

Identification of Subspecies of Big Sagebrush by Ultraviolet Spectrophotometry

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Abstract

The three subspecies of big sagebrush (*Artemisia tridentata*) are dominant shrubs over much of the Intermountain West. Because the subspecies differ in palatability and habitat requirements, researchers and resource managers have become increasingly concerned with their identification. Subspecies have been identified by leaf morphology, ultraviolet (UV) fluorescence, or chromatography. Fluorescence of leaf extracts under short-wave UV light provides a convenient technique for distinguishing between *A.t. vaseyana* and the other two subspecies, but this technique will not distinguish between *A.t. tridentata* and *A.t. wyomingensis*. Chromatographic techniques can differentiate between all of the subspecies, but the methods are tedious. We describe a technique for distinguishing all three subspecies by UV spectrophotometry. Alcohol leaf extracts of the three subspecies produce relative absorbance graphs that differ markedly from one another between 230 and 280 nm.

Shrubs of the big sagebrush (*Artemisia tridentata* Nutt.) complex are dominant in the vegetation over much of the Great Basin and the arid plains and valleys of the Intermountain West. Recent studies have contributed to our understanding of the evolutionary history and phylogenetic relationships of the members of this complex (Beetle 1960, Beetle and Young 1965, Winward 1970, Hanks et al. 1973, McArthur and Plummer 1978) and emphasized the differences in palatability among the various species and subspecies (Brunner 1972, Hanks et al. 1973, Schlatterer 1973, McArthur and Plummer 1978). Within the species *A. tridentata*, three subspecies and three forms of the subspecies *A.t. vaseyana* are generally recognized (Winward 1970, Schlatterer 1973, Winward and Tisdale 1977). These are: *A.t. tridentata*, *A.t. wyomingensis*, *A.t. vaseyana* form *vaseyana*, *A.t. vaseyana* form *spiciformis*, and *A. tridentata* "X" (Winward and Tisdale 1977). Because the subspecies differ markedly in palatability, range managers and researchers have become increasingly concerned with their identification. In some instances, however, the variability in morphology makes subspecies and forms difficult to differentiate (West et al. 1978).

Subspecies of big sagebrush have been identified by leaf morphology (Winward 1970, Brunner 1972, McDonough et al. 1975, Winward and Tisdale 1977), phenology (Winward and Tisdale 1977), or chemotaxonomic methods involving ultraviolet fluorescence (Winward and Tisdale 1969, Stevens and McArthur 1974) or

chromatography (Holbo and Mozingo 1965, Winward 1970, Brunner 1972, Hanks et al. 1973). Fluorescence under long-wave ultraviolet light of leaf extracts in water or alcohol provides a convenient field or laboratory technique for distinguishing *A.t. vaseyana* from either *A.t. wyomingensis* or *A.t. tridentata* but it will not discriminate between the latter two subspecies (Winward and Tisdale 1969). Chromatographic techniques can differentiate between all of the subspecies, but the methods are time-consuming and tedious by comparison, and at least one (Winward 1970) involves the use of benzene, which is thought to be a carcinogen.

Studies in our laboratory indicate that alcohol extracts from leaves of the three subspecies produce characteristic ultraviolet absorbance patterns. This paper describes the spectrophotometric technique and presents typical absorbance spectra for the three subspecies.

Materials and Methods

Fifty-two samples of *A.t. vaseyana* and 125 samples of *A.t. tridentata* were collected from various locations in southeastern Idaho, northern Utah, and southwestern Montana. Over 620 samples of *A.t. wyomingensis* were taken from the Idaho National Engineering Laboratory Site in southeastern Idaho. Additional samples from herbarium specimens of the various subspecies were analyzed. Each field sample consisted of 5–10 g of leafy material,

