the mean of 0.543 and a SD of ± 0.126 for MeOH extracts. In

Table 3. Linear regression equations of MeOH extracts on EtOH extract spectra values of 10 herbarium specimens.

Relative OD at 250 nm: MeOH = 0.39 EtOH + 0.29	($r^2 = 0.33$)
Relative OD at 265 nm: MeOH = 0.40 EtOH + 0.19	($r^2 = 0.66$)
Relative spectral slope from 250 nm to 265 nm: MeOH = 0.93 EtOH + 0.001	($r^2 = 0.0631$)

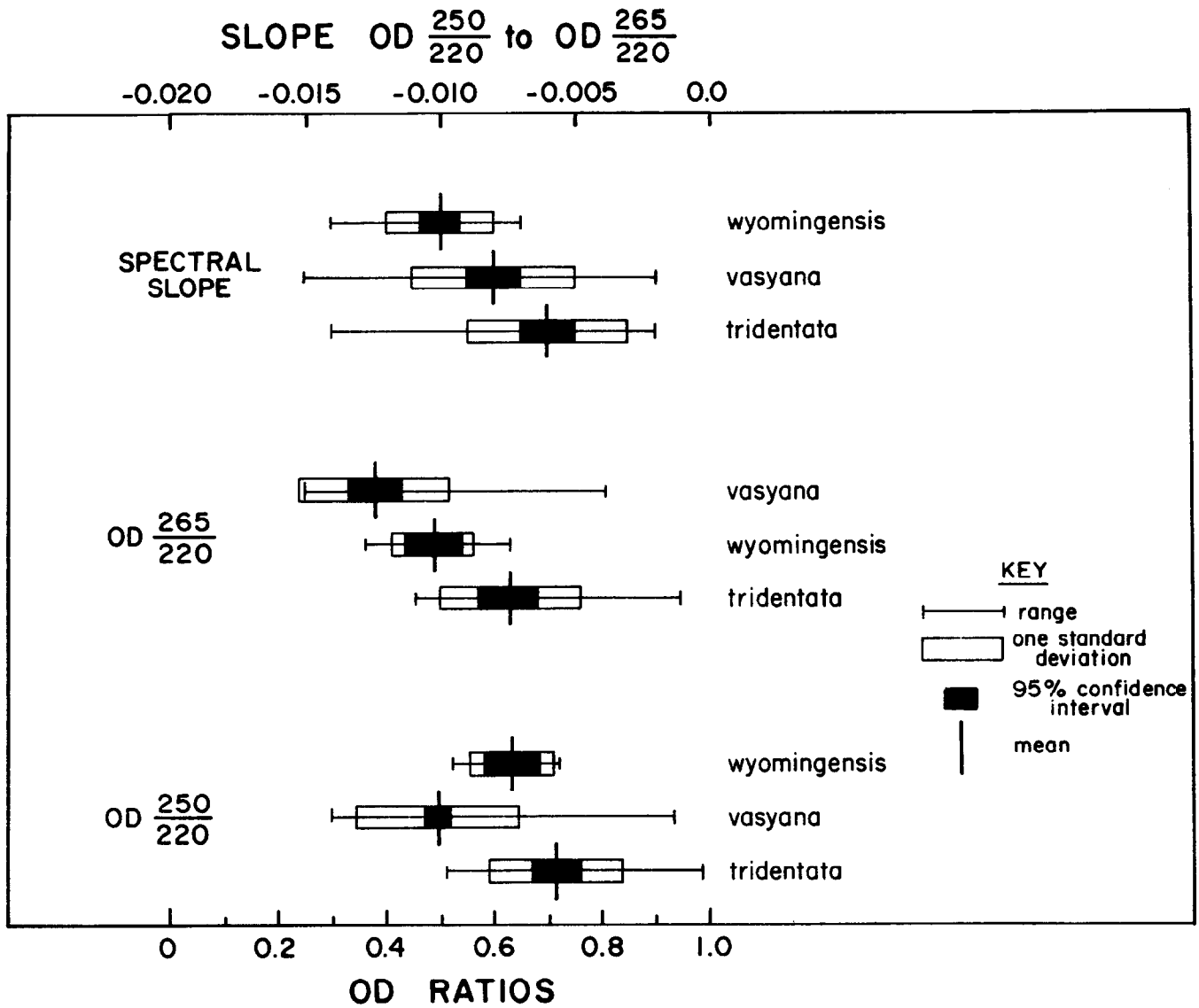

Fig. 1. *Comparisons of combined relative optical density measurements among the 3 subspecies of* Artemisia tridentata. *(N = 23, 31, and 8 for subsp.* tridentata, vaseyana *and* wyomingensis, *respectively.*

both cases SD was about 20% of the mean values. In general, EtOH had higher relative ODs than MeOH extracts (i.e., less difference between ODs at 220 nm and those at 250 nm and 265 nm), but the regression slopes of EtOH vs. MeOH OD's did not prove to be significantly different from 1.00 ($p = 0.95$).

