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EFFECTS OF ORGANIC SOLVENTS ON USE OF TARBUSH BY SHEEP

R.E. ESTELL,* D.M. ANDERSON, and K.M. HAVSTAD

USDA-ARS, Jornada Experimental Range
Las Cruces, New Mexico 88003-0003

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Abstract—Tarbush (*Flourensia cernua* DC) is a native perennial shrub prevalent in many parts of the Chihuahuan Desert. Ruminants have exhibited limited use of tarbush leaves and new leaders as a forage during the summer growing season. Efforts to increase use of tarbush by lambs through spraying with various organic solvents were unsuccessful, probably because of the highly variable response. However, complete immersion of tarbush in organic solvents (acetone and ethanol) increased ($P < 0.01$) tarbush use by ram lambs when compared to unaltered tarbush. Data suggest that removal of secondary compounds from the leaf surface of tarbush using organic solvents enhanced acceptability of tarbush to sheep.

Key Words—*Flourensia cernua*, leaf surface, organic solvents, sheep, herbivory, tarbush.

INTRODUCTION

Many arid rangeland shrubs are used sparingly or seasonally by livestock, if at all (Owen-Smith and Cooper, 1987). Tarbush (*Flourensia cernua* DC) is present throughout the Chihuahuan Desert of North America and has a resinous leaf surface. Tarbush is consumed in limited quantities by sheep and cattle during certain times of year (Nelson et al., 1970; Anderson and Holechek, 1983). Tarbush has a relatively high nutritional value (Nelson et al., 1970), but certain plant parts may be toxic to livestock (Mathews, 1944; Dollahite and Allen, 1975). Kingston et al. (1975) described two sesquiterpenes in tarbush. During short-term, forced use of tarbush by cattle, sheep, and goats, no obvious adverse effects were apparent (Anderson et al., 1991); however, degree of use of indi-

*To whom correspondence should be addressed.

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vidual tarbush plants within the densely stocked paddocks was extremely variable.

Secondary plant metabolites may function as antiherbivory defense mechanisms in many plant species (Freeland, 1991). Various lipophilic compounds, e.g., mono- and sesquiterpenes, are present on the leaf surface of tarbush and are soluble in ethanol (Estell et al., 1994). Schwartz et al. (1980) reported that as volatile oils and terpenoid fractions increased in juniper, preference by deer decreased. Secondary compounds on the leaf surface of shrubs are at the plant–animal interface and, consequently, may dictate whether a plant is consumed or rejected by browsing ruminants. During forced use of tarbush, livestock exhibited a wide range of acceptance of individual plants, and degree of use of individual tarbush plants was related to epicuticular wax content (Estell et al., 1992). In addition, a few individual terpenes were related to individual plant use (R.E. Estell, unpublished data).

We hypothesized that removal of the surface resin and associated secondary chemicals might enhance tarbush acceptance by sheep. We applied organic solvents to tarbush in an attempt to remove surface secondary chemicals and to determine if the rate or extent of tarbush use by sheep could be altered. Five organic solvents were evaluated using two methods of application: spray and immersion.

METHODS AND MATERIALS

Experiment 1. Experiment 1 was designed to examine the effect of spraying with four organic solvents on the acceptability of tarbush to naive lambs. Our hypothesis was that spraying tarbush with organic solvents would rinse off surface chemicals and consequently enhance its acceptability to lambs. Ten tarbush plants were harvested from the Jornada Experimental Range (JER; located in southcentral New Mexico) the day prior to initiation of the study and transported to the laboratory. Five uniform branches were removed from each plant. Branches from one subset of five plants were placed in five bundles containing five branches each, one branch from each plant. This process was repeated for the other set of five plants, resulting in two sets of five "identical" bundles. Construction of bundles in this manner was an attempt to avoid confounding plant genetics and solvent treatment. Bundles were placed in 4-liter tin cans filled with water. Two bundles served as controls (unsprayed), and the other eight were sprayed (0.75 liters) with either 95% ethanol, methanol, ether, or acetone. Each treatment was applied to one bundle from each subset of plants.

Spraying took place the morning the study began. All solvents were reagent grade except acetone (histological grade). The hand sprayer (1 liter) used to apply treatments was thoroughly rinsed between solvents to prevent cross-con-

tamination. Branches were removed from cans, sprayed individually, and then wired into bundles. Branches were misted with the appropriate solvent (except controls) to allow for solubilization of surface compounds and then thoroughly sprayed before they had time to dry. Branches were sprayed from top to bottom (including underside as much as possible) to maximize drainage. After spraying at 0900 hr, all plant bundles in their water-filled tin cans were wired randomly to plywood sheets about 1 m apart on the perimeter of the fence.

Six ram lambs (Polypay × Rambouillet, 8 months of age, mean weight approximately 38.5 kg) with previous grazing experience limited to alfalfa pasture were placed in the pen (84 m^2) at 0900 hr. All plant material was removed from the pen before study initiation. Lambs were fasted for 24 hr prior to initiation of the experiment. Free access to water was available at all times. Percentage of use of each bundle was estimated (ocular estimation) at 1530 hr (6.5 hr after exposure) and at 0730 hr (22.5 hr after exposure) the following morning after removal of lambs. Ocular estimates of use were made to the nearest 5% use class by an experienced observer with no knowledge of the treatments applied. Percentage use data were collected at two times to assess differences in percentage use during the first few hours of exposure and over the entire period.

Experiment 2. Experiment 2 was designed to examine an alternative method (immersion) of applying solvent to tarbush. Our hypothesis was that removal of surface compounds would be more complete and consequently plants would be more acceptable to lambs when immersed in solvent rather than when sprayed with solvent. The lambs, facilities, and protocol used in experiment 1 were also used in experiment 2. Plants (eight plants, four branches of each plant per bundle) were harvested and placed in bundles as in experiment 1.

