Tarbush Leaf Surface Terpene Profile in Relation to Mammalian Herbivory

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Abstract—Cattle, sheep and goats were densely stocked in paddocks containing tarbush (*Flourensia cernua* DC) for six to nine days and defoliation of 160 plants was recorded daily during two years. Plants were separated into high, moderate or low defoliation categories. Leaves were collected from plants during the same stage of maturity during the third year. Leaf surface compounds were extracted with ethanol and mono- and sesquiterpenes were analyzed using gas chromatography/ion trap mass spectrometry. A set of 11 variables was identified that appeared most closely related to plant defoliation categories: dry matter, ash, limonene, camphor, borneol, *α*-copaene, *β*-caryophyllene, *α*-pinene, *p*-cyrene, *cis*-jasmine and *caryophyllene* oxide concentrations. This group distinguished among all three defoliation categories (P < 0.03) when subjected to multivariate analysis. These data support the hypothesis that leaf surface chemistry is related to degree of defoliation of individual tarbush plants by livestock.

Land managers need environmentally safe and economically viable remediation technologies for shrub dominated areas. Managing large herbivores to deter shrub invasion while using shrubs as forage is a desirable option. An understanding of the role of secondary chemistry in mediating interactions of grazing ruminants and desert shrubs should yield useful information for manipulating diet selection. Antiherbivore compounds in desert shrubs are primarily terpenoids and phenolics (Meyer and Karasov 1991). Volatile plant substances are particularly effective herbivory defense mechanisms because herbivores are repelled before plant damage occurs (Levin 1976). Yabann and others (1985) reported that sheep selected individual sagebrush plants with a lower total monoterpenoid concentration. Morrison and others (1987) reported compounds present in the volatile fraction altered cattle preference for three *Panicum* spp. Other workers have reported relationships of individual terpenes, subgroups (for example, oxygenated monoterpenes) and/or total terpenoid concentration with herbivory by various mammals (Schwartz and others 1980; Reichardt and others 1985; Elliott and Loudon 1987; Bucyanayandi and others 1990; Goralka and Langenheim 1991; Zhang and States 1991).

Differential defoliation of tarbush (*Flourensia cernua* DC) when browsed simultaneously by cattle, sheep and goats in a previous study at this location was related to concentration of epicuticular wax and two unidentified terpenes (Estell and others 1994a). Tarbush contains several classes of secondary compounds (Kingston and others 1975; Dillon and others 1976; Bohlmann and Grenz 1977; Arengullin-Gallardo 1985). While relatively unpalatable, tarbush leaves can be consumed safely in moderate amounts for several weeks by sheep (Fredrickson and others 1994; King and others 1996). However, tarbush is acutely toxic to several mammalian herbivores during flowering (Mathews 1944; Hailey and others 1966; Dollahite and Allen 1975).

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

Materials and Methods

Study Site

The study site was located on the Jornada Experimental Range (JER) in southern New Mexico in an area heavily infested with tarbush. This area had been exposed to light to moderate stocking rates during the 75 years prior to the study. Annual precipitation for 1989, 1990 and 1991 was 239, 259 and 395 mm, respectively, for site 1 and 337, 275 and 390 mm, respectively, for site 2. The total growing season (July, August and September) precipitation for 1989, 1990 and 1991 was 164, 175 and 238 mm, respectively, for site 1 and 223, 172 and 223 mm, respectively, for site 2. Long term (1915 to 1993) mean annual and growing season precipitation for the area is 245 and 131 mm, respectively.

Sampling Protocol

This study was conducted in conjunction with a study of tarbush utilization by livestock (Anderson and others 1991). The study consisted of high density stocking (cattle, sheep
and goats) of eight paddocks (0.6 ha) at two sites (four adjacent paddocks per site; sites approximately 1.6 km apart) for six to nine days (depending on forage availability) during two periods (approximately two weeks apart; four randomly selected paddocks browsed each period) in each of two years (1989 and 1990). Twenty plants in each paddock (10 in period 1 for 1989) on permanently marked transects were randomly selected and daily defoliation of each plant was recorded (ocular estimation to the nearest 5% defoliation class by an experienced observer) during the browsing period each year.

