Chromatographic Recognition of some Palatable and Unpalatable Subspecies of Rubber Rabbitbrush in and around Utah

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Highlight: *Paper chromatography is useful in identifying palatable and unpalatable subspecies of rubber rabbitbrush in Utah. Methanol extraction of dried foliar material followed by two-dimensional (n-butanol:acetone:water, 4:1:3; acetic acid:water, 15:85) ascending paper chromatography reveals distinctive patterns for* Chrysothamnus nauseosus *subspecies* albicaulis, salicifolius, graveolens, *and* consimilis. *Patterns for rarer subspecies* junceus *and* hololeucus *are not as well defined. Gene exchange between subspecies is demonstrated by chemical markers, the chromatographic spots, in putative hybrids and introgressents. Range utilization of current growth by browsing animals is highest for subspecies* albicaulis *and* salicifolius, *less for subspecies* graveolens, *and least for subspecies* consimilis. *Populations of other subspecies introgressed by* consimilis *receive limited browsing.*

Paper chromatography will make it possible to select palatable subspecies of rubber rabbitbrush for range improvement projects and nonpalatable subspecies for areas where animal use is to be discouraged.

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Rubber rabbitbrush (*Chrysothamnus nauseosus*), is widely distributed in western North America. It is most abundant in the central portion of its range, the Great Basin area including all of Utah, where it is a common shrub on plains and foothills and in mountain valleys (Hall and Clements, 1923). The many subspecies, ecotypes, and biotypes of the rubber rabbitbrush species complex occur on a wide variety of soils and sites; these range from heavy clay and alkali soils of salty lowlands to more neutral to acid soils of higher sagebrush, juniper-pinyon, and ponderosa pine zones (Plummer et al., 1968).

Rubber rabbitbrush is a rapidly maturing, nutritious shrub (Leach, 1956; Dietz et al., 1962; Sampson and Jespersen, 1963); however, its forage value may vary with subspecies, ecotype, and season. Big game and livestock use it slightly to moderately throughout much of the summer. During late summer and fall, most classes of livestock and game will browse flowers and, occasionally, a few leaves and more tender stems. On fall and winter ranges, the current growth of some subspecies is often heavily utilized (Fig. 1), whereas other forms are browsed much less, if at all (Plummer et al., 1968).

Rubber rabbitbrush is noted for infraspecific variation (Anderson, 1966). Hall, the latest monographer, recognized 22 subspecies (Hall and Clements, 1923), but also noted (Hall, 1919):

> Every autumnal excursion into a new district brings to light one or more forms not previously described. The only limits set to the number of new species or varieties which might be set up lie in one's ability to visit all parts of the field during the flowering period and the failure or disinclination to recognize minute variations.

Nevertheless, most Utah populations of rubber rabbitbrush can be referred to the subspecies *albicaulis, graveolens, consimilis,* and *salicifolius.* Subspecies

consimilis characteristically grows on drier lowlands where drought and alkali may be restrictive to the other three subspecies. However, *consimilis* also occurs on foothills with *graveolens* and, less frequently, with *albicaulis*. In many dry desert sites, it is found closely associated with subspecies of yellowbrush (*C. viscidiflorus*). Subspecies *albicaulis* is fundamentally a foothill type that meets and intergrades with *salicifolius* at higher elevations and with *graveolens* and *consimilis* on lower sites. Subspecies *salicifolius* generally occurs on mountain areas and is most prevalent in mountain brush and ponderosa pine zones. Unlike other subspecies, it exhibits some tolerance to shade. All forms establish abundantly on disturbed areas and are constituents of adjoining stabilized cover.