The major differences between experiments 1 and 2 were the washing technique and the solvents used. A shallow pan (approximately 30 × 60 × 8 cm) containing about 5 cm of solvent was used to wash branches; each branch was immersed individually in solvent, with constant agitation and rotation, for approximately 2 min while using pressure to immerse as much of the branch at once as possible without damaging the branch. The immersion process was conducted immediately prior to initiation of the study. Only three solvents (acetone, ethanol, and denatured ethanol) were compared to the control (no washing). Based on results of experiment 1, ether and methanol were eliminated from the comparison in experiment 2 because of similarities with other treatments. All four branches in each bundle were washed in the container, with replacement of solvent as necessary to maintain a constant fluid level. The pan was then emptied, rinsed with the solvent to be used next, and the process repeated for the four branches in the next bundle. Lambs were placed in the pen at approximately 0800 hr. Lambs were allowed to browse until 0730 hr the following morning. Visual estimates of percentage of use of each bundle were obtained

at 1430 hr (6.5 hr after exposure) and 0730 hr (23.5 hr after exposure) the following morning.

Statistical Analysis. Percentage use data were subjected to arcsine transformation (Steel and Torrie, 1960), and analysis of variance was conducted using GLM procedures of SAS (1989). Use at each time was analyzed using a randomized block design with block and treatment (organic solvent) in the model. Blocks consisted of plant bundles composed of the same plants (i.e., two blocks, each consisting of five bundles constructed from the same five plants in experiment 1 and four bundles from four plants in experiment 2). Means were separated using predicted difference (SAS, 1989) when significant F values ($P < 0.05$) were detected for overall model and treatment. Data presented in Tables 1 and 2 below were analyzed after arcsine transformation, but are presented as actual percentages of use.

RESULTS AND DISCUSSION

Experiment 1. No differences ($P > 0.05$) were noted for degree of use by lambs at either time (Table 1) when the four organic solvents were sprayed on tarbush; however, the variability between bundles within treatment was generally large. For the control, acetone, and ether treatments, one bundle exhibited relatively high use the following morning while the other bundle was used only slightly. The ethanol and methanol rinsed bundles exhibited fairly consistently low use. Very little use had occurred on any plants after 6.5 hr of exposure, suggesting that none of the solvent treatments dramatically altered plant palatability. Ethanol was chosen as a solvent because previous research (Goatcher

TABLE 1. PERCENTAGE USE BY RAM LAMBS OF TARBUSH SPRAYED WITH ORGANIC SOLVENTS

	Use (%)[b]	
Treatment[a]	6.5 hr	22.5 hr
Control[c]	8	43
Acetone[c]	8	45
Ether[c]	5	38
Methanol[c]	6	13
Ethanol[c]	3	13

[a]Treatment refers to the solvent used as spray treatment.
[b]Percent use based on ocular estimation after 6.5 and 22.5 hr of exposure.
[c]Least square means, $N = 2$; pooled standard error = 2.7 and 12.3 for use at 6.5 and 22.5 hr, respectively.

and Church, 1970) indicated sheep have a relatively high rejection threshold for ethanol (8.3 ml/100 ml). The fact that lambs were naive to browsing tarbush might account for the lack of consumption even after 22.5 hr. In general, no clear treatment effects were evident. The within-treatment variability may have been due to our inability to apply the spraying technique in a consistent manner.

Experiment 2. Differences ($P < 0.01$) among treatments were noted for percentage use (Table 2) after 23.5 hr of exposure. Ethanol- and denatured ethanol-immersed tarbush were used more than acetone-rinsed and control tarbush, while acetone-immersed tarbush was used to a greater extent than control bundles. At 6.5 hr after exposure, tarbush use was not different ($P > 0.05$) among treatments and was more variable than at 23.5 hr after exposure. Short-term use was generally low except for one bundle each from the ethanol and denatured ethanol treatments. Bundles composed of the same plants should have eliminated any confounding effects due to differences in plant genetics. Kainulainen et al. (1992) indicated that the terpene composition of conifers is under genetic control. No block effect was observed ($P > 0.05$) at 23.5 hr, suggesting that bundles from the two subsets of plants were not differentially used.

The consistent treatment response across bundles at 23.5 hr is in contrast to observations in experiment 1. Data would suggest that if tarbush use is improved in response to removal of surface secondary compounds, the immersion method may more completely (or uniformly) remove surface compounds. These results are substantiated by previous findings that the epicuticular wax (chloroform extraction of surface compounds) concentration of individual tarbush plants was related to the degree of use by livestock (Estell et al., 1992). Furthermore, these results imply a role for leaf surface secondary chemicals that are soluble in organic solvents as deterrents to use of tarbush by ruminants.

TABLE 2. PERCENTAGE USE BY RAM LAMBS OF TARBUSH IMMERSED IN ORGANIC SOLVENTS

	Use (%)[b]	
Treatment[a]	6.5 hr	23.5 hr
Control[c]	6	8[d] a
Acetone[c]	8	45 b
Ethanol[c]	40	88 c
DnEthanol[c]	40	85 c

[a] Treatment refers to the solvent used as immersion treatment (DnEthanol = denatured ethanol).
[b] Percent use based on ocular estimation after 6.5 and 23.5 hr of exposure.
[c] Least square means, $N = 2$; pooled standard error = 15.6 and 6.6 for use at 6.5 and 23.5 hr, respectively.
[d] Means in a column followed by different letters differ ($P < 0.01$).

Information obtained regarding relationships of secondary chemistry and herbivory could ultimately be exploited to modify consumption of shrubs by browsing herbivores.

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