Individual plants were separated into high, moderate or low defoliation categories (HD, MD, or LD, respectively) based on daily defoliation patterns (HD: ≥50% defoliation at period midpoint; MD: <50% defoliation at midpoint and ≥50% defoliation at period end; LD: <50% defoliation at period end). For periods with an odd number of days, the midpoint was defined as midpoint plus one half day.

Livestock were excluded from paddocks during 1988 through 1991 except during the short browsing periods described previously. Tarbush leaf samples were collected from each plant (n = 154, six plants were not sampled) during the third week of August, 1991. Timing of collection was selected to coincide with the mature leaf stage when tarbush was browsed during 1989 and 1990. Approximately 50 g of leaves (entire current seasons growth removed and placed in plastic bags) were removed from each plant, immediately placed on dry ice and stored at −10 °C. A voucher specimen of tarbush was placed in the JER herbarium in Las Cruces, NM.

**Chemical Analyses**

Mature leaves (including petiole) of uniform size and appearance from the midpoint of the current seasons growth were subjected to chemical analyses. Dry matter was analyzed in duplicate using 10 whole leaves. Epicuticular wax (modification of the gravimetric procedure of Mayeaux and others 1981) was analyzed in duplicate by extracting 10 whole leaves with 20 ml of chloroform for 20 sec. Surface compounds from five whole thawed leaves were extracted and analyzed with modified procedures of Estell and others (1994b).

Leaves were extracted with 5 ml of 95% ethanol for 5 min and filtered through a glass fiber filter. An internal standard (2-carene, 10 ng/μl) was added and samples were refrigerated. Surface mono- and sesquiterpenes were analyzed using gas chromatography/ion trap mass spectrometry (electron impact ionization source, DB-5 column, 5% phenyl, 95% methyl silicone, 30 m, 0.32 mm i.d., 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 and positive identification of leaf surface terpenes were described previously (Estell and others 1994b).

**Statistical Analyses**

Relationships of plant chemistry and herbivory were based on the 1990 tarbush defoliation categories. Univariate analysis of variance was conducted using the MANOVA procedure of SAS (1985) to examine relationships of plant defoliation categories with individual variables (dry matter, ash, epicuticular wax and 22 mono- and sesquiterpenes). Means were separated using Least Significant Difference when the overall model was significant (P < 0.10; SAS 1985).

A variable selection procedure (M. Mahrt, Experimental Statistics Department, New Mexico State University, personal communication) was used to examine each pair of categories prior to multivariate analysis. This procedure was used to reduce the number of variables subjected to multivariate analysis because of computational limitations. This selection process examined all possible combinations of variables for each number of variables and selected a set of variables based on the point at which removing a variable substantially reduced the ability to distinguish between two categories. This process was conducted separately for each category comparison (HD versus LD, HD versus MD and MD versus LD), resulting in a set of variables best separating each pair of categories. With few exceptions, variables important for separating MD plants from either HD or LD plants were also contained in the set separating HD and LD categories. Moreover, variables distinguishing between HD and LD were assumed most likely to have biological meaning. Thus, this group was subjected to multivariate analysis of variance using the MANOVA procedure of SAS (1985). The Wilks’ Lambda test statistic was used to separate categories (P < 0.05).

**Results and Discussion**

Least square means for dry matter, ash, epicuticular wax and 22 mono- and sesquiterpenes by category appear in Table 1. Four terpenes initially thought to be present in tarbush were not found: nerol, cis-nerolidol, cis-verbenol and o-cymene. Four terpenes were present but not quantified due to analytical difficulties: tricyclene, trans-jasmone, γ-gurjunene and globulol.