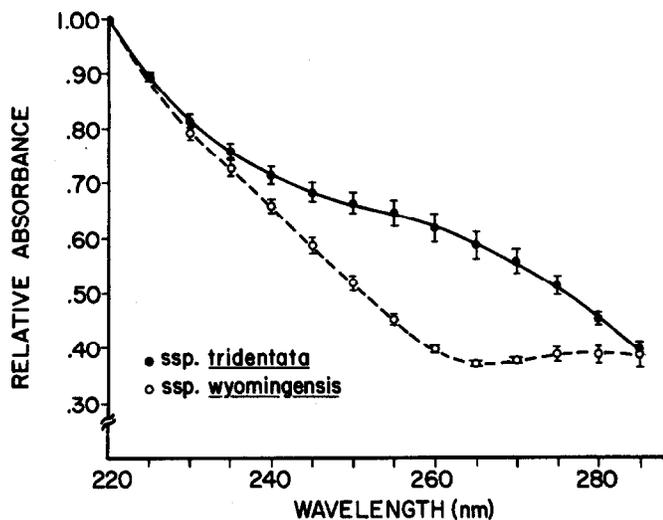


Fig. 1. Relative absorbance as a function of wavelength for leaf extracts of *Artemisia tridentata* tridentata and *A.t. wyomingensis*. Vertical bars indicate 95% confidence intervals based on standard errors for 30 samples chosen at random from the total pool of samples that were analyzed for each subspecies.

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collected by clipping one or more peripheral branchlets from an individual sagebrush plant. Samples were first identified by morphological analysis (Brunner 1972, Hanks et al. 1973, McDonough et al. 1975, Winward and Tisdale 1977) and the fluorescence technique (Winward and Tisdale 1969).

For spectrophotometric analysis, approximately 0.1 g of leaf material was ground with a mortar and pestle, mixed with 10 ml of 70% ethanol, and allowed to stand for 15 minutes. The mixture was then filtered through Whatman No. 1 filter paper in a Büchner funnel. Approximately 0.3 ml of the green- to yellow-colored extract was diluted with 10 ml of 70% ethanol. Absorbance was then measured at 5-nm increments from 220–285 nm inclusive with a Beckman DU spectrophotometer equipped with a Gilford Model 220 optical density converter and absorbance indicator. Absorbance values varied depending upon the concentration of the extract. Therefore, relative absorbance values were calculated by setting the maximum observed value for each sample equal to one.

Results and Discussion

Samples of *A.t. tridentata*, *A.t. wyomingensis*, and *A.t. vaseyana* consistently produced relative absorbance graphs that differed markedly from one another (Figs. 1 and 2). We have

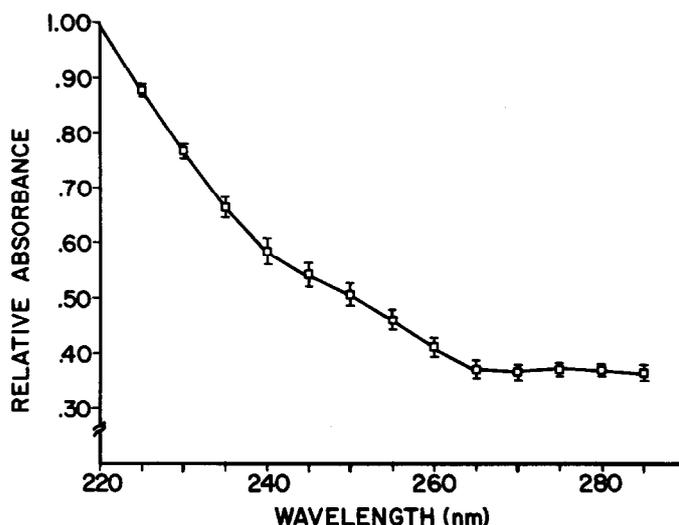


Fig. 2. Relative absorbance as a function of wavelength for leaf extracts of *Artemisia tridentata vaseyana*. Vertical bars indicate 95% confidence intervals as described for Figure 1.

