Effect of light intensity and wavelength on concentration of plant secondary metabolites in the leaves of *Flourensia cernua*

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A B S T R A C T

Flourensia cernua (tarbush) is a shrub that has encroached into grasslands in many areas of the northern Chihuahuan Desert and contains high levels of carbon-based secondary compounds. Concentrations of secondary compounds are affected by numerous biotic and abiotic influences, including amount and wavelength of solar radiation. However, responses to shade and ultraviolet light restriction are inconsistent among plant species and compound class. We conducted a three-year study to evaluate the effect of shade and UV light restriction on total phenolic and terpene concentrations in tarbush. Sixty plants were randomly assigned to one of three treatments (control, UV light restriction, or 50% incident light restriction). Mean concentrations of total phenolics and total volatiles in tarbush were 82.4 and 12.5 mg/g DM, respectively. Total phenolics did not differ between UV-restricted and control plants, but were lower in shaded plants than the other treatments ($P < 0.05$). Total volatiles tended to be greater for the UV-restricted treatment than control plants ($P = 0.056$), with shaded plants not different from either treatment. Treatment effects were detected for 18 individual compounds ($P < 0.05$). Our results partially support the hypothesis that UV restriction and shading alter carbon-based secondary chemical concentrations.

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1. Introduction

Rangelands worldwide have experienced degradation during the last two centuries, generally resulting in a transition from grassland to shrubland. *Flourensia cernua* DC (tarbush) has increased in the northern Chihuahuan Desert in areas that were previously grasslands (Gibbens et al., 2005).

Secondary chemistry of desert shrubs represents a basic mechanism for plant competitiveness and appears to be especially important for adaptation to harsh, resource-limited environments (Freeland, 1991). Not only are there thousands of plant secondary metabolites (PSM) representing several classes, but their concentrations differ temporally and spatially among and within species, and their proportions relative to other compounds (both primary and secondary) are in constant flux. Their presence and concentration in a given plant are influenced by genetics (Heyworth et al., 1998) and a host of biotic

and abiotic environmental factors, including phenology (Byrd et al., 1999), plant age (Elger et al., 2009), leaf age (Gershenzon and Croteau, 1991), location within plant (Shelton, 2000), time of day (Komenda and Koppmann, 2002), soil moisture (Llusià et al., 2006), nutrient availability (Powell and Raffa, 1999), temperature (Llusià et al., 2006), light intensity and wavelength (Baraza et al., 2004; Xu and Sullivan, 2010), herbivory (Gershenzon and Croteau, 1991), mechanical damage (Danell et al., 1997), and CO_2 level (Kuokkanen et al., 2003).

Both light intensity and ultraviolet light have been reported to alter concentration of secondary compounds in a variety of plants, although responses vary among species and with class of PSM (Graglia et al., 2001; Semerdjieva et al., 2003; Izaguirre et al., 2007). For example, elevated concentrations of several individual flavonoids and phenolic acids were observed in silver birch (*Betula pendula*) in response increased UV-B radiation (Lavola et al., 1998; de la Rosa et al., 2001), and Thines et al. (2007) observed increased flavonoid concentrations in sagebrush (*Artemisia tridentata*) exposed to increased UV-B radiation, whereas terpene concentrations were unaffected. Hartley et al. (1995) reported lower total phenolics and condensed tannins and increased monoterpene concentrations in shaded Sitka spruce (*Picea sitchensis*), and Johnson et al. (1997) reported lower volatile terpene concentrations in shaded ponderosa pine (*Pinus ponderosa*) needles than those in full sun. Baraza et al. (2004) observed decreased condensed tannins and total phenolics in shaded oak seedlings (*Quercus pyrenaica Willd*.) vs those in full light.

The mechanisms by which UV and partial shade affect PSM differ. Ultraviolet radiation is thought to trigger production of phenolics with antioxidant properties involved in plant protection from photodamage (Close and McArthur, 2002; Xu and Sullivan, 2010). Reduced light intensity via shading can affect allocation of photosynthate into carbon-based PSM because of tradeoffs that exist between growth and defense and the influence of resource availability (including light) on these tradeoffs (Herms and Mattson, 1992; Herms, 1999). In high-light/low-nutrient environments, PSM in woody plants are typically carbon-based, and light stress would be expected to decrease carbon-based secondary compound concentrations (Coley et al., 1985; Bryant et al., 1992).

Tarbush extracts exhibited antifeedant (Estell et al., 2001), phytotoxic, allelopathic (Dayan and Tellez, 1999), antitermitic, antifungal, and algicidal properties (Tellez et al., 2001). Little is known about environmental influences on tarbush chemistry. Leaf surface terpenes on tarbush are highly variable from plant to plant (Estell et al., 1994) and among locations within a plant (Estell et al., 2013). Many individual compounds are also affected by leaf age (Estell et al., 2013). Tarbush regrowth contained a greater concentration of total terpenes than intact plants (Fredrickson et al., 2007). Total phenolics varied with season, and both total phenolics and condensed tannins varied among years (Estell et al., 1996).