A set of 11 variables from plant samples collected in 1991 which best distinguished between HD and LD categories in 1990 were subjected to multivariate analysis (dry matter, ash, limonene, camphor, borneol, α-copaene, β-caryophyllene, α-pinene, p-cymene, cis-jasmone, and caryophyllene oxide concentrations). While certain variables important for separating MD plants from HD and/or LD plants during pairwise comparisons were not included in this set, the separation of all categories was possible using only the above set of compounds (P < 0.0001, P < 0.001 and P < 0.03 for HD versus LD, MD versus LD and HD versus MD comparisons, respectively). This fact is not surprising given the number of variables common to all three pairwise separations.

Also, variables suggested to be important based on univariate analysis appeared in the subset selected for multivariate analysis. Separation of HD and LD categories
Table 1—Tarbush leaf surface chemistry and relationship to 1990 defoliation categories based on univariate analysis.a

<table>
<thead>
<tr>
<th>Variable</th>
<th>RTb</th>
<th>HDc</th>
<th>MDc</th>
<th>LDc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>65.9 (2.0)</td>
<td>66.6 (1.6)</td>
<td>71.6 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Ash, % of dry matter</td>
<td>11.5 (0.2)</td>
<td>11.2 (0.1)c</td>
<td>10.8 (0.2)d</td>
<td></td>
</tr>
<tr>
<td>Epicuticular wax, % of dry matter</td>
<td>8.6 (0.4)</td>
<td>8.1 (0.3)</td>
<td>8.5 (0.6)</td>
<td></td>
</tr>
<tr>
<td>α Pinene</td>
<td>621</td>
<td>136.2 (23.0)</td>
<td>99.5 (18.1)</td>
<td>214.3 (30.2)d</td>
</tr>
<tr>
<td>Camphene</td>
<td>669</td>
<td>70.2 (8.9)</td>
<td>60.0 (7.0)</td>
<td>71.9 (11.7)</td>
</tr>
<tr>
<td>Sabinene</td>
<td>748</td>
<td>12.6 (1.6)</td>
<td>9.5 (1.2)</td>
<td>13.3 (2.0)</td>
</tr>
<tr>
<td>β Pinene</td>
<td>764</td>
<td>16.1 (2.7)</td>
<td>10.9 (2.1)</td>
<td>18.3 (3.5)</td>
</tr>
<tr>
<td>Myrcene</td>
<td>810</td>
<td>29.5 (3.3)</td>
<td>27.1 (2.6)</td>
<td>28.6 (4.3)</td>
</tr>
<tr>
<td>3-Carene</td>
<td>868</td>
<td>21.9 (3.7)</td>
<td>25.6 (2.9)</td>
<td>25.2 (4.8)</td>
</tr>
<tr>
<td>m-Cymene</td>
<td>907</td>
<td>0.57 (0.12)</td>
<td>0.49 (0.09)</td>
<td>0.85 (0.16)</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>928</td>
<td>5.3 (0.7)</td>
<td>5.4 (0.6)</td>
<td>3.7 (0.9)</td>
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<tr>
<td>Limonene</td>
<td>937</td>
<td>84.4 (11.1)</td>
<td>94.3 (8.7)</td>
<td>97.3 (14.6)</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>950</td>
<td>25.5 (6.6)</td>
<td>24.6 (5.2)</td>
<td>20.2 (8.6)</td>
</tr>
<tr>
<td>Camphor</td>
<td>1331</td>
<td>3.4 (0.6)</td>
<td>3.2 (0.5)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td>Borneol</td>
<td>1412</td>
<td>272.3 (41)</td>
<td>192.2 (32)</td>
<td>283.8 (54)</td>
</tr>
<tr>
<td>cis-Jasmone</td>
<td>2063</td>
<td>55.5 (5.9)d</td>
<td>42.9 (4.6)d</td>
<td>26.0 (7.7)</td>
</tr>
<tr>
<td>α Copaene</td>
<td>2016</td>
<td>5.3 (0.7)</td>
<td>3.8 (0.6)</td>
<td>4.9 (1.0)</td>
</tr>
<tr>
<td>α Gurgunene</td>
<td>2100</td>
<td>0.13 (0.06)</td>
<td>0.24 (0.04)</td>
<td>0.16 (0.07)</td>
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<td>β Caryophyllene</td>
<td>2133</td>
<td>93.9 (11.1)</td>
<td>81.8 (8.7)</td>
<td>76.9 (14.5)</td>
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<td>Calarene</td>
<td>2162</td>
<td>1.7 (0.2)</td>
<td>1.3 (0.2)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>α Humulene</td>
<td>2228</td>
<td>20.3 (2.1)</td>
<td>16.7 (1.6)</td>
<td>16.5 (2.7)</td>
</tr>
<tr>
<td>Ledene</td>
<td>2322</td>
<td>0.56 (0.11)</td>
<td>0.33 (0.09)</td>
<td>0.40 (0.15)</td>
</tr>
<tr>
<td>trans-Nerolid</td>
<td>2505</td>
<td>0.65 (0.09)</td>
<td>0.39 (0.07)d</td>
<td>0.35 (0.12)d</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>2549</td>
<td>36.8 (5.4)</td>
<td>47.2 (4.3)</td>
<td>37.3 (7.1)</td>
</tr>
<tr>
<td>Flourensadiol</td>
<td>3196</td>
<td>2425.8 (285)</td>
<td>2579.0 (224)</td>
<td>3431.5 (373)d</td>
</tr>
</tbody>
</table>

