Journal of Chemical Ecology, Vol. 24, No. 1, 1998

RELATIONSHIP OF TARBUSH LEAF SURFACE TERPENE PROFILE WITH LIVESTOCK HERBIVORY[1]

R. E. ESTELL,[2,*] E. L. FREDRICKSON,[2] D. M. ANDERSON,[2] K. M. HAVSTAD,[2] and M. D. REMMENGA[3]

[2]*USDA-ARS, Jornada Experimental Range*
Las Cruces, New Mexico 88003-0003
[3]*University Statistics Center, New Mexico State University*
Las Cruces, New Mexico 88003-0003

(Received July 15, 1996; accepted August 24, 1997)

Abstract—Tarbush (*Flourensia cernua* DC.) is a Chihuahuan Desert shrub with a resinous leaf surface containing terpenes that may affect livestock herbivory. Cattle, sheep, and goats were densely stocked in paddocks containing tarbush in two consecutive years for six to nine days and defoliation of 160 plants was recorded daily. Plants were categorized as exhibiting high or low defoliation. Leaves were collected from these plants the third year for chemical analysis. A selection procedure was used to generate two variable sets closely related to defoliation category. One set contained 14 variables (dry matter, ash, α-pinene, sabinene, 3-carene, *p*-cymene, limonene, camphor, borneol, *cis*-jasmone, β-caryophyllene, α-humulene, ledene, and flourensadiol) and the other set contained 14 unidentified compounds. When subjected to multivariate analysis, each group distinguished between the two defoliation categories ($P < 0.001$ and $P < 0.0019$ for known and unknown variable sets, respectively). These data support the hypothesis that leaf surface chemistry of individual tarbush plants is related to extent of defoliation by livestock.

Key Words—*Flourensia cernua*, leaf surface chemistry, diet selection, epicuticular wax, monoterpenes, sesquiterpenes.

INTRODUCTION

Desert grasslands are being replaced by shrubs in many arid regions of the world. This transition is often rapid and signifies serious deterioration of affected lands.

*To whom correspondence should be addressed.
[1]Preliminary findings of this research were presented in the 1995 Wildland Shrub Symposium.

0098-0331/98/0100-0001$15.00/0

Remedial technologies that are both environmentally beneficial and economically viable are needed. A potential method to control expansion of invading shrubs is by manipulating dietary preferences of domestic herbivores to increase selective pressures on shrubs while reducing pressures on grasses and associated grassland species. Chemical deterrents of herbivory in desert shrubs are primarily terpenoids and phenolics (Meyer and Karasov, 1991). Monoterpene content and/or profile have been related to dietary preferences of various mammals (Schwartz et al., 1980; Reichardt et al., 1985; Yabann et al., 1985; Elliott and Loudon, 1987; Bucyanayandi et al., 1990; Zhang and States, 1991). A clearer understanding of the role of these compounds in mediating interactions of grazing animals and desert shrubs should yield useful information for manipulating dietary preferences.

Currently we are using tarbush (*Flourensia cernua* DC.) to study the role of shrub chemistry in diet selection by livestock. Tarbush is an increasingly spreading species in many productive Chihuahuan Desert sites (Buffington and Herbel, 1965) with a high production potential (Estell et al., 1996), for which a biological control mechanism is desired. Tarbush is nutrient dense and contains more nitrogen than most desert shrubs (Nelson et al., 1970; Estell et al., 1996). However, depending on season and availability of other forages, tarbush is consumed by free-ranging livestock only in limited amounts (Nelson et al., 1970; Anderson and Holechek, 1983). Differential defoliation of tarbush by livestock (cattle, sheep, and goats) in a previous study at this location (Estell et al., 1994b) was related to the concentration of epicuticular wax and two unidentified terpenes. Tarbush contains several classes of secondary compounds (Kingston et al., 1975; Dillon et al., 1976; Bohlmann and Grenz, 1977; Aregullin-Gallardo, 1985), some of which may affect diet selection. Immersing tarbush in acetone or ethanol increased consumption by lambs (Estell et al., 1994a), suggesting leaf surface secondary compounds reduced tarbush acceptability to sheep. Although relatively unpalatable, tarbush leaves can be consumed safely in moderate amounts for several weeks by domestic ruminants (Fredrickson et al., 1994; King et al., 1996). However, certain growth stages of tarbush may be acutely toxic for some mammalian herbivores [i.e., flowering stage (Mathews, 1944; Dollahite and Allen, 1975)].