shown the three patterns on two graphs in order to depict the confidence intervals for each subspecies clearly and to emphasize the striking contrast between *A.t. tridentata* and *A.t. wyomingensis*. *A.t. tridentata* always produced a plateau in relative absorbance between 240 and 270 nm and then descended rapidly beyond 270 nm. In contrast, relative absorbance for *A.t. wyomingensis* decreased almost linearly from 220 to 265 nm and remained almost constant beyond 265 nm (Fig. 1). Values for *A.t. vaseyana* decreased more rapidly than those for *A.t. wyomingensis* between 220 and 240 nm but did not differ significantly from those for *A.t. wyomingensis* beyond 240 nm (Figs. 1 and 2). Relative absorbance values did vary somewhat from sample to sample, but as shown by the confidence intervals, the pattern was consistent for each subspecies. The results were the same regardless of whether fresh or dried leaf material was used. Likewise, use of ephemeral or persistent leaves yielded similar absorbance graphs.

All samples produced consistent patterns for their respective subspecies, as identified by leaf morphology and the ultraviolet fluorescence test, except for five of 33 samples from a stand of what was tentatively identified as *A.t. vaseyana* in Curlew Valley, Idaho.

These samples produced absorbance patterns that were similar to *A.t. tridentata* from 220 to 245 nm, but the absorbance then decreased rapidly, becoming constant after 265 nm as did *A.t. wyomingensis* and *A.t. vaseyana* (Fig. 3). In addition to the unusual relative absorbance patterns, these Curlew Valley samples had leaf morphologies that made positive identification difficult, and they showed a very pale bluish-cream fluorescence under ultraviolet light, rather than the intense bluish-cream characteristic of other samples of *A.t. vaseyana*. These observations suggest that these five samples were from hybrids between *A.t. tridentata* and *A.t. vaseyana*.

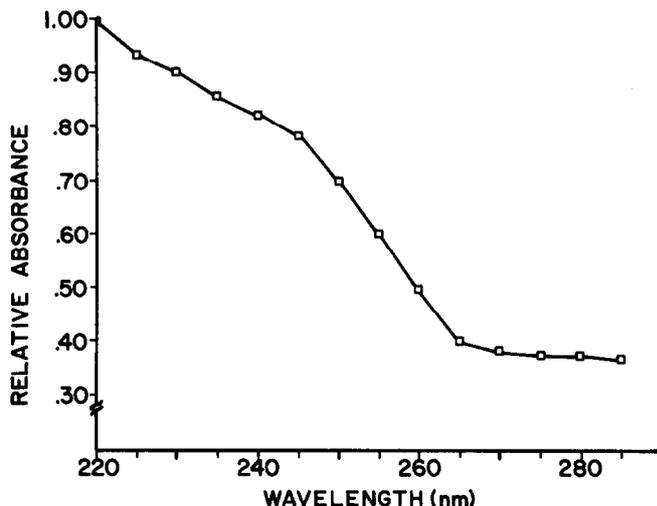


Fig. 3. Pattern of relative absorbance for samples of *Artemisia tridentata* which are thought to be hybrids between *A.t. vaseyana* and *A.t. tridentata*.

Stevens and McArthur (1974) attempted to quantify the fluorescence nature of sagebrush by using a spectrophotometer to measure transmittance through leaf extracts at 364 nm. Their test revealed no differences between *A.t. tridentata* and *A.t. wyomingensis* at that wavelength. We obtained similar results at wavelengths greater than 320 nm; however, the patterns for those subspecies between 240 and 280 nm are strikingly different (Fig. 1). Thus, the spectrophotometric technique appears to be particularly useful in discriminating between *A.t. tridentata* and *A.t. wyomingensis*, the two taxa that are most difficult to separate by any other technique.

An experienced technician can prepare a sample and measure its absorbance values in 20 to 30 minutes using a manual spectrophotometer. We conclude that the technique provides a convenient and rapid method for differentiating between the three subspecies of *A. tridentata*.

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