In many plant species, PSM concentrations and volatile emissions have implications for several biotic processes, including herbivory (Harborne, 2001), plant—plant communication and herbivore/predator interactions (Dicke et al., 1993), pollination (Piechulla and Pott, 2003), litter dynamics (Kraus et al., 2003), and allelopathy (Vaughan and Ord, 1991). Their concentrations could be impacted by climate change, as both temperature and UV radiation affect carbon-based PSM in some woody plants (Lavola et al., 1998; Llusià et al., 2006). Our objective was to examine the effect of UV light restriction and partial shade on concentrations of terpenoids and phenolics in tarbush. Our hypothesis was that UV restriction and partial shade would reduce these carbon-based secondary compounds in tarbush.

2. Materials and methods

2.1. Site description

The study was conducted on the Jornada Experimental Range in south-central New Mexico in an area containing a dense stand of tarbush. A 60×60 m exclosure was enclosed with wire fencing prior to the study to exclude livestock and large wildlife. The site is characterized by gently undulating (1—5% slope) deep, well-drained, sandy clay loam soils of the Doña Ana—Reagan association (SCS, 1980). Long-term mean monthly temperatures for the coldest (January) and warmest (July) months are 6 and 26 °C, respectively. Long-term (1978—1997) mean annual precipitation is 256 mm. Annual and growing season (July to September) precipitation at the site was 164.1 and 105.4 mm (1995), 285.2 and 197.1 mm (1996), and 340.9 and 174.0 mm (1997), respectively. Tarbush and *Scleropogon brevifolius* Phil (burrograss) are the dominant vegetation. Tarbush is a deciduous, root-sprouting shrub that remains dormant until after summer rainfall (Fredrickson et al., 2007).

2.2. Sample collection

Prior to active growth in spring of 1995, 60 dormant tarbush plants were randomly selected within the exclosure and labeled at the base with an aluminum tag. Plants were randomly assigned to one of three treatments: control, ultraviolet light restriction, or partial shade (50% of incident light restriction). In early summer of year 1 (June 28th, 1995), metal frames ($1.5 \times 1.5 \times 1.2$ m) made of 5.1 cm angle iron were placed over each of the 60 plants. The top surface of 20 frames were covered with clear plastic film to block UV light penetration and 20 frames were covered with black shade cloth that restricted 50% of incident light. Twenty control plants had only the metal frame. These open-sided shelters remained intact for the duration of the three year study.

Samples were collected from all 60 plants each year (1995, 1996, and 1997; $n = 20$ tarbush per treatment) in late September each year (near the endpoint of active current year's growth, just prior to flowering). Leaves were collected from the middle third of leaders in the outer canopy. Approximately 100 leaves (including petiole) were removed from all sides of

each plant with forceps, placed in plastic bags (two bags containing either 40 or 60 leaves) on dry ice, and transported to the laboratory. Forty leaves were stored at −20 °C for subsequent DM and terpene analysis and 60 leaves were immediately freeze dried for total phenolic analysis and stored at room temperature.

2.3. Laboratory analysis

After thawing, five whole leaves of uniform size and appearance from each plant were weighed in duplicate and extracted at room temperature for 5 min in 5 ml of 100% ethanol containing an internal standard (2-carene, Aldrich Chemical Co., Milwaukee, WI; 5 ηg/μl) with occasional shaking. The extract was filtered through a fiberglass (Fisherbrand G8) filter (2.5 cm o.d.) with a disposable plastic syringe into 20-ml vials and stored at 4 °C until analysis (Tellez et al., 1997). Blanks were prepared as described above without tarbush. Ten leaves were also subjected to dry matter analysis in duplicate at 100 °C for 24 h.

Freeze-dried leaves were ground in liquid N with a mortar and pestle, mixed thoroughly, and stored in a plastic bag at −20 °C in a dessicant-filled container for subsequent analysis of total phenolics in duplicate (Folin-Denis procedure; tannic acid as standard; AOAC, 1990). Due to limited sample, the procedure was modified to utilize 0.05 g samples. A tarbush standard sample was analyzed with each run for validation of the sample size modification and to assure assay consistency among days. Freeze-dried samples were also analyzed for dry matter (AOAC, 1990) modified to use approximately 0.10 g samples. Due to limited amounts for some samples, an average DM value was used to convert total phenolics to a DM basis. A total of 12, 14, and 30 samples were analyzed in duplicate for year 1, 2, and 3, respectively. Average DM values for years 1, 2, and 3 were 92.88 (±0.68), 91.54 (±0.53), and 92.43 (±1.05), respectively. Though absolute values may differ slightly from actual DM, differences among treatment variables should not be affected.