Our objective was to examine the relationship of tarbush leaf surface terpene profile with defoliation by livestock. Our hypothesis was that tarbush plants defoliated to a lesser extent during the previous year would contain greater concentrations of one or more leaf surface terpenes than highly defoliated plants.

METHODS AND MATERIALS

Study Site. The study was conducted on the Jornada Experimental Range (JER) in southcentral New Mexico in an area where tarbush has increased dramatically. The area was excluded from livestock grazing in 1988 and exposed to light to moderate stocking with cattle during the 75 years prior to livestock exclusion. Mean annual precipitation on study sites for 1989, 1990, and 1991 was 288, 267, and 393 mm, respectively. Growing season (July, August, and September) precipitation for 1989, 1990, and 1991 was 194, 174, and 231 mm, respectively. Long-term (1915–1933) mean annual and growing season precipitation for the area was 245 and 131 mm, respectively.

Sampling Protocol. This study was conducted in conjunction with a two-year study of tarbush utilization by livestock (Anderson et al., 1991). Eight paddocks (0.6 ha) at two sites (four adjacent paddocks per site; sites approximately 1.6 km apart) were densely stocked (cattle, sheep, and goats) for six to nine days (depending on forage availability) during one of two periods (approximately two weeks apart; four randomly selected paddocks browsed each period) in each of two years (1989 and 1990). High-density stocking was accomplished using cattle, sheep, and goats in a ratio of 8:20:19 and 8:23:16 per paddock in 1989 and 1990, respectively. Twenty plants in each paddock (10 in period 1 for 1989) were randomly selected and individual plant defoliation was recorded daily (visual estimation to the nearest 5% defoliation class by an experienced observer) during the browsing interval each year. Individual plants were grouped into high (HD) or low (LD) defoliation categories each year based on daily defoliation patterns (HD: $\geq$50% defoliation at period end; LD: $<$50% defoliation at period end).

Livestock were excluded from paddocks between 1988 and 1991 except for the short browsing interval each year (1989 and 1990). Leaf samples were collected from each plant during 1991 (N = 154, six plants were not sampled) in late August to coincide with the mature leaf stage when plants were browsed during 1989 and 1990. Approximately 50 g of leaves were collected from each plant by removing the current year's leader growth from the outer canopy at several locations on each plant. Intact leaders were immediately placed in plastic bags, frozen on dry ice, and stored at $-10°C$. A voucher specimen of tarbush was placed in the JER herbarium located in Las Cruces, New Mexico.

Chemical Analyses. Mature leaves (including petiole) of uniform size and appearance from the midpoint of the current year's growth were subjected to chemical analyses. Dry matter was analyzed for each plant from 10 whole leaves per duplicate. Epicuticular wax [modification of the gravimetric procedure of Mayeaux et al. (1981)] was analyzed for each plant on 10 whole leaves per duplicate by extraction with 20 ml of chloroform for 20 sec. Leaf surface mono- and sesquiterpenes were extracted and analyzed by modified procedures of Estell et al. (1994c). Five whole leaves from each plant were thawed and extracted at room temperature with 5 ml of 95% ethanol for 5 min. The extract was filtered through a glass-fiber filter and refrigerated. 2-Carene (10 ng/μl) was added as an internal standard. Extracts were subjected to gas chromatography–ion trap mass spectrometry (electron impact ionization source, DB-5, 5% phenyl, 95%

methyl silicone, 30 m, 0.32 mm ID, 0.25-μm film thickness, helium as carrier gas at 1 ml/min, 300-sec filament multiplier delay time, 220°C injector temperature, 260°C transfer line temperature, initial column temperature of 50°C, 1°C/min ramp to 60°C, 3°C/min ramp to final column temperature of 240°C, 5 min isothermal, 75 min total run time, 1 μl injection volume). Tentative identification of leaf surface terpenes was based on comparison of unidentified peak spectra to the spectral library assembled by Adams (1989). Positive identification of compounds was based on comparison of retention times and spectra to those of standards. Concentrations of unidentified peaks were estimated using peak area ratios relative to the internal standard.

Statistical Analyses. All analyses relating plant chemistry to the extent of defoliation were conducted using 1990 defoliation categories because chemistry data were available for all 160 plants. Relationships of concentration of dry matter, ash, epicuticular wax, mono- and sesquiterpenes, and unidentified compounds with plant defoliation categories were examined individually (univariate analysis) using analysis of variance (MANOVA procedures) (SAS, 1989).