The foliage of subspecies *consimilis* and *graveolens* is green to yellow green. Subspecies *consimilis* has almost threadlike leaves less than 1.0 mm in width. In contrast, the leaves of *graveolens* are 1.0 to 3.0 mm wide. The foliage of *albicaulis* is light green to white; its pubescence varies greatly between ecotypes. Subspecies *salicifolius* specimens range from bluish green to light green and appear to intergrade into *albicaulis*. Subspecies *salicifolius* leaves are 3.0 to 10.0 mm wide, whereas those of *albicaulis* are usually 0.5 to 1.5 mm wide, rarely as wide as 3.0 mm. Two less common Utah subspecies are *junceus* and *hololeucus*. Subspecies *junceus* is a nearly leafless, yellow-green subspecies adapted to sandy areas in the Colorado River drainage. Subspecies *hololeucus* is a Great Basin foothill and valley form. Only cryptic flower and achene differences distinguish it from subspecies *albicaulis*.

Subspecific identification is complicated by (1) apparent intermediate forms and (2) deterioration and/or loss of floral structures from late winter until the next flowering cycle in late summer and autumn. This study reports the development of a laboratory method that correlates chromatographic patterns with browsing preferences of big game and livestock and identifies the subspecies of rubber rabbitbrush most common in Utah. The success of such an approach in sagebrush has

Fig. 1. *Sheep browsing* Chrysothamnus nauseosus *subspecies* albicaulis *near Ephraim, Utah, during the winter of 1961.*

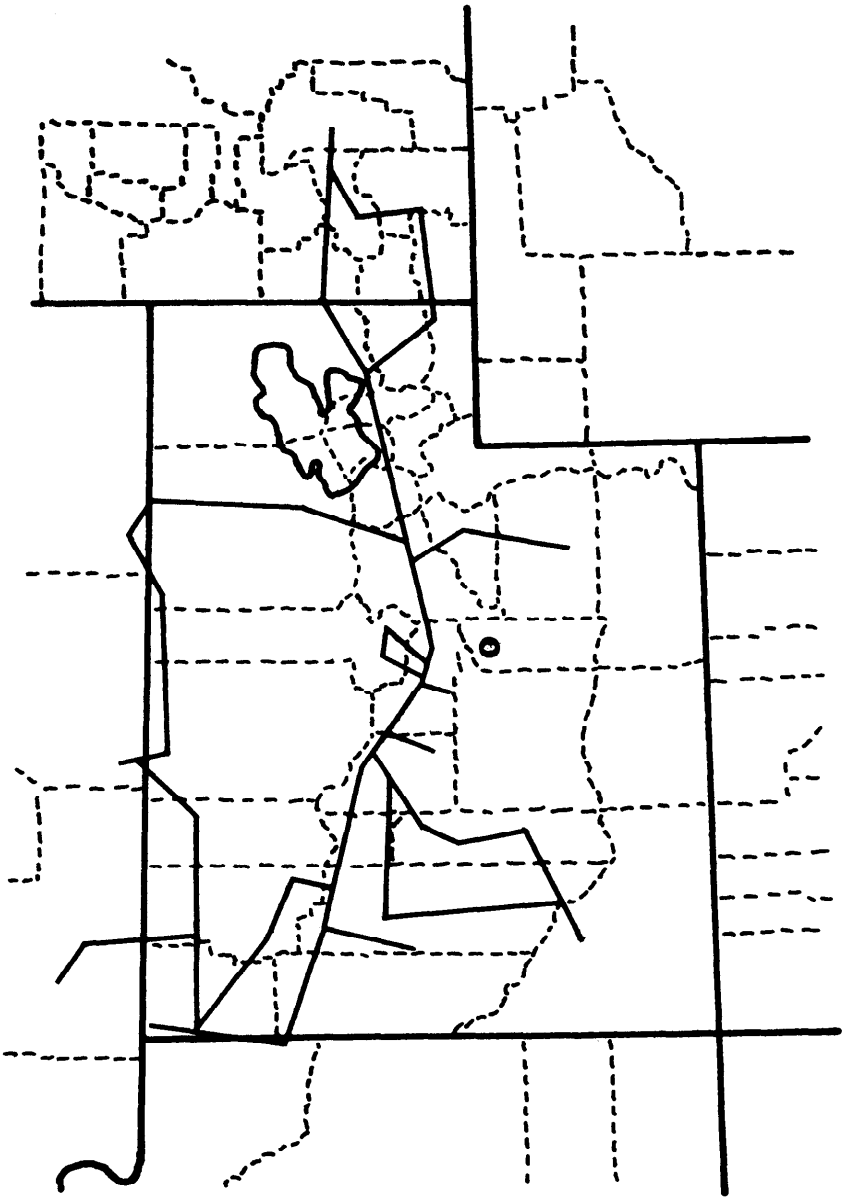

Fig. 2. *Lines show approximate collection transects for rubber rabbitbrush populations studied. The* ⊖ *is deer winter range transplant site.*

Table 1. Chromatographic spot properties and occurrence.