Ethanol extracts were analyzed for leaf surface terpenes with gas chromatography-mass spectrometry using a Finnigan ion trap mass spectrometer (EI, 70 eV; Thermoelectron Corporation, Waltham, MA) in conjunction with a Varian model 3400 gas chromatograph equipped with a CTC-A200s autosampler and a DB-5 column (30 m, 0.25 mm i.d., fused silica capillary column, film thickness 0.25 μm, 5% phenyl-methylpolysiloxane coating; J&W Scientific, Santa Clara, CA). Helium served as the carrier gas (1 ml/min), split flow was 20 ml/min (ratio 20:1), injection volume was 1 μl (duplicate extractions; single injection per extraction), with a programmed temperature run (injector temp. 220 °C, transfer line temp. 240 °C, initial column temp. 60 °C, final column temp. 240 °C, 3 °C/min, detector temperature 260 °C) (Adams, 1995; Tellez et al., 1997). Volatile compounds were identified by comparing mass spectra and retention time with authentic compounds when available. Otherwise, compounds were tentatively identified with spectral libraries (Adams, 1995) and Kovats retention indices. Individual terpenoid concentrations (averaged across duplicate injections before statistical analysis) were estimated using the internal standard and total volatile concentration was estimated by summing individual volatile concentrations within a sample.

2.4. Statistical analysis

Data were analyzed using repeated measures linear mixed effects models (PROC MIXED; SAS V9.4; SAS Institute, Cary NC) to model effects of treatment, year and their interaction on total phenolics and total and individual terpene concentrations. Models were applied separately for each variable. Treatment was a fixed effect and year was a repeated effect with plant as the subject. An unstructured temporal covariance was used for each model. The Kenward-Roger method was used for computing denominator degrees of freedom for fixed effects. Means were separated with LSD when a significant F test was detected ($P < 0.05$).

3. Results

Total phenolics averaged 82.4 mg/g DM across all years and treatments. Total estimated volatile concentration averaged 12.5 mg/g DM across all years and treatments. A total of 102 individual compounds (including 19 unknowns) were present on tarbush leaves. The largest compounds (>100 μg/g of DM) were camphene, ymogi alcohol, artemisia alcohol, borneol, germacrene D, β-eudesmol, selin-11-en-4-α-ol, cryptomeridiol, flourensiadiol, and 10 unknowns (Table 1). The largest components in tarbush in previous studies were artemisia alcohol, borneol, β-eudesmol, flourensadiol, and two unknowns (Fredrickson et al., 2007) and camphene, artemisia alcohol, borneol, (Z)-methyl jasmonate, selin-11-en-4-α-ol, flourensadiol, and three unknowns (Estell et al., 2013). As in previous studies, many of the unknowns eluted late and were probably diterpenes.

No year × treatment interactions were detected for any variable ($P > 0.05$). Year effects were detected for almost every variable (data not shown). Total phenolics did not differ between year 1 and 2, but both had greater concentrations than year 3 ($P < 0.05$). Total volatiles differed ($P < 0.05$) among years (year 2 > year 1 > year 3). A year effect was detected for nearly all individual compounds (n = 97). For 45 of these compounds, concentration in year 2 was greater ($P < 0.05$) than in year 1 or 3, which did not differ. Another 29 compounds exhibited greater concentrations in year 2 than the other years, but year 3 was also greater than year 1 ($P < 0.05$).

A treatment effect was detected for total phenolics, with the shade treatment lower ($P < 0.05$) than control and UV restriction, which did not differ (Table 1). Total volatiles tended to differ ($P = 0.056$; Table 1), with the UV restriction treatment greater than controls, while the shade treatment did not differ from either of the other treatments. Treatment effects were

Table 1

Effect of UV restriction and partial shade on leaf terpenes and total phenolics in tarbush.[a]