Discussion and Conclusions

The results above indicate that, similar to many taxonomic features, UV absorbance is probably too variable to be used alone for positive subspecific identification of individual specimens in *Artemisia tridentata.* The variation may be due to phenotypic expression in the extracted materials (terpenes) in response to current local conditions. Thus the wide geographic and seasonal diversity represented in herbarium specimens could also represent a wide variety of growing conditions and phenotypic responses. Phenotypic variability could also explain the differences found between 2 Snake River Canyon populations, which, at any one time, usually experience rather different habitat conditions.

On the other hand, there were definite trends in values as seen in the significantly different subspecific means. Consequently, UV absorbance could be a valuable taxonomic tool when used in conjunction with other features for comparing populations in adjacent habitats, or perhaps for identifying and separating whole populations. In individual cases, however, it may be best to use a combination of morphological features coupled with UV spectra for reliable identification, as suggested by Goodrich et al. (1985) and Winward (pers. comm.). It may also be possible to obtain more definitive UV absorption spectra by refining and separating the extracts before examining the spectra. Certainly more work in this area seems warranted.

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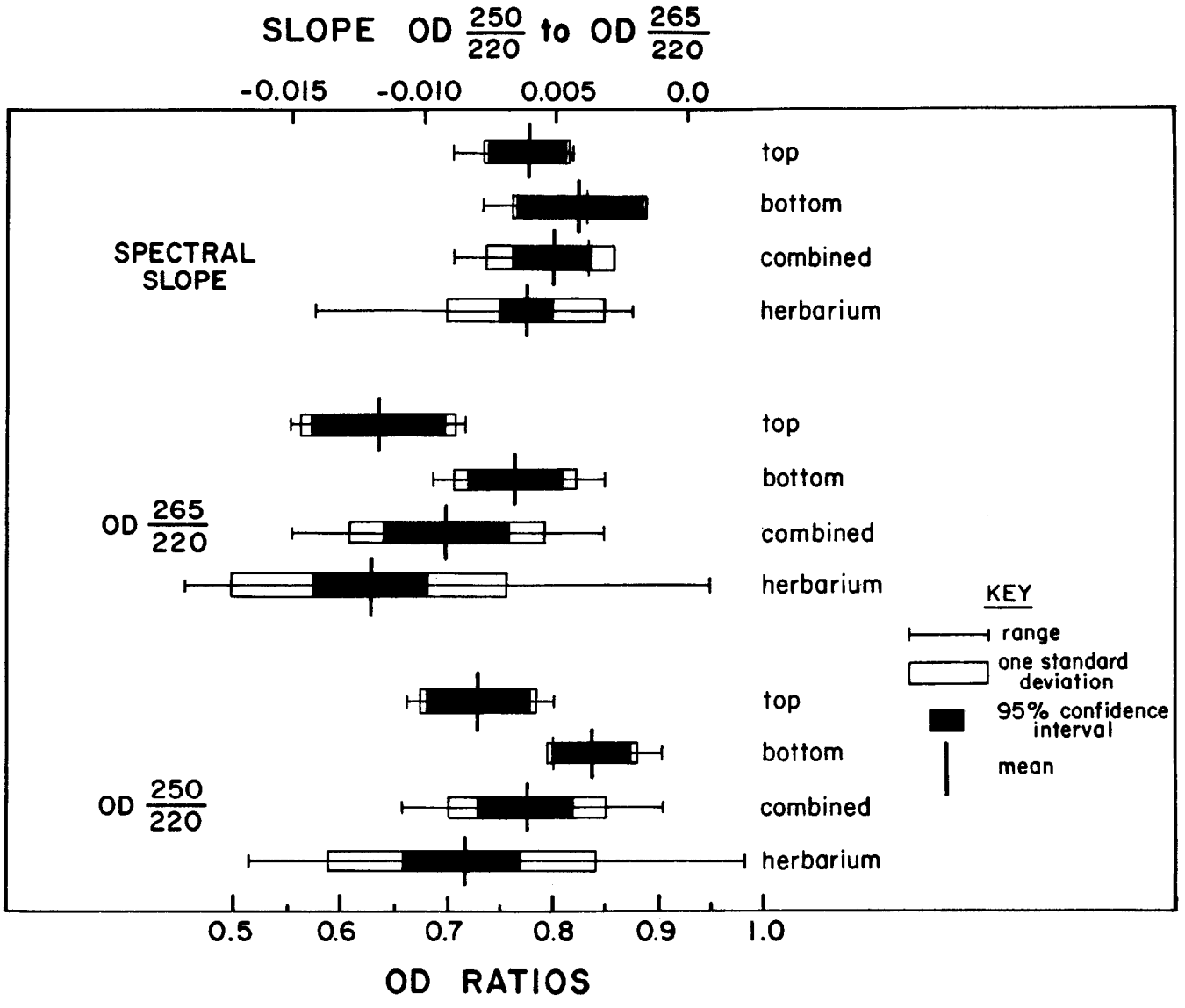

Fig. 2. *Comparisons of relative optical density measurements from fresh tissues and (dry) herbarium material of* A. tridentata *subsp.* tridentata. *Fresh material represents 2 populations (N = 5 each), one each at the top and bottom of the Snake River canyon near Lewiston, Id. Results are presented for the populations individually and combined for comparison with herbarium samples (N = 23).*

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