Spot no.	R_{f_1} (×100)[1]	R_{f_2} (×100)[2]	Color: Ultraviolet light	Color: Ultraviolet light + ammonia vapor	Color: Visible light + ammonia vapor	Frequency of occurrence[3]: *Chna*[a]	*Chna*[g]	*Chna*[c]	*Chna*[s]	*Chna*[j]	*Chna*[h]
20	46 ± 04	08 ± 02	gold	gold	–	0.87	0.35	0.00	0.35	0.17	1.00
27	64 ± 04	04 ± 02	gold	gold	–	.05	.21	.52	.10	.17	.40
30	78 ± 06	03 ± 03	gold	gold	–	.35	.07	.29	.15	.17	.20
31	87 ± 06	08 ± 03	–	blue	–	.21	.30	.29	.15	.00	.00
32	93 ± 02	07 ± 01	–	blue green	–	.02	.16	.33	.10	.00	.40
33	78 ± 03	17 ± 03	whitish	tan	–	.00	.12	.74	.05	.00	.00
34	92 ± 03	14 ± 05	violet	brown	yellow	.02	.19	.60	.05	.17	.00
35	84 ± 04	23 ± 04	–	light blue	–	.19	.05	.17	.05	.00	.00
40	31 ± 03	61 ± 04	pink	pink	–	.98	1.00	1.00	1.00	1.00	1.00
50	41 ± 05	44 ± 03	violet	gold	yellow	.97	.93	.86	1.00	.17	1.00
51	52 ± 03	37 ± 03	blue	yellow green	grey	1.00	1.00	1.00	1.00	1.00	1.00
52	50 ± 03	52 ± 04	blue	yellow green	grey	1.00	.96	.98	.90	1.00	.80
54	56 ± 04	57 ± 04	violet	gold	yellow	.50	.74	.88	.75	.50	.20
55	66 ± 07	52 ± 06	–	violet	–	.07	.11	.31	.00	.50	.00
60	82 ± 02	61 ± 05	blue	blue	–	.23	.43	.35	.25	.33	.20
61	87 ± 04	66 ± 03	blue green	blue green	–	.50	.53	.64	.40	.83	.40
70	18 ± 04	85 ± 04	–	blue	–	.07	.19	.14	.10	.17	.20
71	28 ± 04	84 ± 03	–	blue	–	.87	.98	1.00	1.00	1.00	1.00
80	39 ± 04	73 ± 02	blue	yellow green	yellow brown	1.00	1.00	1.00	1.00	1.00	1.00
81	43 ± 05	80 ± 04	blue	blue green	–	1.00	.95	.98	1.00	1.00	1.00
82	54 ± 03	75 ± 03	blue	blue	–	.94	.98	1.00	.95	1.00	1.00
83	55 ± 04	86 ± 04	blue	blue	–	.34	.58	.74	.50	.50	.40
90	70 ± 04	78 ± 04	–	blue green (diffuse)	–	.16	.30	.29	.40	.50	.60
91	90 ± 04	82 ± 04	violet	dark brown	–	.44	.30	.33	.55	.00	.60

[1] N-butanol:acetone:water, 4:1:3 (v/v/v).
[2] 15% acetic acid.
[3] Abbreviations for *Chrysothamnus nauseosus* subspecies: *Chna*[a] = *albicaulis* (62 samples); *Chna*[g] = *graveolens* (57 samples); *Chna*[c] = *consimilis* (42 samples); *Chna*[s] = *salicifolius* (20 samples); *Chna*[j] = *junceus* (6 samples); and *Chna*[h] = *hololeucus* (5 samples).

been demonstrated (Hanks et al., 1971; Hanks and Jorgensen, 1973; Hanks et al., 1973).