Chemical[b,c]	RT[d]	Control	UV restriction	50% shade	P-value
Total phenolics[e]		84.4 ± 1.7[g]	83.9 ± 1.7[g]	78.9 ± 1.7[h]	0.035
Total volatiles[e,f]		11.5 ± 0.8	14.1 ± 0.8	11.9 ± 0.8	0.056
Santolina triene[c,e]	279	1.4 ± 0.5	2.8 ± 0.5	2.3 ± 0.5	0.080
Tricyclene[e]	303	6.6 ± 1.0	10.1 ± 1.0	8.5 ± 1.0	0.056
α-Thujene[c,e]	305	2.4 ± 0.5	3.7 ± 0.5	3.1 ± 0.5	0.197
α-Pinene[e]	319	41.4 ± 7.1	59.9 ± 7.1	55.6 ± 7.1	0.160
Camphene[e]	338	123.8 ± 19.0[g]	226.9 ± 19.0[h]	207.2 ± 19.0[h]	0.001
Sabinene[e]	378	13.3 ± 2.2[g]	20.1 ± 2.2[h]	20.7 ± 2.2[h]	0.037
β-Pinene[e]	383	18.3 ± 2.9[g]	31.5 ± 2.9[h]	30.3 ± 2.9[h]	0.003
Myrcene[e]	405	22.6 ± 1.4	21.2 ± 1.4	21.5 ± 1.4	0.771
Mesitylene[c,e]	411	2.3 ± 1.1	3.9 ± 1.1	5.1 ± 1.1	0.220
Yomogi alcohol[c,e]	419	93.6 ± 8.5	106.1 ± 8.5	106.0 ± 8.5	0.494
3-Carene[e]	443	29.8 ± 1.8	31.1 ± 1.8	28.6 ± 1.8	0.632
α-Terpinene[e]	457	1.2 ± 0.2[g]	1.9 ± 0.2[h]	1.5 ± 0.2[g,h]	0.026
p-Cymene[e]	469	7.4 ± 1.0	10.3 ± 1.0	9.2 ± 1.0	0.143
Limonene[e]	478	50.2 ± 3.1	51.1 ± 3.1	47.4 ± 3.1	0.680
1,8-Cineole	484	25.7 ± 5.7	40.1 ± 5.7	27.5 ± 5.7	0.152
(Z)-β-Ocimene[e]	495	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.241
(E)-β-Ocimene[c,e]	521	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	0.337
trans-Decahydronaphthalene[c,e]	532	0.8 ± 5.7	0.8 ± 5.7	10.7 ± 5.7	0.364
γ-Terpinene + Artemisia ketone[c,e]	544	3.3 ± 0.5	4.6 ± 0.5	4.7 ± 0.5	0.103
cis-Sabinene hydrate[e]	563	22.6 ± 5.0	37.7 ± 5.0	24.9 ± 5.0	0.075
Artemisia alcohol[c,e]	594	436.6 ± 64.0	647.8 ± 64.0	580.8 ± 64.0	0.065
Terpinolene[e]	609	4.0 ± 0.5	4.3 ± 0.5	4.4 ± 0.5	0.868
trans-Sabinene hydrate[e]	634	11.8 ± 1.8	17.3 ± 1.8	12.9 ± 1.8	0.069
cis-p-Menth-2-en-1-ol[c,e]	681	7.3 ± 1.0	9.0 ± 1.0	8.8 ± 1.0	0.404
α-Campholenal[c,e]	692	2.0 ± 0.5	3.1 ± 0.5	3.2 ± 0.5	0.135
trans-Pinocarveol[c,e]	730	7.8 ± 2.1	13.0 ± 2.1	14.2 ± 2.1	0.074
Camphor + trans-Verbenol[c,e]	740	9.5 ± 1.7	13.7 ± 1.7	13.2 ± 1.7	0.154
Isoborneol[c,e]	768	1.5 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	0.081
cis-Chrysanthenol[c] + Pinocarvone[c,e]	781	72.2 ± 28.4[g]	174.5 ± 28.4[h]	106.9 ± 28.4[g,h]	0.039
Borneol[e]	790	290.8 ± 43.0[g]	524.1 ± 43.0[h]	478.5 ± 43.0[h]	0.000
Terpin-4-ol[e]	824	5.3 ± 0.5	6.3 ± 0.5	5.7 ± 0.5	0.407
m-Cymen-8-ol[c,e]	831	3.5 ± 0.6	3.3 ± 0.6	4.0 ± 0.6	0.644
