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Volatile Compounds on the Leaf Surface of Intact and Regrowth Tarbush (*Flourensia cernua* DC) Canopies

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Abstract Shrub expansion into desert grasslands is a serious problem resulting in loss of forage and rangeland productivity. *Flourensia cernua* DC (tarbush) is one such shrub contributing to the decline of Chihuahuan Desert grasslands. Our previous research has shown tarbush consumption by sheep and goats to be negatively related to leaf surface concentration of individual terpenes and epicuticular wax. Concentrations of compounds such as terpenes often change with plant age and phenology. Our objective was to examine the effect of altering the vegetative state of tarbush on volatile chemicals. Ninety tarbush plants were randomly selected, and all biomass within 10 cm of the soil surface was removed from 45 plants during winter dormancy. Leaves were collected the following summer during active growth from the canopy of intact controls and resprouts. Leaf surface volatiles were analyzed by gas chromatography-mass spectroscopy and subjected to univariate analysis of variance and stepwise discriminate analysis. Of the 87 compounds present on tarbush leaves, 35 were greater in canopy samples and 16 were greater in regrowth samples based on univariate analysis ($P<0.05$). Mean concentration of total volatiles on canopy leaves tended to be less ($P=0.062$) than that of regrowth (3,642 vs. 4,684 μg/g DM). Nine compounds in the discriminant analysis (α-muurolene, iso-borneol, unknown#6, *p*-cymen-8-ol, unknown#7, sabinene, β-caryophyllene, δ-cadinene, and α-copaene) explained 95% of the variation between canopy and regrowth samples. Lower cumulative concentration of volatile compounds in canopy than regrowth samples suggests repsrouts may be less vulnerable to herbivory than intact tarbush.

Keywords Tarbush · *Flourensia cernua* · Regrowth · Terpenes · Plant secondary metabolites

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Introduction

The transition of desert grasslands to shrub-dominated states is detrimental in areas where lands are valued for forage production for livestock. This transition, often accompanied by degraded conditions (e.g., soil erosion, decreased biodiversity, altered spatial complexity), is typically termed desertification (Pickup 1988; Peters et al. 2004) and has complex causes and effects (Reynolds and Stafford Smith 2002; Bestelmeyer 2005). *Flourensia cernua* DC (tarbush) is one shrub that is increasing in dominance at the expense of desert grasslands in the northern Chihuahuan Desert (Buffington and Herbel 1965). Dominance of shrubs in areas grazed by livestock is typically related to their palatability (Leopold 1924). Tarbush consumption by sheep and goats is negatively related to leaf surface concentration of individual terpenes and epicuticular wax (Estell et al. 1994a, b, 1998a). Herbivory/pruning of woody plants during dormancy can cause a reversion to juvenile growth form and increase the proportion of the plant that is current year growth, both of which are typically more heavily chemically defended (Bryant et al. 1991). Swihart and Bryant (2001) compiled 128 studies that examined a wide variety of herbivores and woody plants and found mature plants typically were preferred over juvenile plants. Mature woody plants have been reported to contain less camphor (white spruce; Sinclair et al. 1988), mono- and diterpenes (red cedar; Vourc'h et al. 2002), and total phenolics (13 African tree species; Rooke et al. 2004) than immature plants. Pisani et al. (2001) fed old and new growth from two *Prosopis* species (new growth was from plants that were topped 10 months earlier) and found that goats preferred old growth vs. new growth of *P. caldenia*, and that this difference was not explained by concentration of total phenolics or condensed tannins. No difference in preference for old or new growth was observed for the lesser preferred *P. flexuosa* in their study. Gowda (1997) observed no difference in concentration of total phenolics or condensed tannins in *Acacia tortilis* that were pruned (removal of outer 20 cm of all twigs) vs. intact plants, although physical defense (spine production) increased with pruning. Although several simulated browsing studies exist, few studies have examined effects of complete mechanical biomass removal on secondary chemical content in regrowth of woody species. Rea and Gillingham (2001) measured regrowth of Scouler's willow (*Salix scouleriana*) at different times after mechanical brushing (biomass removal 10–15 cm aboveground) and found tannin content was unaffected or lower than in intact plants. Our objective was to compare chemical profiles of the leaf surface of intact tarbush and regrowth. Our hypothesis was that regrowth would invest resources into defense compounds and contain greater terpene concentrations than intact plants.