Materials and Methods

About 200 samples of rubber rabbitbrush were collected during the early summer of 1972. Populations were sampled on a roughly north-south transect running through Utah from Bingham County, Idaho, to Mohave County, Ariz. Smaller transects were run off from the main transect (Fig. 2). A few samples were collected from mature transplants growing in experimental plots west of Helper and north of Ephraim, Utah.[1] The taxonomic keys of Hall (Hall and Clements, 1923) and Anderson (1973) were used to identify specimens.

Animal use of the current year's growth was determined for the bushes selected for chromatographic studies. This use was determined in the field at the time of collection by ocular estimate of the green weight of forage removed. A use-scale was employed in assessing the degree of browsing: 0 = < 10% use; 1 = 10-20% use; and 9 = 90-100% use.

Plant foliage from representative individual bushes was placed in paper bags and allowed to air dry. Only mature leaves on mature plants were sampled; studies on a variety of plant groups indicate that younger plants and/or leaves often produce different chromatographic patterns than mature leaves on mature plants (Urban, 1959; El-Basycuni and Towers, 1964; Asker and Frōst, 1972). Samples were adequately ventilated while drying to prevent extensive discoloration of foliage. Methanol extraction of phenolic compounds was effected by the method described by Hanks et al. (1971). Twenty-five μl of the methanol extract was spotted on 23-cm squares of Whatman No. 3 MM chromatography paper and chromatogrammed by means of the two-dimensional ascending method. The solvent for the first dimension was n-butanol:acetone:water, 4:1:3 (v/v/v); that for the second dimension was acetic acid:water, 15:85 (v/v). Chromatograms were observed under longwave ultraviolet light and spots were characterized according to R_f values (R_{f_1} = first dimension, R_{f_2} = second dimension) and physical appearance before and after exposure to ammonia vapor. Calculations of mean R_f values and accompanying standard deviations were based on approximately 15 measurements per spot (range = 6 to 26). Frequency of occurrence for each spot was calculated for each subspecies. Spots were numbered according to their position on the finished chromatogram. The numbering system used begins at the origin (bottom right of the chromatogram); the numbers increase from right to left in three series from the bottom to the top of the chromatograms (Fig. 3, Table 1).

Results and Discussion

Frequency of spot occurrence can be helpful in identifying the subspecies of rubber rabbitbrush (*albicaulis, graveolens, consimilis,* and *salicifolius*) most common in Utah (Table 1). Chromatographic patterns and nonfloral morphology are adequate for identification of the common subspecies. Some chromatographic variation is apparent within each subspecies that is attributable to ecotypic, edaphic, climatic, and developmental differences (Ball et al., 1967; Taylor, 1971).

A brilliant gold spot, number 20, in the bottom center of a chromatogram characterized most analyzed populations of subspecies *albicaulis* (Fig. 3). Of the few populations lacking spot 20, virtually all included a combination of spots 30, 35, and 91; all lacked spot 33, and only one exhibited spot 34.

A cluster of spots in the lower left corner characterized subspecies

[1] Respectively, deer winter range owned by the Utah Division of Wildlife Resources; Snow Field Station maintained cooperatively by Snow College and the Utah Agricultural Experiment Station.

consimilis chromatograms. Two or more of spots 27, 30, 31, 32, 33, or 34 were present in all but three sampled accessions. In fact, an average of three spots in the lower left corner was recorded for subspecies *consimilis.* Spots 27, 33, 34, and 54 are particularly characteristic of this subspecies (Table 1). None of the other subspecies exhibited the number or color-intensity of spots in the lower left corner of chromatograms. However, chromatograms of the yellowbrush (*C. viscidiflorus*) species complex are strikingly similar to those of subspecies *consimilis* (1972-73 *Chrysothamnus viscidiflorus* chromatography, D. L. Hanks and E. D. McArthur, on file at Great Basin Experimental Area, Ephraim, Utah). Subspecies *consimilis* may be the connecting link between the rubber rabbitbrush and yellowbrush (or low rabbitbrush) species complexes.