p-Cymen-8-ol[c,e]	837	2.9 ± 1.4	3.8 ± 1.4	6.2 ± 1.4	0.248
α-Terpineol[e]	855	2.9 ± 0.8	4.2 ± 0.8	4.5 ± 0.8	0.283
Myrtenal[c,e]	868	1.1 ± 0.2	1.7 ± 0.2	1.4 ± 0.2	0.065
Myrtenol[c,e]	870	1.9 ± 0.3	2.9 ± 0.3	2.7 ± 0.3	0.089
cis-Chrysanthenyl acetate[c,e]	1038	1.5 ± 0.4	2.4 ± 0.4	2.3 ± 0.4	0.225
Bornyl acetate[c,e]	1100	1.3 ± 0.2[g]	2.2 ± 0.2[h]	1.9 ± 0.2[h]	0.001
Carvacrol[c,e]	1142	1.7 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	0.231
α-Cubebene[c,e]	1274	4.3 ± 7.5	5.9 ± 7.5	17.9 ± 7.5	0.381
Eugenol[e]	1281	2.2 ± 1.0	1.9 ± 1.0	3.6 ± 1.0	0.429
Cyclosativene[c,e]	1317	3.6 ± 0.4	3.7 ± 0.4	3.9 ± 0.4	0.850
α-Copaene[e]	1340	9.1 ± 1.2	11.5 ± 1.2	12.2 ± 1.2	0.165
β-Bourbonene[c,e]	1364	10.1 ± 0.8	11.0 ± 0.8	12.1 ± 0.8	0.246
β-Cubebene[c,e]	1372	13.7 ± 1.6	15.3 ± 1.6	17.8 ± 1.6	0.196
(Z)-Jasmone[e]	1393	29.9 ± 3.0[g]	40.0 ± 3.0[h]	41.4 ± 3.0[h]	0.013
(E)-Caryophyllene[e]	1452	90.0 ± 7.7	91.4 ± 7.7	90.9 ± 7.7	0.992
α-Humulene[e]	1530	43.5 ± 3.9	43.0 ± 3.9	44.6 ± 3.9	0.957
Allo-Aromadendrene[c,e]	1551	4.1 ± 0.6	5.7 ± 0.6	5.4 ± 0.6	0.135
Drima-7,9(11)-diene[c,e]	1576	7.5 ± 0.8[g]	11.1 ± 0.8[h]	9.5 ± 0.8[g,h]	0.006
γ-Muurolene[c,e]	1588	14.4 ± 2.8	17.8 ± 2.8	22.5 ± 2.8	0.132
Germacrene D[c,e]	1599	211.7 ± 17.0	194.9 ± 17.0	199.2 ± 17.0	0.769
β-Selinene[c,e]	1620	16.8 ± 2.1	20.8 ± 2.1	19.4 ± 2.1	0.407
epi-Cubebol[c,e]	1637	15.5 ± 2.1	20.9 ± 2.1	20.4 ± 2.1	0.139
Bicyclogermacrene[c,e]	1639	7.0 ± 1.1	6.8 ± 1.1	8.2 ± 1.1	0.579
α-Muurolene[c,e]	1651	9.0 ± 1.0	10.5 ± 1.0	10.1 ± 1.0	0.544
Unknown-01[e]	1668	46.5 ± 6.2	45.5 ± 6.2	32.0 ± 6.2	0.190
γ-Cadinene[c,e]	1684	36.4 ± 5.2	47.2 ± 5.2	40.7 ± 5.2	0.339
cis-Calamenene[c,e]	1702	5.5 ± 0.5	5.9 ± 0.5	5.8 ± 0.5	0.826
Δ-Cadinene[c,e]	1704	4.7 ± 0.6	4.0 ± 0.6	4.1 ± 0.6	0.677
Cadina-1,4-diene[c,e]	1726	21.4 ± 3.1	27.5 ± 3.1	29.8 ± 3.1	0.148
Elemol[c,e]	1764	43.0 ± 3.3[g,h]	51.5 ± 3.3[g]	37.4 ± 3.3[h]	0.011
Longicamphenylone[c,e]	1790	13.0 ± 3.2	7.8 ± 3.2	7.0 ± 3.2	0.352
Ledol[c,e]	1808	53.9 ± 6.3[g]	80.5 ± 6.3[h]	65.2 ± 6.3[g,h]	0.014
Germacrene D-4-ol[c,e]	1831	30.3 ± 2.6	23.9 ± 2.6	25.9 ± 2.6	0.215
Spathulenol[c,e]	1833	25.2 ± 2.2	21.5 ± 2.2	25.0 ± 2.2	0.419
Caryophyllene oxide[e]	1843	58.5 ± 5.8	65.9 ± 5.8	49.8 ± 5.8	0.153
Unknown-02[e]	1865	400.7 ± 69.2	391.4 ± 69.2	267.5 ± 69.2	0.319