Methods and Materials

Study Site The study was conducted on the Jornada Experimental Range in south-central New Mexico, USA. Soils on the site are deep, well-drained, Doña Ana-Reagan association and vary from sandy loam to loam (SCS 1980). The site is gently undulating with 1 to 5% slope. Long-term (1915 to present) mean annual and growing season (July–September) precipitation for the area is 247 and 131 mm, respectively. Long-term mean monthly temperatures for the coldest (January) and warmest (July) months are 6 and 26°C, respectively. Dominant vegetation on the study site is *Scleropogon brevifolius* Phil (burrograss) and tarbush (a deciduous, root-sprouting shrub). Study plants were located in a 7.5-ha paddock within a dense stand of tarbush from which livestock had been excluded for 5 yr before the study.

Sampling Protocol Ninety tarbush plants in the paddock were selected randomly and labeled at the base with an aluminum tag. All above-ground biomass (10 cm above soil surface) was removed at one time with lopping shears from 45 randomly selected plants during winter dormancy and removed from the site. Leaves were collected the following August during active growth from both regrowth and intact controls. Approximately 75 leaves (including petiole) were collected from a minimum of five leaders of current year's growth from each plant. Leaves were sampled from the middle one-third of leaders with forceps, placed on dry ice, and subsequently stored at −20°C.

Laboratory Analyses Five leaves from each sample were extracted in duplicate for 5 min at room temperature in 5 ml of 100% ethanol containing 5 ng/μl of 2-carene (internal standard) with occasional shaking, followed by filtration through a fiberglass (Fisherbrand G8) filter and stored at 4°C. Dry matter was measured in duplicate for each sample (10 leaves per duplicate) at 100°C for 24 hr. Leaf surface volatiles were analyzed by gas chromatography coupled to mass spectrometry, using a Finnigan ion trap mass spectrometer (EI, 70 eV) in conjunction with a Varian model 3400 gas chromatograph equipped with a DB-5 column (30 m × 0.25 mm fused silica capillary column, film thickness 0.25 μm) with helium as carrier gas (1 ml/min), 1 μl injection size, and a programmed (injector temperature 220°C, transfer line temperature 240°C, initial column temperature 60°C, final column temperature 240°C, 3°C/min) temperature run (Adams 1995; Tellez et al. 1997). Identification of volatile compounds was performed by a comparison of mass spectra with authentic compounds when available or literature data (Adams 1995), and by a comparison of their relative retention times with those of authentic compounds or by comparison of their retention indices with those in the literature (Adams 1995). Concentrations of individual compounds were estimated by using the internal standard, and total volatile concentration was estimated from the cumulative concentrations of all compounds within a treatment.

Statistical Analysis Data were transformed to natural logarithms and subjected to univariate analysis of variance to compare mean concentration between resprouts and canopy for each compound and stepwise discriminate analysis to determine a subset of compounds that could discriminate between resprouts and canopy (SAS/STAT® 2002–2003).

Results

Of the 87 compounds present on tarbush leaves, 35 were greater in intact canopy samples and 16 were greater in regrowth samples based on univariate analysis ($P<0.05$; Table 1). The largest components (>100 μg/g of dry matter) were flourensadiol, unknown #1, artemisia alcohol, borneol, β-eudesmol, and unknown #4 for intact tarbush, and flourensadiol, unknown #7, unknown #6, artemisia alcohol, unknown #5, and β-eudesmol for tarbush resprouts (Table 1). Mean concentration of total volatiles on canopy leaves tended to be less ($P=0.062$) than that of regrowth (3642 vs. 4684 μg/g DM).

In the discriminate analysis, nine compounds (α-muurolene, iso-borneol, unknown #6, *p*-cymen-8-ol, unknown #7, sabinene, β-caryophyllene, δ-cadinene, and α-copaene) explained nearly 95% of the variation between canopy and regrowth samples, and α-muurolene alone explained almost 65% of the variation (Table 2). This subgroup was composed primarily of compounds in low concentrations (<20 μg/g of dry matter) with the exception of unknowns #6 and #7.