A variable selection procedure (M. Mahrt and D. W. Smith, personal communication) was used to select a subset of the 26 known variables (dry matter, ash, wax, identified terpenes) to subject to multivariate analysis. This procedure examines all possible subsets of variables and determines the best set for predicting membership in the two categories. The generalized squared distance function (D^2; distance between centroids of categories) is used to measure the distance between the two categories. A variable set providing maximum D^2 was determined for each number of variables, i.e., all possible combinations of 1–26 variables were examined. Maximum D^2 achieved between categories gradually decreased as the number of variables was reduced. This selection procedure identifies the subset of variables that provides the greatest D^2 for a given number of variables; however, selecting a set of variables of an appropriate size to subject to multivariate analysis is somewhat subjective. Our goal was to minimize the number of variables used to discriminate between the two defoliation categories without eliminating important variables or compromising the ability to distinguish between categories with a reasonable level of confidence. The 14-variable subset was selected for multivariate analysis because eliminating 12 variables reduced D^2 by less than 10% compared to inclusion of all 26 variables. This procedure was repeated to determine the best possible combination of the 24 unidentified variables. Removal of 10 variables resulted in a subset of 14 unidentified variables that reduced D^2 by less than 10% from the original 24 variables. The procedure was conducted separately for the known and unknown compound groups because of the complexity of the data set. These sets that distinguished HD from LD plants were subjected to multivariate analysis of variance (knowns and unknowns separately) in the MANOVA procedure of SAS (1989). The Wilks' lambda test statistic was used to test for differences between categories ($P < 0.05$).

RESULTS

Effective precipitation in 1989 and 1990 was similar to the long-term mean, while 1991 precipitation was above average. Much of the 1991 precipitation occurred in December, after samples were collected. Conditions were generally dry during the first several weeks of the growing season in both 1990 and 1991. Insect damage (primarily due to *Zygograma tortuosa* Rogers) was extensive in 1990, but was not evaluated in 1989. Insect damage was prevalent in 1991, with many shrubs exhibiting severe defoliation. The number of plants classified as HD and LD was 92 and 28 in 1989 and 132 and 28 in 1990. Of the 120 shrubs evaluated both years, 82 plants were HD both years, 13 were LD both years, 15 plants shifted from LD in 1989 to HD in 1990, and 10 plants shifted from HD in 1989 to LD in 1990.

Least square means for dry matter, ash, epicuticular wax, and individual compound concentrations are presented in Table 1. When individual compounds were subjected to univariate analysis, several leaf surface components were related to degree of herbivory the previous year (Table 1). α-Pinene and flourensadiol concentrations were greater and *cis*-jasmone concentration was lower in LD plants ($P < 0.05$). Dry matter tended to be greater and ash and *p*-cymene concentrations tended to be lower in LD plants ($P < 0.10$). The concentration of unknown 23 was greater and unknowns 2 and 12 were lower in LD plants ($P < 0.05$). Unknowns 22 and 24 tended to be lower in LD plants ($P < 0.10$).

The set of identified compounds in plants in 1991 that best distinguished between HD and LD categories in 1990 contained 14 variables (dry matter, ash, α-pinene, sabinene, 3-carene, *p*-cymene, limonene, camphor, borneol, *cis*-jasmone, β-caryophyllene, α-humulene, ledene, and flourensadiol concentrations). The set of unidentified compounds distinguishing between categories contained 14 variables (estimated concentrations of unknowns 2, 4, 7, 9, 12, 13, 15, 16, 17, 18, 19, 22, 23, and 24). Separation of the two categories was possible using the above set of known ($P < 0.001$) or unknown ($P < 0.0019$) compounds.

DISCUSSION

Results of the univariate analysis indicated dry matter and ash content, two hydrocarbon monoterpenes (α-pinene and *p*-cymene), a green leaf volatile (*cis*-jasmone), and an oxygenated sesquiterpene (flourensadiol) were related to defoliation categories. Variables identified for inclusion into the set subjected to multivariate analysis included dry matter and ash concentration, five hydrocarbon monoterpenes (α-pinene, sabinene, 3-carene, *p*-cymene, and limonene), two oxygenated monoterpenes (camphor and borneol), *cis*-jasmone, three hydrocarbon sesquiterpenes (α-humulene, ledene, and β-caryophyllene), and one oxygenated sesquiterpene (flourensadiol).