Table 1 (*continued*)

Chemical[b,c]	RT[d]	Control	UV restriction	50% shade	P-value
Unknown-03[e]	1892	62.6 ± 6.3	82.3 ± 6.3	73.7 ± 6.3	0.089
β-Oplopenone[c,e]	1903	13.7 ± 2.3	17.7 ± 2.3	19.1 ± 2.3	0.235
Unknown-04[e]	1941	5.4 ± 0.8	5.3 ± 0.8	3.6 ± 0.8	0.204
1-epi-Cubenol[c,e]	1957	88.6 ± 5.0	82.4 ± 5.0	85.0 ± 5.0	0.677
Hinesol[c,e]	1969	7.3 ± 0.8	8.1 ± 0.8	6.0 ± 0.8	0.211
Caryophylla-4(14),8(15)-dien-5a-ol[c] + epi-α-Muurolol[c,e]	1983	4.0 ± 0.7	5.0 ± 0.7	4.4 ± 0.7	0.619
(Z)-methyl jasmonate[c,e]	1996	12.7 ± 1.0[g]	13.2 ± 1.0[g]	16.4 ± 1.0[h]	0.018
β-Eudesmol[c,e]	1999	611.1 ± 42.0	518.8 ± 42.0	615.0 ± 42.0	0.192
Selin-11-en-4-α-ol[c,e]	2010	133.0 ± 12.9	164.8 ± 12.9	134.4 ± 12.9	0.150
Unknown-05[e]	2027	49.5 ± 10.1	50.9 ± 10.1	58.2 ± 10.1	0.805
Bulnesol[c,e]	2045	21.7 ± 2.4	23.7 ± 2.4	16.8 ± 2.4	0.129
(Z)-Methyl epi-jasmonate[c]	2068	12.4 ± 1.3	11.8 ± 1.3	13.6 ± 1.3	0.592
α-Bisabolol[c,e]	2083	69.4 ± 7.5	82.7 ± 7.5	86.2 ± 7.5	0.256
Eudesma-4(15),7-dien-1-β-ol[c,e]	2094	8.4 ± 2.2	10.7 ± 2.2	10.9 ± 2.2	0.671
Unknown-06	2156	7.1 ± 21.6	8.7 ± 21.6	44.9 ± 21.6	0.381
Unknown-07[e]	2168	9.7 ± 2.2[g]	19.1 ± 2.2[h]	10.1 ± 2.2[g]	0.004
Oplopanone[c,e]	2194	13.5 ± 1.4	16.2 ± 1.4	17.3 ± 1.4	0.160
Unknown-08	2216	720.1 ± 187.5	495.2 ± 187.5	526.6 ± 187.5	0.657
Xanthorrhizol[c,e]	2233	5.9 ± 0.9	6.8 ± 0.9	6.0 ± 0.9	0.746
β-Acoradienol[c,e]	2249	45.0 ± 3.7[g]	61.4 ± 3.7[h]	48.0 ± 3.7[g]	0.005
Nootkatone[c,e]	2336	17.8 ± 2.2	20.4 ± 2.2	17.7 ± 2.2	0.635
Cryptomeridiol[c,e]	2355	146.3 ± 19.0	180.7 ± 19.0	126.3 ± 19.0	0.128
Flourensiadiol[e]	2472	3069.8 ± 354.9[g]	4630.5 ± 354.9[h]	3662.3 ± 354.9[g,h]	0.009
Unknown-09[e]	2591	38.3 ± 4.1[g]	55.8 ± 4.1[h]	44.4 ± 4.1[g,h]	0.012
Unknown-10[e]	2624	126.5 ± 16.4[g,h]	171.4 ± 16.4[g]	99.8 ± 16.4[h]	0.010
Unknown-11[e]	2751	234.3 ± 17.8	235.9 ± 17.8	228.1 ± 17.8	0.948
Unknown-12[e]	2803	74.1 ± 5.6[g]	99.9 ± 5.6[h]	73.0 ± 5.6[g]	0.001
Unknown-13[e]	2881	1007.0 ± 98.7	976.6 ± 98.7	798.1 ± 98.7	0.274
Unknown-14	3104	8.0 ± 2.8	12.8 ± 2.8	8.2 ± 2.8	0.381
Unknown-15[e]	3226	171.2 ± 35.2	112.6 ± 35.2	88.1 ± 35.2	0.233
Unknown-16[e]	3286	1551.9 ± 185.4	1778.4 ± 185.4	1351.3 ± 185.4	0.269
Unknown-17[e]	3331	150.3 ± 40.5	137.4 ± 40.5	131.1 ± 40.5	0.944
Unknown-18[e]	3417	140.7 ± 20.0	175.1 ± 20.0	124.6 ± 20.0	0.192
Unknown-19[e]	3452	177.3 ± 25.0	126.1 ± 25.0	106.1 ± 25.0	0.120

[a] Means ± SEM; n = 60; mg/g DM for total phenolics and total volatiles, individual compounds are μ/g DM.
[b] Individual compounds were identified using kovats indices and mass spectral libraries; estimated concentrations were based on relative proportions of internal standard (2-carene).
[c] Tentatively identified based on Adams (1995); identity of other compounds verified with authentic standards.
[d] Retention time.
[e] A year effect (P < 0.05) was observed.
[f] Total volatiles = cumulative estimated concentrations of individual compounds.
[g] Means with different superscripts differ (P < 0.05).
[h] Means with different superscripts differ (P < 0.05).

detected for 18 (P < 0.05) individual compounds (Table 1). Concentrations of camphene, sabinene, β-pinene, borneol, bornyl acetate, and Z-jasmone were greater for UV-restricted and partial shade treatments than controls (P < 0.05). Concentrations of α-terpinene, cis-chrysanthenol + pinocarvone, drima-7,9(11)-diene, ledol, flourensadiol and unknown 09 were greater (P < 0.05) with UV restriction than for controls. Concentrations of unknown 07, β-acoradienol, and unknown 12 were greater with UV restriction than controls and shaded plants (P < 0.05), while elemol and unknown 10 concentrations were greater (P < 0.05) for UV restriction than the partial shade treatment. Only one compound (Z-methyl jasmonate) was greater (P < 0.05) in shaded plants than the other treatments.

4. Discussion

Differences among years may have been caused by variation in amount and timing of precipitation. Rainfall during the growing season was 105.4, 197.1, and 174.0 mm in year 1, 2, and 3, respectively. Rainfall occurred late in the growing season during 1995 and earlier in the growing season in 1996 and 1997. The fact that the greatest rainfall occurred early in the growing season in year 2 may explain the elevated total volatiles in year 2 for control plants. Insect damage from *Zygograma tortuosa* larvae was generally light during 1995 and 1996, and much heavier in 1997 (anecdotal observations only). Whether precipitation patterns and/or insect damage (i.e., induction) occurred are speculative. Induction can stimulate secondary compound synthesis in response to insect and mechanical damage (i.e., our clipping protocol) (Holopainen and Gershenzon, 2010). However, the fact that control plants in year 3 had lowest concentrations of total phenolics and lower total volatiles than year 2 would suggest induction was not a factor in yearly responses. It is not surprising that no indication of induction was observed from year to year, given that induced PSM synthesis can occur rapidly in response to biotic stresses such as herbivory or mechanical damage (Kost and Heil, 2006) and is often short-lived (Gershenzon, 1994).