Table 1 Concentration and univariate analysis of volatile leaf surface chemicals on intact (control) and resprouted tarbush[a]

	Intact	Resprout	*P*
Total volatiles[b]	3643±358	4684±421	0.062
Santolina triene[c]	4.20±0.53	1.36±0.14	0.001
Tricyclene	13.34±1.61	3.04±0.50	0.001
α-thujene[c]	6.40±0.90	3.20±0.52	0.001
α-pinene	22.64±3.64	25.85±3.37	0.339
Camphene	80.29±11.27	81.96±12.02	0.607
Sabinene	3.42±0.83	6.17±1.18	0.016
β-pinene	13.24±2.27	17.78±3.12	0.202
Myrcene	20.70±4.91	21.19±5.55	0.468
Mesitylene[c]	3.53±0.57	2.57±0.49	0.064
Yomogi alcohol[c]	91.51±10.03	14.43±3.24	0.001
δ-3-carene	19.88±3.55	18.51±4.85	0.331
α-terpinene	3.04±0.40	1.22±0.17	0.001
p-cymene	9.57±2.03	7.81±0.95	0.868
Limonene	23.82±4.73	25.05±6.17	0.756
1,8-cineole	28.59±8.01	29.33±5.17	0.659
(*Z*)-β-ocimene	2.29±5.16	0.60±0.07	0.001
(*E*)-β-ocimene[c]	1.45±0.25	0.76±0.22	0.001
Trans-decahydronaphthalene[c]	2.10±0.39	0.49±0.05	0.001
γ-terpinene + artemisia ketone[c]	6.38±1.02	2.49±0.35	0.001
Cis-sabinene hydrate	10.56±2.49	13.23±2.46	0.408
Artemisia alcohol[c]	191.48±29.37	142.64±24.99	0.046
Terpinolene	2.49±0.53	1.23±0.22	0.001
Trans-sabinene hydrate	7.08±1.48	8.04±1.10	0.185
Cis-*p*-menth-2-en-1-ol[c]	10.25±1.22	3.61±0.59	0.001
α-campholenal[c]	3.32±0.55	1.13±0.40	0.001
Trans-pinocarveol[c]	5.99±0.96	1.67±0.47	0.001
Camphor + *trans*-verbenol[c]	8.31±1.20	2.55±0.41	0.001
Isoborneol[c]	3.03±0.27	0.42±0.06	0.001
Cis-chrysanthenol[c] + pinocarvone[c]	59.70±14.64	37.99±8.33	0.114
Borneol	168.94±25.48	74.26±15.82	0.001
Terpin-4-ol	11.27±1.51	3.44±0.77	0.001
m-cymen-8-ol[c]	2.10±0.20	0.38±0.06	0.001
p-cymen-8-ol[c]	3.82±0.44	0.52±0.08	0.001
α-terpineol	4.61±0.59	1.10±0.29	0.001
Myrtenal[c]	1.55±0.30	0.47±0.08	0.001
Myrtenol[c]	1.97±0.27	0.19±0.02	0.001
Cis-chrysanthenyl acetate[c]	3.85±0.55	1.07±0.19	0.001
Bornyl acetate[c]	3.94±0.51	2.30±0.36	0.009
Carvacrol[c]	5.38±0.60	0.80±0.12	0.001
α-cubebene[c]	2.41±0.32	2.66±0.44	0.855
Eugenol	2.51±0.39	0.38±0.07	0.001
Cyclosativene[c]	3.83±0.54	2.52±0.28	0.201
α-copaene	4.62±0.58	7.87±1.42	0.265
β-bourbonene[c]	8.54±0.62	7.19±0.76	0.072
β-cubebene[c]	6.72±0.60	4.97±0.60	0.034
(*Z*)-jasmone	23.33±2.80	23.64±3.15	0.544
Methyl eugenol	1.90±0.31	0.28±0.06	0.001
β-caryophyllene	17.65±1.50	11.72±1.24	0.001

Table 1 (continued)