TABLE 1. TARBUSH LEAF SURFACE CHEMISTRY AND RELATIONSHIP TO 1990 DEFOLIATION CATEGORIES BASED ON UNIVARIATE ANALYSIS[a]

Variable	RT[b]	HD[b]	LD[b]
Dry matter (DM), (%)		66.3 (1.2)*[c]	71.6 (2.6)†
Ash (% of DM)		11.3 (0.1)*	10.8 (0.2)†
Epicuticular wax (% of DM)		8.3 (0.3)	8.5 (0.6)
Individual compounds (μg/g DM)			
α-Pinene	621	113.5 (14.3)‡	214.3 (30.2)§
Camphene	669	63.9 (5.5)	71.9 (11.7)
Sabinene	748	10.7 (1.0)	13.3 (2.0)
β-Pinene	764	12.9 (1.7)	18.3 (3.5)
Myrcene	810	28.0 (2.0)	28.6 (4.2)
3-Carene	868	24.2 (2.3)	25.2 (4.8)
m-Cymene	907	0.52 (0.07)	0.65 (0.16)
p-Cymene	928	5.3 (0.4)*	3.7 (0.9)†
Limonene	937	90.5 (6.9)	97.3 (14.6)
1,8-Cineole	950	24.9 (4.1)	20.2 (8.6)
Camphor	1331	3.3 (0.4)	4.5 (0.8)
Borneol	1412	222.7 (25.4)	283.8 (53.8)
cis-Jasmone	2063	46.9 (3.7)‡	26.0 (7.8)§
α-Copaene	2016	4.4 (0.5)	4.9 (1.0)
α-Gurjunene	2100	0.20 (0.03)	0.16 (0.07)
β-Caryophyllene	2133	86.4 (6.8)	76.9 (14.5)
Calarene	2162	1.4 (0.1)	1.3 (0.3)
α-Humulene	2228	18.1 (1.3)	16.5 (2.7)
Ledene	2322	0.42 (0.07)	0.40 (0.15)
trans-Nerolidol	2505	0.49 (0.06)	0.35 (0.12)
Caryophyllene oxide	2549	43.2 (3.4)	37.3 (7.1)
Flourensadiol	3196	2520.6 (175.5)‡	3431.5 (372.3)§
Unknown 1[d]	1110	714.1 (58.5)	700.1 (124.0)
Unknown 2	2292	253.1 (22.1)‡	133.6 (46.9)§
Unknown 3	2355	92.0 (13.7)	60.6 (29.0)
Unknown 4	2577	234.0 (80.4)	338.6 (170.5)
Unknown 5	2676	157.6 (22.1)	124.7 (46.8)
Unknown 6	2720	91.6 (13.4)	71.8 (28.5)
Unknown 7	2729	73.6 (11.2)	91.9 (23.7)
Unknown 8	2923	148.3 (44.8)	74.6 (95.0)
Unknown 9	3086	147.5 (21.1)	93.3 (44.7)
Unknown 10	3198	2158.2 (328.1)	1855.0 (696.0)
Unknown 11	3356	105.1 (19.1)	137.7 (40.6)
Unknown 12	3474	196.8 (21.6)‡	68.0 (45.7)§
Unknown 13	3527	137.0 (21.3)	92.7 (45.3)
Unknown 14	3581	667.1 (90.4)	770.8 (191.8)
Unknown 15	3777	0.38 (0.19)	0.0 (0.41)
Unknown 16	3844	23.5 (5.4)	17.7 (11.4)
Unknown 17	3936	1603.0 (199.0)	1267.0 (422.1)
Unknown 18	3993	1084.9 (164.7)	1269.5 (349.3)

TABLE 1. Continued

Variable	RT[b]	HD[b]	LD[b]
Unknown 19	4040	3.0 (1.3)	0.39 (2.8)
Unknown 20	4048	177.7 (36.0)	161.8 (76.4)
Unknown 21	4120	193.7 (22.6)	187.3 (48.0)
Unknown 22	4147	199.7 (29.1)*	80.1 (61.8)†
Unknown 23	4386	139.4 (19.9)‡	272.6 (42.3)§
Unknown 24	4444	35.9 (7.8)*	4.8 (16.5)†

[a]Least square means (standard error) of chemical concentrations in 1991; $N = 126$ and 28 for HD and LD categories, respectively.
[b]RT = retention time (sec); HD and LD = high and low defoliation categories, respectively.
[c]*,†Defoliation categories with different superscripts differ ($P < 0.10$). ‡,§ Defoliation categories with different superscripts differ ($P < 0.05$).
[d]Tentatively identified as artemisia alcohol.