Subspecies *graveolens* exhibited the most variable chromatograms of the subspecies studied (Table 1). Spot 20 was present in most sampled populations from southeastern Utah (Wayne, Garfield, and San Juan Counties), but was usually absent in other sampled populations. Populations from northeastern Mohave County, Ariz., and southeastern Washington County, Utah, had a unique series of large, blue spots, visible under ultraviolet light, along the left side of the chromatogram. Because of their limited distribution, these spots are not shown in Figure 2 or Table 1. They are number 39, $R_{f1} = 0.93$, $R_{f2} = 0.30$; number 66, $R_{f1} = 0.95$, $R_{f2} = 0.54$; number 92, $R_{f1} = 0.84$, $R_{f2} = 0.80$. Some subspecies *graveolens* accessions show signs of hybridization or introgression with *consimilis,* as is evidenced by the presence of spots 27, 33, and 34. Other *graveolens* accessions indicate affinities with subspecies *albicaulis* by the presence of spot 20.

Spots 90 and 91 occurred more frequently in subspecies *salicifolius* chromatograms than in those of any other subspecies common in Utah. As in the case of *albicaulis, salicifolius* accessions lacked the preponderance of spots in the 30 series that characterized *consimilis* chromatograms (Table 1). The chromatographic patterns of the northern Utah (Cache and Box Elder Counties) collections had spot 20, but

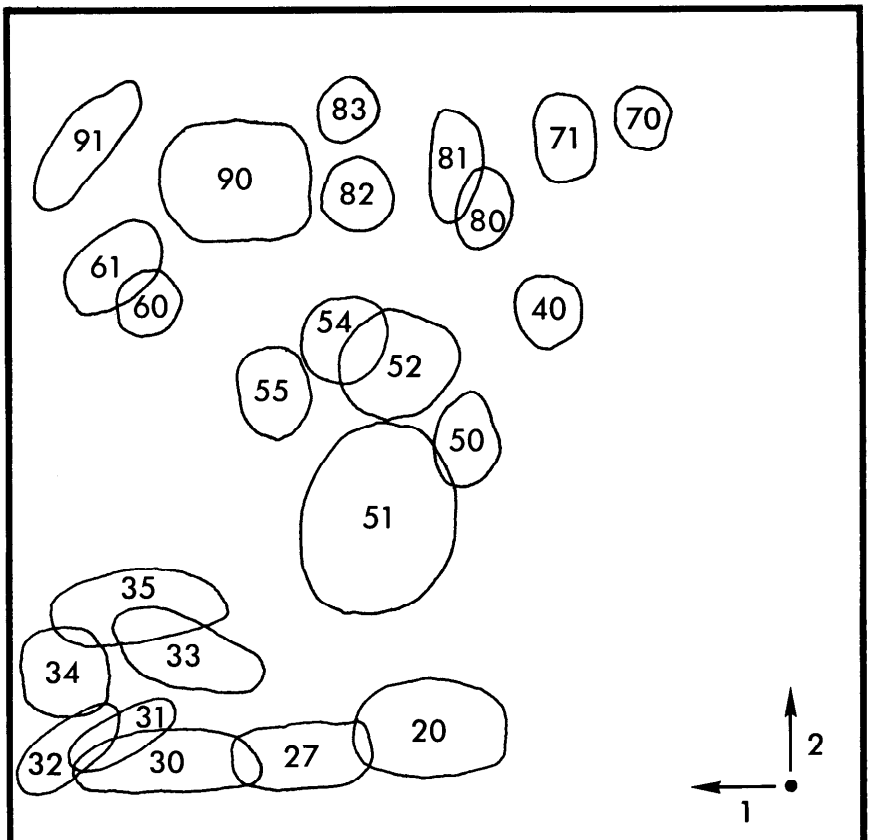

Fig. 3. *Composite two-dimensional chromatogram of methanol-soluble extracts from the leaves of rubber rabbitbrush. Spots are shown in relative size and position.*

accessions of other *salicifolius* populations show this bright gold spot only occasionally.