	Intact	Resprout	*P*
α-humulene	11.84±1.23	5.91±0.69	0.001
Allo-aromadendrene[c]	2.29±0.37	3.09±0.53	0.511
Drima-7,9(11)-diene[c]	9.10±1.35	5.90±0.52	0.967
γ-muurolene[c]	3.16±0.66	4.03±0.45	0.007
Germacrene D[c]	27.82±3.77	19.20±2.61	0.167
β-selinene[c]	8.39±1.58	12.43±1.16	0.001
Epi-cubebol[c]	4.74±1.30	22.71±4.48	0.001
Bicyclogermacrene[c]	1.21±0.27	1.33±1.00	0.007
α-muurolene[c]	1.62±0.46	9.48±0.82	0.001
γ-cadinene[c]	10.86±1.77	31.47±6.94	0.012
Cis-calamenene[c]	1.65±0.37	6.13±0.95	0.001
δ-cadinene[c]	2.54±0.75	9.47±1.41	0.001
Cadina-1,4-diene[c]	6.78±1.33	3.46±1.83	0.116
Elemol[c]	9.73±1.81	7.67±1.60	0.263
Ledol[c]	48.65±9.47	37.49±4.31	0.852
Germacrene D-4-ol[c]	15.35±2.01	15.19±1.39	0.101
Spathulenol[c]	18.63±1.24	9.30±1.10	0.001
Caryophyllene oxide	29.99±3.99	27.22±4.58	0.423
Unknown#1	325.93±96.14	119.12±38.15	0.003
Unknown#2	38.79±3.77	30.4±3.47	0.055
β-oplopenone[c]	5.39±0.96	6.77±0.89	0.022
1-epi-cubenol[c]	23.42±1.79	35.40±2.85	0.002
Epi-α-muurolol[c]	6.30±1.16	3.54±0.59	0.094
(*Z*)-methyl jasmonate[c]	9.44±3.92	35.06±10.15	0.346
β-eudesmol[c]	140.61±9.88	118.35±11.10	0.080
Selin-11-en-4-α-ol[c]	76.33±9.82	28.83±3.95	0.001
Unknown#3	34.17±6.45	26.66±6.28	0.229
Bulnesol[c]	3.71±0.39	4.21±0.57	0.562
(*Z*)-methyl epi-jasmonate[c]	14.09±1.38	10.39±1.06	0.086
α-bisabolol[c]	8.24±1.93	40.39±6.35	0.001
Oplopanone[c]	3.30±0.49	6.73±0.78	0.001
Unknown#4	116.03±3.35	69.56±21.89	0.147
β-acoradienol[c]	21.28±1.44	8.75±1.01	0.001
Nootkatone[c]	7.39±1.04	12.41±1.76	0.003
Cryptomeridiol[c]	67.45±9.04	86.80±12.15	0.207
Flourensiadiol	1505.47±186.26	2238.83±294.24	0.786
Unknown#5	21.54±4.18	62.91±13.97	0.150
Unknown#6	29.32±13.62	197.91±18.58	0.001
Unknown#7	32.81±10.68	687.67±108.77	0.001

[a] Compounds were identified using kovats indices and mass spectral libraries; concentrations (± SEM; μg/g DM) were estimated from relative proportions of internal standard (2-carene); *N*=44 and 45 for regrowth and intact plants, respectively. Statistical analyses were conducted on natural logarithms of concentration means.

[b] Total volatiles are the cumulative estimated concentration of all compounds within a treatment.

[c] Tentatively identified based on Adams (1995)

Table 2 Stepwise discriminate analysis of volatile chemicals in intact (control) and resprouted tarbush

Order entered	Chemical	R^2
1	α-muurolene	0.645
2	Isoborneol	0.818
3	Unknown#6	0.874
4	*p*-cymen-8-ol	0.901
5	Unknown# 7	0.913
6	Sabinene	0.924
7	β-caryophyllene	0.933
8	δ-cadinene	0.942
9	α-copaene	0.948