Several compounds that were significant univariates or in the variable set subjected to multivariate analysis in our study have been related to diet selection in other systems. Elliott and Loudon (1987) found red deer rejected a pelleted diet when exposed to the odor of five monoterpenes, including α-pinene, limonene, and borneol. Zhang and States (1991) reported the concentration of sabinene was greater in ponderosa pines avoided by Abert squirrels. Limonene concentration was greater in the bark of conifer species without meadow vole damage (Bucyanayandi et al., 1990). Sinclair et al. (1988) reported camphor extracted from white spruce was a feeding deterrent for snowshoe hares when added to rabbit chow. Personius et al. (1987) indicated that *p*-cymene and camphor were related to mule deer selection among and within sagebrush taxa. Riddle et al. (1996) identified specific monoterpenes correlated either positively (cymene and camphor) or negatively (α-pinene, sabinene plus β-pinene, myrcene, limonene) with juniper intake by goats.

In contrast, compounds not important in our study have been related to herbivory in other species. Reichardt et al. (1985) reported that cineole negatively affected feeding preference of hares, and Snyder (1992) reported that ponderosa pine trees selected by Abert squirrels contained greater concentrations of β-pinene in the xylem oleoresin. Myrcene concentration was negatively related to conifer use by Abert squirrels (Zhang and States, 1991) and meadow voles (Bucyanayandi et al., 1990). Nolte et al. (1994) reported no effect on feed preference when guinea pigs were fed pellets containing limonene. We are not aware of data regarding relationships of 3-carene, *cis*-jasmone, α-humulene, ledene, β-caryophyllene, or flourensadiol with herbivory in mammals.

In a previous study at this location, dry matter, ash, and epicuticular wax concentrations were negatively related to amount of defoliation by livestock (Estell et al., 1994b). A positive relationship between water content and defoliation was also observed in the present study, and dry matter was in the set of variables used to separate the two categories. A positive relationship between plant water content and grazing preference was described earlier by Archibald et al. (1943). Components of ash such as sodium can have positive or negative influences on animal preferences, depending on the postingestive consequences of previous dietary choices (Grovum and Chapman, 1988). However, ash content was negatively related to herbivory in the previous study and positively related in this study (based on univariate analysis). Ash was also in the set of variables subjected to multivariate analysis. Methodological differences or environmental factors may explain differences between studies.

The fact that epicuticular wax was negatively related to the degree of defoliation in the previous study and unrelated to defoliation category in this study with univariate analysis was somewhat surprising. However, the fact that epicuticular wax was not in the subset of variables subjected to multivariate analysis was expected, because the analysis takes into account the interrelationships among variables, and epicuticular wax contains many of the compounds measured. Several compounds in the variable set were not significant univariate variables, probably because of inherent differences between statistical analyses (only one variable is considered at a time during univariate analysis while multivariate analysis considers all variables and their interrelationships simultaneously). We recognize that a large type I error is an unavoidable consequence of the univariate statistical analyses because of the number of tests performed. Multivariate analyses were subsequently conducted in part to address these concerns. Our goal was to identify chemicals exhibiting possible relationships with intensity of defoliation, and further bioassay studies will be conducted to evaluate promising variables.

The number of variables related to extent of defoliation with multivariate analyses illustrates the complexity of relationships among plant chemicals driving plant–animal interactions and the likelihood of synergistic and antagonistic relationships among phytochemicals involved in diet selection. We examined volatile leaf surface compounds because tarbush has a resinous exudate containing several volatile terpenes present at the plant–animal interface and because a relationship of epicuticular wax and diet selection was observed previously (Estell et al., 1994b). Other constituents (internal leaf chemicals, other classes of secondary compounds, and concentration of various nutrients) undoubtedly affect diet selection as well, which further increases the complexity of the issue.

During the browsing study, rate and extent of individual tarbush defoliation were highly variable. Because visual estimates of defoliation for individual plants were recorded daily during that study, leaf samples were not collected from these plants during 1989 and 1990. By exploring relationships of tarbush chemistry in 1991 with defoliation of the same plants in the previous year, we assumed that heavy browsing during 1989 and 1990 did not induce plant secondary chemistry changes that persisted in 1991. Tarbush exists in a high-light, low-nutrient environment, which would be expected to limit growth to a greater extent than photosynthesis and favor accumulation of carbon-based secondary chemicals (Bryant et al., 1985). The high carbon–nitrogen ratio typical of resource-poor desert environments should favor chemical defense rather than biomass production, and slow growth rates should favor constitutive rather than induced defense (Bryant et al., 1983; Coley et al., 1985). Furthermore, mono- and sesquiterpenes are under strong genetic control relative to environmental influences in some plant species (Kainulainen et al., 1992; Michelozzi et al., 1995) and are generally less responsive to environmental influences than many phytochemicals, particularly in leaf tissue (Gershenzon and Croteau, 1991).