aLeast square means(standard error) of chemical concentrations in 1991; n = 48, 78 and 28 for HD, MD, and LD categories, respectively.
bRT = retention time (sec).
HC, MD and LD refer to high, moderate and low defoliation categories, respectively.
cOverall model significant (P < 0.05).
dOverall model significant (P < 0.10).
eDefoliation categories differ (P < 0.05).
fDefoliation categories differ (P < 0.10).

is most critical in terms of practical application and design of future studies. While the procedure for selecting the group of variables to enter into multivariate analysis was somewhat subjective and based solely on compounds selected for separating HD and LD plants, this group distinguished among all three categories (P < 0.03).

When individual compounds were subjected to univariate analysis, several tarbush leaf surface components were related to defoliation category the previous year (table 1). Ash content was less (P < 0.05) for LD than HD plants, with MD plants not different than LD or HD plants. Flourensadiol and α pinene concentrations were greater for LD plants (P < 0.05), with no difference between HD and MD groups. Cis-jasmone concentration tended to be less (P < 0.10) for LD plants, with no difference between HD and MD plants. Trans-nerolidol concentration was greater (P < 0.05) for HD plants, with no difference between MD and LD plants.

Several variables not in the subset subjected to multivariate analysis may be related to herbivory. For example, sabinene, α humulene and flourensadiol were selected during the variable selection procedure as important for discriminating the MD category from both HD and LD categories.

Trans-nerolidol was selected for separating HD and MD plants, and was a significant univariate variable (table 1). Camphene, 1,8-cineole and α gurgunene were selected for separating HD and MD plants, while β pinene, 3-carene, calarene (β gurgunene) and ledene (viridiflorene) were selected for separating MD and LD categories. Epicuticular wax, myrcene and m-cymene were not selected for discriminating among any categories.

Variables in the set subjected to multivariate analysis included dry matter and ash concentration, three hydrocarbon monoterpenes (limonene, α pinene and p-cymene), three oxygenated monoterpenes (camphor, borneol and cis-jasmone), two hydrocarbon sesquiterpenes (α copaene and β caryophyllene) and one oxygenated sesquiterpene (caryophyllene oxide). Results of univariate analysis indicated ash content, one hydrocarbon monoterpene (α pinene), no hydrocarbon sesquiterpenes, one oxygenated monoterpene (cis-jasmonone) and two oxygenated sesquiterpenes (trans-nerolidol and flourensadiol) were related to plant categories.