Discussion

Concentration of carbon-based secondary metabolites in woody shrub species are well documented to be highly variable and affected by a number of factors, including plant species, class of compound, leaf age, plant age, season of year, induced responses from mechanical damage or herbivory, as well as a host of other environmental factors such as soil nutrients and light intensity (Cedarleaf et al. 1983; Bryant et al. 1991; Gershenzon and Croteau 1991; Powell and Raffa 1999; Massei et al. 2000; Ward and Young 2002). Pruning or herbivory during dormancy may cause woody plants to revert to juvenile growth stage and increase the proportion of current years growth, both of which are typically more chemically defended (Bryant et al. 1991). A number of theories have been forwarded regarding the allocation of plant resources to secondary metabolites, but a common theme is that tradeoffs exist between growth and defense (Herms and Mattson 1992). Young tissue is typically highly defended in slow-growing woody plants in low resource environments that rely predominately on constitutive carbon-based defenses (Bryant et al. 1991). Because regrowth from the crown and roots of shrubs represents a critical tissue that is paramount to survival of the plant, our hypothesis was that it would contain higher concentrations of secondary metabolites. However, our anecdotal observations suggest an alternative hypothesis because tarbush leaves from plants previously cut near the soil surface (roadgrader blading, etc.) are larger and more succulent in appearance, lighter in color, and less resinous and fragrant than leaves from uncut plants (visual observations only), suggesting they might contain lower concentrations of secondary compounds on the leaf surface. Increased palatability of regrowth after browsing has been reported for woody species in some cases (e.g., Danell et al. 1985; du Toit et al. 1990). Tarbush is a deciduous root sprouter, with new growth for both intact plants and regrowth occurring after summer rains. Both tissues were sampled on the same day; thus, leaf age and season were the same for this study, and any differences in leaf chemistry should be caused by the biomass removal treatment. Although plant age could not be specifically addressed, the 90 plants used in this study were fairly uniform in size and shape, and were assumed to be approximately the same age.

Because secondary metabolite concentration is closely related to consumption of shrubs by livestock and wildlife, the amount of defensive chemicals has implications for herbivory. Three of the first nine compounds to enter the discriminant analysis (sabinene, β-caryophyllene, and α-copaene) were unrelated to intake by lambs at concentrations found in tarbush (Estell et al. 1998b, 2000, 2005). Furthermore, none of the compounds tested (N=25) that did affect intake (α-pinene, camphene, caryophyllene oxide, and camphor) at

concentrations present in tarbush (Estell et al. 1998b, 2002) differed for regrowth with the univariate analysis (except camphor, which was inseparable from *trans*-verbenol in the present study). However, the total amount of volatile chemicals (cumulative estimated concentration of all volatiles within treatment) present on the leaf surface tended to differ (P=0.062) between intact and resprouted tarbush (Table 1). Intake of tarbush and other shrubs by ruminants has been related to total volatile concentration (Schwartz et al. 1980; Estell et al. 1994b; Riddle et al. 1996); thus, resprouts might be less palatable for livestock. However, it cannot be ruled out that other individual compounds that differed between intact and regrowth plants could affect palatability.

A number of studies have examined mechanical shrub removal in the context of shrub control and nutritional value (e.g., Kituku et al. 1992; Reynolds et al. 1992), but few report effects on secondary metabolite concentrations. Pisani et al. (2001) and Rea and Gillingham (2001) both reported lower concentrations of phenolics and/or tannins in regrowth of woody shrubs. This response is not completely surprising as these classes of compounds frequently increase in conjunction with structural components associated with maturity. Tannin content of blackbrush acacia regrowth 34 weeks after burning was greater than unburned controls, but honey mesquite and spiny hackberry regrowth tannin content did not differ from controls (Schindler et al. 2004). We are not aware of any studies that have examined terpenes in response to above-ground biomass removal for woody plants. Whereas mowing agronomic crops and pastures is well documented to reduce maturity and improve forage quality, less is known about the effects of altered growth stage in woody species. Our goal was to examine chemical changes associated with altering the vegetative state of tarbush by clipping intact plants and generating resprouts. Lower cumulative concentration of volatile compounds in intact plants than regrowth suggests repsrouts might be less vulnerable to herbivory than intact tarbush. From a practical standpoint, there would appear to be no advantage to altering growth stage as a means to improve tarbush consumption and reduce competition with grasses, although animal studies are needed for confirmation. Our findings support the general observations that young plants are more defended than older plants, in contrast to anecdotal observations mentioned above. Mowing tarbush and grazing the regrowth may not be an appropriate mechanism to promote grasses in tarbush-dominated desertified areas.

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