Too few accessions of less common subspecies *junceus* and *hololeucus* were chromatogrammed to permit conclusions. However, subspecies *junceus* is distinctive morphologically. Also, chromatograms showed spot 61 to be prominent and more common in *junceus* than in other subspecies. Chromatographic patterns observed among collections of subspecies *hololeucus* were similar to those of *albicaulis* in that spot 20 was prominent; however, the frequency of occurrence of other spots was different (Table 1).

Observations on browsing are preliminary. They were made over large areas where browsing pressure would not be expected to be uniform. However, all collections were made from rangelands under some browsing pressure by wild and/or domestic animals. These observations indicate the relative palatability of the common Utah subspecies of rubber rabbitbrush. Summer utilization of current growth reveals a marked preference for subspecies *albicaulis* and *salicifolius* (Table 2). Conversely, utilization of subspecies *consimilis* reveals low preference by browsing animals. Few accessions of subspecies *graveolens* show browsing, but those that do often reveal considerable utilization. Plant growth habits and field observations in all seasons indicate that subspecies *salicifolius* and *albicaulis* generally receive more intensive use than *graveolens* and vastly more use than *consimilis.* Although our data (Table 2) reflect utilization during the summer, the season of lowest use, additional observations made in all seasons over a period of years show subspecific preferences are constant.

A detailed study of mixed populations (*albicaulis-consimilis* and *graveolens-consimilis*) revealed considerable intergrading of plant color, leaf characteristics, and palatability. These characteristics are correlated with an intermixing of chromatographic patterns, particularly the presence of spots 33 and 34, which

Table 2. Summer (1972) browsing of current growth of subspecies and hybrids of rubber rabbitbrush in field conditions.

Use	Subspecies of *Chrysothamnus nauseosus*				
	albicaulis	*salicifolius*	*graveolens*	*consimilis*	*consimilis* introgressents
Percent showing use of current growth	29	30	9	5	22
Amount of use on those showing use (0–9 scale):					
Range	2–9	3–9	3–9	2	0–2
Mean	6.6	6.7	6.0	2.0	1.0
Sample size	62	20	57	42	9

are characteristically *consimilis* spots in plants morphologically similar to other subspecies. A notable example of intergraded characters can be observed in an *albicaulis* planting on the Utah Division of Wildlife Resources deer winter range west of Helper, Utah. There, among plants from one source, considerable variation of morphological characters and of utilization by deer was observed. Variations in morphological characteristics included color, leaf size, and pubescence that might be expected from an *albicaulis-consimilis* hybrid swarm. A plant-by-plant estimation of the degree of browsing by mule deer and observations on morphology correlated well with a chromatographic analysis; therefore, additional evidence that introgression had occurred between these two subspecies was provided. Chromatograms of shrubs browsed lightly and rated at $\leqq 2$ on the use scale contained spot 33, whereas those rated > 4 lacked this spot and most other spots that characterized *consimilis*.

Browsing preference in the subspecies studied seems to be correlated with the presence of the *consimilis*-characterizing spots in the 30 series, particularly spot 33 and, to a lesser degree, spot 34. Perhaps the phenolics associated with these spots are directly inhibitory. Longhurst et al. (1968, 1969) indicated that aversion to phenolic compounds could be a basis for grazing preference of deer. In any case, the absence of these spots in rubber rabbitbrush is linked closely to palatability. Rubber content may have an effect on palatability. Hall and Goodspeed (1919) found a relatively high rubber content in taxa referable to subspecies *consimilis* contrasted with a low rubber content in *albicaulis* (called *Chrysothamnus nauseosus* variety *speciosus* by Hall and Goodspeed).

This chromatographic study presents a relatively easy method of distinguishing the common subspecies of rubber rabbitbrush. Particular chromatographic patterns are associated with the palatability of subspecies and permit easier selection for specific purposes. Subspecies *salicifolius* and *albicaulis* would be preferred for range improvement or restoration projects, whereas *consimilis* should be chosen for roadside planting and other areas where animal density and/or traffic is to be discouraged.

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