Although not analyzed statistically, the individual plant defoliation category was generally consistent across years. In fact, 79.2% of the plants had the same classification in 1989 and 1990 and 12.5% changed from LD to HD, while 8.3% changed from HD to LD. The fact that only 10 of 120 plants were HD in 1989 (no previous browsing) and LD in 1990 (after forced heavy browsing in 1989) suggests that chemical induction was minimal and/or short term or that differences in plant chemistry between HD and LD plants were great enough that differences existed in spite of induction. Category shifts may also reflect slight differences in patterns of tarbush consumption resulting from year to year differences in availability of other plant species.

Long-term induction responses to herbvivory cannot be ruled out, particularly given the prevalence of insect damage. Induction and release of terpenes and other volatile compounds by agronomic crops in response to insect herbivory have been demonstrated (Turlings and Tumlinson, 1992; Dicke et al., 1993; Loughrin et al., 1995). Bryant and Raffa (1995) suggested that for woody plant species, induction is a more important defense mechanism against insects, while constitutive defense is more important for mammals. Biotic stresses such as herbivory can rapidly induce terpene synthesis in many plant species (Carroll and Hoffman, 1980; Gershenzon and Croteau, 1991). However, because induction of secondary compounds is often short-lived (Faeth, 1992; Furstenburg and van Hoven, 1994; Gershenzon, 1994), long-term effects on herbivory may be less of a concern.

Leaf age and plant age can also affect terpene levels in plants, with terpene concentrations typically greater in young leaves than in mature leaves (Gershenzon and Croteau, 1991). Moreover, chemically defended plant species are generally most defended in the juvenile stage (Sinclair et al., 1988; Bryant et al., 1991). We attempted to minimize effects of leaf age by sampling at the same phenological stage as during previous browsing periods. Paddocks were located in areas that have been infested with tarbush for a number of years, and plant age effects were assumed to be minimal.

In summary, leaf surface compounds of tarbush appeared to be related to diet selection in the preceding year. Variable sets containing 14 known or 14 unknown compounds were identified that distinguished between the two defoliation categories when subjected to multivariate analysis. Other secondary chemicals in tarbush leaves (e.g., phenolics) and/or nutrient composition may also influence degree of tarbush herbivory by livestock.

Acknowledgments—The authors wish to thank Dr. R. P. Gibbens for estimating tarbush defoliation, Dr. D. G. I. Kingston for providing a sample of flourensadiol, and Ariceli Gonzalez, Linda Saar, and Antonio Garcia for assistance with sample collection and laboratory analyses.

REFERENCES

ADAMS, R. P. 1989. Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press, San Diego, California, 302 pp.

ANDERSON, D. M., and HOLECHEK, J. L. 1983. Diets obtained from esophageally fistulated hiefers and steers simultaneously grazing semidesert tobosa rangeland. *Proc. West. Sect. Anim. Sci.* 34:161–164.

ANDERSON, D. M., GIBBENS, R. P., HULET, C. V., HAVSTAD, K. M., and ESTELL, R. E. 1991. Browsing arid rangeland shrubs under multispecies management strategies, pp. 22–26, *in* P. Daget and M. Kernick (eds.). *Procedings, 4th International Rangeland Congress*, Montpellier, France (abstract).

ARCHIBALD, J. G., BENNETT, E., and RITCHIE, W. S. 1943. The composition and palatability of some common grasses. *J. Agric. Res.* 66:341–347.

AREGULLIN-GALLARDO, M. 1985. Chemical and biological significance of benzofurans and benzopyrans in the *Asteraceae*. PhD dissertation. University of California, Irvine, 204 pp.

BOHLMANN, F., and GRENZ, M. 1977. Über inhaltsstoffe der gattung *Flourensia*. *Chem. Ber.* 110:295–300.

BRYANT, J. P., and RAFFA, K. F. 1995. Chemical antiherbivore defense, pp. 365–381, *in* B. L. Gartner (ed.). Plant Stems: Physiology and Functional Morphology. Academic Press, San Diego, California, 440 pp.

BRYANT, J. P., CHAPIN, F. S., III, and KLEIN, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368.

BRYANT, J. P., CHAPIN, F. S., III, CLAUSEN, T. P., and REICHARDT, P. R. 1985. Effect of resource availability on woody plant-mammal interaction, pp. 3–8, *in* F. D. Provenza, J. T., Flinders, and E. D. McArthur (eds.). *Proceedings, Symposium on Plant-Herbivore Interactions.* Intermountain Research Station, USDA, Forest Service, Ogden, Utah, 179 pp.