Plant age, leaf age, season, and location within a plant also affect PSM concentrations in tarbush (Fredrickson et al., 2007; Estell et al., 2013). However, the sampling protocol in this study controlled for effects of all of these factors but plant age. Plant age was not specifically accounted for in this study, but the plants were in close proximity and reasonable similar in size and shape, and presumably comparable in age.

In our study, total phenolics were lower for shaded plants than control or UV-restricted plants. Rousseaux et al. (2004) reported that reduced UV radiation increased gallic acid but reduced a flavonoid aglycone in southern beech tree (*Nothofagus antarctica*) branches. Furthermore, supplemental UV increased carbon-based PSM (phenolics and/or flavonoids) in silver birch (Lavola et al., 1998; de la Rosa et al., 2001), sagebrush (Thines et al., 2007) and two *Nicotania* species (Izaguirre et al., 2007). Phenolic compounds and/or condensed tannins were lower in shade for Sitka spruce (Hartley et al., 1995), oak (*Quercus crispula*) (Nabeshima et al., 2001) and oak (*Q. pyrenaica*) seedlings (Baraza et al., 2004). Although the reduction in total phenolics with shading in our study was anticipated, the lack of effect of UV restriction on total phenolics was counter to our expectations.

Total volatiles tended to be elevated by UV restriction, but shaded plants did not differ from control plants in our study. In contrast, Thines et al. (2007) observed no effect of UV radiation on terpene concentrations in sagebrush. Shade was reported to increase monoterpene concentration in Sitka spruce (Hartley et al., 1995), but decreased terpene concentrations in ponderosa pine needles (Johnson et al., 1997). However, Rinnan et al. (2011) reported no effect of shading on volatile emissions from three species of arctic shrubs. While neither the effect of UV restriction nor the lack of effect of shade support our hypotheses, the lack of conformity to our hypotheses is not completely surprising given the variable responses among plant species and class of compounds to both shading and UV exposure discussed earlier (Graglia et al., 2001; Hansen et al., 2006; Izaguirre et al., 2007).

Carbon-based secondary compounds have important ecological roles in numerous plant species. Volatiles can serve as signals to herbivores and predators and for plant to plant communication (Dicke et al., 1993; Holopainen, 2004). Phenolics are also involved in ecological processes, including herbivory (Harborne, 2001), allelopathy (Vaughan and Ord, 1991), and litter decomposition (Kraus et al., 2003). While the interactions of tarbush PSM with ecological processes are poorly understood, compounds from tarbush have been shown to exhibit allelopathic and phytotoxic properties (Dayan and Tellez, 1999; Mata et al., 2003).

In conclusion, UV light restriction increased total volatile concentrations and had no effect on total phenolics in tarbush. In contrast, partial shade decreased total phenolic concentrations and had no effect on volatile concentrations. These findings may have implications for a number of ecological processes.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois.

AOAC, 1990. Official Methods of Analysis, fifteenth ed. Association of Official Analytical Chemists, Washington DC, USA.

Baraza, E., Gómez, J.M., Hódar, J.A., Zamora, R., 2004. Herbivory has a greater impact in shade than in sun: response of *Quercus pyrenaica* seedlings to multifactorial environmental variation. Can. J. Bot. 82, 357—364.

Bryant, J.P., Reichardt, P.B., Clausen, T.P., 1992. Chemically mediated interactions between woody plants and browsing mammals. J. Range Manag. 45, 18—24.

Byrd, D.W., McArthur, E.D., Wang, H., Graham, J.H., Freeman, D.C., 1999. Narrow hybrid zone between two subspecies of sagebrush, *Artemisia tridentata* (Asteraceae). VIII. Spatial and temporal pattern of terpenes. Biochem. Syst. Ecol. 27, 11—25.

Close, D.C., McArthur, C., 2002. Rethinking the role of many plant phenolics — protection from photodamage not herbivores? Oikos 99, 166—172.

Coley, P.D., Bryant, J.P., Chapin III, F.S., 1985. Resource availability and plant antiherbivore defense. Science 230, 895—899.

Danell, K., Haukioja, E., Huss-Danell, K., 1997. Morphological and chemical responses of mountain birch leaves and shoots to winter browsing along a gradient of plant productivity. Ecoscience 4, 296—303.

Dayan, F.E., Tellez, M.R., 1999. Phytotoxicity of tarbush (*Flourensia cernua* DC) leaf extracts. Allelopath. J. 6, 1—12.

de la Rosa, T.M., Julkunen-Tiitto, R., Lehto, T., Aphalo, P.J., 2001. Secondary metabolites and nutrient concentrations in silver birch seedlings under five levels of daily UV-B exposure and two relative nutrient addition rates. New Phytol. 150, 121—131.