Specific terpenes (especially oxygenated monoterpenes), subclasses of terpenes and/or total terpene concentration in various plants have been associated with degree of herbivory.
in several mammalian species. In deer, negative relationships have been reported for diet selection and crude terpenoid content (Longhurst and others 1968), volatile odor concentration (Elliott and Loudon 1987) and concentrations of oxygenated monoterpenes and total volatile oil or monoterpenes concentration (Schwartz and others 1980; Goralka and Langenheim 1991). Zhang and States (1991) reported tree avoidance by Abert squirrels was related to total concentration and diversity of terpenes, as well as presence and concentration of specific terpenes.

Monoterpenes have also been associated with diet selection for voles (Bucanayandide and others 1990) and hares (Reichardt and others 1985). While this relationship has rarely been evaluated in domestic ruminants, Yabann and others (1985) reported that sheep selected individual sagebrush plants with lower total monoterpane concentrations, and Morrison and others (1987) found compounds present in the volatile fraction of three Panicum spp. affected preference of cattle.

In a previous study at this location, concentrations of dry matter, ash, epicuticular wax and two unidentified terpenes were all related to the amount of defoliation by livestock (Estell and others 1994a). A positive relationship between water content and defoliation was observed in that study. Dry matter also was in the set of variables used to separate the three categories with multivariate analysis. A positive relationship between plant water content and grazing preference was described earlier by Archibald and others (1943). Components of ash such as sodium can have either a positive or negative influence on animal preferences depending on the post-ingestive consequences of previous dietary choices (Groven and Chapman 1988). However, ash content was negatively related to level of herbivory in the previous study and positively related in this study (based on univariate analysis). Ash was also in the set of variables used during multivariate analysis for separating the three categories. Methodological differences (HD and LD plants were sampled in the previous study) or environmental factors may explain differences between studies.

The fact that epicuticular wax was related to defoliation in the previous study but not in this study with univariate analysis was 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 of variables, and wax contains many of the compounds measured. Inherent differences in univariate and multivariate analyses can provide conflicting results because only one variable is considered at a time with univariate analysis while multivariate analysis considers all variables and their interrelationships simultaneously. The number of variables related to degree of defoliation with multivariate analysis illustrates the complexity of relationships among plant chemicals driving plant-animal interactions and the potential for synergistic and antagonistic relationships among phytochemicals involved in the diet selection process.

During the browsing study, degree of defoliation of individual plants was highly variable. Because ocular estimates of defoliation for individual plants were recorded daily during that study, we were unable to collect leaves from 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 which persisted in 1991.

Shrubs in a high-light, low-nutrient environment would be expected to exhibit slow growth and constitutive, carbon-based defense (Bryant and others 1985; Coley and others 1985). However, secondary compound production for chemical defense may be induced by herbivory in plant-herbivore systems (Gershenzon and Croteau 1991), and induction effects of biotic stresses such as herbivory on terpene synthesis may be very rapid (Carroll and Hoffman 1980; Gershenzon and Croteau 1991). Long-term induction and subsequent effects on herbivory may be less pronounced, because induction of secondary compounds is often short-lived (Faeth 1992; Furstenburg and van Hoven 1994).

For plants with defoliation data from both years, 65 plants were in the same category in 1989 and 1990, 31 plants were in a higher category in 1990 and 24 plants were in a lower category in 1990. Three plants in the LD category in 1989 were in the HD category in 1990 and no plants in the HD category in 1989 were in the LD category in 1990. The arbitrary nature of this classification system and the continuous nature of daily plant defoliation curves may account for some of the shifts among categories between years.

These shifts may also reflect slight differences in patterns of tarbush consumption resulting from year to year differences in availability of other plant species. However, the fact that only 24 plants were in a lower defoliation category in 1990 (after forced heavy browsing in 1989) than in 1989 (no previous browsing), and particularly that no plant in the HD category in 1989 was in the LD category in 1990, 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.

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References


Mathews, F. P. 1944. The toxicity of the ripe fruit of blackbrush or tarbush (Flourensia cernua) for sheep and goats. Texas Agricultural Experiment Station Bulletin, No. 664. 16 p.