BRYANT, J. P., KUROPAT, P. J., REICHARDT, P. B., and CLAUSEN, T. P. 1991. Controls over the allocation of resources by woody plants to chemical antiherbivore defense, pp. 83–102, *in* R. T. Palo and C. T. Robbins (eds.). Plant Defenses Against Mammalian Herbivory. CRC Press, Boca Raton, Florida, 192 pp.

BUCYANAYANDI, J. D., BERGERON, J. M., and MENARD, H. 1990. Preference of meadow voles (*Microtus pennsylvanicus*) for conifer seedlings: Chemical components and nutritional quality of bark of damaged and undamaged trees. *J. Chem. Ecol.* 16:2569–2579.

BUFFINGTON, L. C., and HERBEL, C. H. 1965. Vegetational changes on a semidesert grassland range. *Ecol. Monogr.* 35:139–164.

CARROLL, C. R., and HOFFMAN, C. A. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata*. *Science* 209:414–416.

COLEY, P. D., BRYANT, J. P., and CHAPIN, F. S., III. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.

DICKE, M., VAN BAARLEN, P., WESSELS, R., and DIJKMAN, H. 1993. Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: Extraction of endogenous elicitor. *J. Chem. Ecol.* 19:581–599.

DILLON, M. O., MABRY, T. J., BESSON, E., BOUILLANT, M. L., and CHOPIN, J. 1976. New flavonoids from *Flourensia cernua*. *Phytochemistry* 15:1085–1086.

DOLLAHITE, J. M., and ALLEN, T. J. 1975. The toxicity of the fruit of *Fluorensia cernua* (tarbush) (blackbrush). *Southwest. Vet.* 28:113–117.

ELLIOTT, S., and LOUDON, A. 1987. Effects of monoterpene odors on food selection by red deer calves (*Cervus elaphus*). *J. Chem. Ecol.* 13:1343–1349.

ESTELL, R. E., ANDERSON, D. M., and HAVSTAD, K. M. 1994a. Effects of organic solvents on use of tarbush by sheep. *J. Chem. Ecol.* 20:1137–1142.

ESTELL, R. E., FREDRICKSON, E. L., ANDERSON, D. M., MUELLER, W. F., and REMMENGA, M. D. 1994b. Relationship of tarbush leaf surface secondary chemistry to livestock herbivory. *J. Range Manage.* 47:424–428.

ESTELL, R. E., HAVSTAD, K. M., FREDRICKSON, E. L., and GARDEA-TORRESDEY, J. L. 1994c. Secondary chemistry of the leaf surface of *Flourensia cernua*. *Biochem. Syst. Ecol.* 22:73–77.

ESTELL, R. E., FREDRICKSON, E. L., and HAVSTAD, K. M. 1996. Chemical composition of *Flourensia cernua* at four growth stages. *Grass Forage Sci.* 51:434–441.

FAETH, S. H. 1992. Interspecific and intraspecific interactions via plant responses to folivory: An experimental field test. *Ecology* 73:1802–1813.

FREDRICKSON, E., THILSTED, J., ESTELL, R., and HAVSTAD, K. 1994. Effects of chronic ingestion of tarbush (*Flourensia cernua*) on ewe lambs. *Vet. Hum. Toxicol.* 36:409–415.

FURSTENBURG, D., and VAN HOVEN, W. 1994. Condensed tannin as antidefoliate agent against browsing by giraffe (*Giraffa camelopardalis*) in the Kruger National Park. *Comp. Biochem. Physiol.* 107A:425–431.

GERSHENZON, J. 1994. The cost of plant chemical defense against herbivory: A biochemical perspective, pp. 105–173, *in* E. A. Bernays (ed.). Insect-Plant Interactions, Vol. 5. CRC Press, Boca Raton, Florida, 240 pp.

GERSHENZON, J., and CROUTEAU, R. 1991. Terpenoids, pp. 165–219, *in* G. Rosenthal and M. R. Berenbaum (eds.). Herbivores. Their Interactions with Secondary Plant Metabolites, Vol. 1, The Chemical Participants. Academic Press, San Diego, California, 468 pp.

GROVUM, W. L., and CHAPMAN, H. W. 1988. Factors affecting the voluntary intake of food by sheep. 4. The effect of additives representing the primary tastes on sham intakes by oesophageal-fistulated sheep. *Br. J. Nutr.* 59:63–72.

KAINULAINEN, P., OKSANEN, J., PALOMÄKI, V., HOLOPAINEN, J. K., and HOLOPAINEN, T. 1992. Effect of drought and waterlogging stress on needle monoterpenes of *Picea abies*. *Can. J. Bot.* 70:1613–1616.

KING, D. W., ESTELL, R. E., FREDRICKSON, E. L., HAVSTAD, K. M., WALLACE, J. D., and MURRAY, L. W. 1996. Effects of *Flourensia cernua* ingestion on intake, digestion, and ruminal fermentation of sheep consuming tobosa. *J. Range Manage.* 49:325–330.

KINGSTON, D. G. I., RAO, M. M., SPITTLER, T. D., PETTERSEN, R. C., and CULLEN, D. L. 1975. Sesquiterpenes from *Flourensia cernua*. *Phytochemistry* 14:2033–2037.

LOUGHRIN, J. H., MANUKIAN, A., HEATH, R. R., and TUMLINSON, J. H. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21:1217–1227.

MATHEWS, F. P. 1944. The toxicity of the ripe fruit of blackbrush or tarbush (*Flourensia cernua*) for sheep and goats. *Tex. Agric. Exp. Stn. Bull.* 664:16 pp.

MAYEAUX, H. S., JR., JORDAN, W. R., MEYER, R. E., and MEOLA, S. M. 1981. Epicuticular wax on goldenweed (*Isocoma* spp.) leaves: Variation with species and season. *Weed Sci.* 29:389–393.

MEYER, M. W., and KARASOV, W. H. 1991. Chemical aspects of herbivory in arid and semiarid habitats, pp. 167–187, *in* R. T. Palo and C. T. Robbins (eds.). Plant Defenses Against Mammalian Herbivory. CRC Press, Boca Raton, Florida, 192 pp.

MICHELOZZI, M., WHITE, T. L., SQUILLACE, A. E., and LOWE, W. J. 1995. Monoterpene composition and fusiform rust resistance in slash and loblolly pines. *Can. J. For. Res.* 25:193–197.

NELSON, A. B., HERBEL, C. H., and JACKSON, H. M. 1970. Chemical composition of forage species grazed by cattle on an arid New Mexico range. *N.M. Agric. Exp. Etn. Bull.* 561:33 pp.

NOLTE, D. L., MASON, J. R., and LEWIS, S. L. 1994. Tolerance of bitter compounds by an herbivore, *Cavia porcellus*. *J. Chem. Ecol.* 20:303–308.

PERSONIUS, T. L., WAMBOLT, C. L., STEPHENS, J. R., and KELSEY, R. G. 1987. Crude terpenoid influence on mule deer preference for sagebrush. *J. Range Manage.* 40:84–88.

REICHARDT, P., CLAUSEN, T., and BRYANT, J. 1985. Plant secondary metabolites as feeding deterrents to vertebrate herbivores, pp. 37–42, *in* F. D. Provenza, J. T. Flinders, and E. D. McArthur (eds.). *Proceedings, Symposium on Plant–Herbivore Interactions.* Intermountain Research Station, USDA, Forest Service, Ogden, Utah, 179 pp.

RIDDLE, R. R., TAYLOR, C. A., JR., KOTHMANN, M. M., and HUSTON, J. E. 1996. Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish goats. *J. Range Manage.* 49:35–41.

SAS. 1989. SAS/STAT User's Guide, Vol. 2, Version 6, 4th ed. Statistical Analysis System Institute, Inc., Cary, North Carolina, 846 pp.

SCHWARTZ, C. C., REGELIN, W. L., and NAGY, J. G. 1980. Deer preference for juniper forage and volatile oil treated foods. *J. Wildl. Manage.* 44:114–120.

SINCLAIR, A. R. E., JOGIA, M. K., and ANDERSEN, R. J. 1988. Camphor from juvenile white spruce as an antifeedant for snowshoe hares. *J. Chem. Ecol.* 14:1505–1514.

SNYDER, M. A. 1992. Selective herbivory by Abert's squirrel mediated by chemical variability in ponderosa pine. *Ecology* 73:1730–1741.

TURLINGS, T. C. J., and TUMLINSON, J. H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U.S.A.* 89:8399–8402.

YABANN, W. K., BURRITT, E. A., and MALECHEK, J. C. 1985. Sagebrush (*Artemisia tridentata*) monoterpenoid concentrations as factors in diet selection by free-grazing sheep, pp. 64–70, *in* F. D. Provenza, J. T. Flinders, and E. D. McArthur (eds.). *Proceedings, Symposium on Plant–Herbivore Interactions.* Intermountain Research Station, USDA Forest Service, Ogden, Utah, 179 pp.

ZHANG, X., and STATES, J. S. 1991. Selective herbivory of ponderosa pine by Abert squirrels: A re-examination of the role of terpenes. *Biochem. Syst. Ecol.* 19:111–115.