Dicke, M., Bruin, J., Sabelis, M.W., 1993. Herbivore-induced plant volatiles mediate plant-carnivore, plant-herbivore, and plant-plant interactions: talking plants revisited. In: Schultz, J., Raskin, I. (Eds.), Plant Signals in Interactions with Other Organisms. Amer. Soc. Plant Physiol, Rockville, Maryland, pp. 182—196.

Elger, A., Lemoine, D.G., Fenner, M., Hanley, M.E., 2009. Plant ontogeny and chemical defence: older seedlings are better defended. Oikos 118, 767—773.

Estell, R.E., Fredrickson, E.L., Havstad, K.M., 1996. Chemical composition of *Flourensia cernua* at four growth stages. Grass Forage Sci. 51, 434—441.

Estell, R.E., Havstad, K.M., Fredrickson, E.L., Gardea-Torresdey, J.L., 1994. Secondary chemistry of the leaf surface of *Flourensia cernua*. Biochem. Syst. Ecol. 22, 73—77.

Estell, R.E., James, D.K., Fredrickson, E.L., Anderson, D.M., 2013. Within-plant distribution of volatile compounds on the leaf surface of *Flourensia cernua*. Biochem. Syst. Ecol. 48, 144—150.

Estell, R.E., Tellez, M.R., Fredrickson, E.L., Anderson, D.M., Havstad, K.M., Remmenga, M.R., 2001. Extracts of *Flourensia cernua* reduce consumption of alfalfa pellets by sheep. J. Chem. Ecol. 27, 2275—2285.

Fredrickson, E.L., Estell, R.E., Remmenga, M.D., 2007. Volatile compounds on the leaf surface of intact and regrowth tarbush (*Flourensia cernua* DC) canopies. J. Chem. Ecol. 33, 1867—1875.

Freeland, W.J., 1991. Plant secondary metabolites: biochemical coevolution with herbivores. In: Palo, R.T., Robbins, C.T. (Eds.), Plant Defenses against Mammalian Herbivory. CRC Press, Boca Raton, Florida, pp. 61—81.

Gershenzon, J., 1994. The cost of plant chemical defense against herbivory: a biochemical perspective. In: Bernays, E.A. (Ed.), Insect-plant Interactions, vol. 5. CRC Press, Boca Raton, Florida, pp. 105—173.

Gershenzon, J., Croteau, R., 1991. Terpenoids. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), Herbivores. Their Interactions with Secondary Plant Metabolites, The Chemical Participants, vol. 1. Academic Press, New York, pp. 165—219.

Gibbens, R.P., McNeely, R.P., Havstad, K.M., Beck, R.F., Nolen, B., 2005. Vegetation changes in the Jornada Basin from 1858 to 1998. J. Arid Environ. 61, 651—668.

Graglia, E., Julkunen-Tiitto, R., Shaver, G.R., Schmidt, I.K., Jonasson, S., Michelsen, A., 2001. Environmental control and intersite variations of phenolics in *Betula nana* in tundra ecosystems. New Phytol. 151, 227—236.

Hansen, A.H., Jonasson, S., Michelsen, A., Julkunen-Tiitto, R., 2006. Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen dwarf shrubs. Oecologia 147, 1—11.

Harborne, J.B., 2001. Twenty-five years of chemical ecology. Nat. Prod. Rep. 18, 361—379.

Hartley, S.E., Nelson, K., Gorman, M., 1995. The effect of fertilizer and shading on plant chemical composition and palatability to Orkney voles, *Microtus arvalis orcadensis*. Oikos 72, 79—87.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67, 283—335.

Herms, D.A., 1999. Physiological and abiotic determinants of competitive ability and herbivore resistance. Phyton 39, 53—64.

Heyworth, C.J., Iason, G.R., Temperton, V., Jarvis, P.G., Duncan, A.J., 1998. The effect of elevated CO_2 concentration and nutrient supply on carbon-based plant secondary metabolites in *Pinus sylvestris* L. Oecologia 115, 344—350.

Holopainen, J.K., 2004. Multiple functions of inducible plant volatiles. Trends Plant Sci. 9, 529—533.

Holopainen, J.K., Gershenzon, J., 2010. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci. 15, 176—184.

Izaguirre, M.M., Mazza, C.A., Svatos, A., Baldwin, I.T., Ballaré, C.L., 2007. Solar ultraviolet-B radiation and insect herbivory trigger partially overlapping phenolic responses in *Nicotiana attenuata* and *Nicotiana longiflora*. Ann. Bot. 99, 103—109.

Johnson, R.H., Young, B.L., Alstad, D.N., 1997. Responses of ponderosa pine growth and volatile terpene concentrations to manipulation of soil water and sunlight availability. Can. J. For. Res. 27, 1794—1804.

Komenda, M., Koppmann, R., 2002. Monoterpene emissions from Scots Pine (*Pinus Sylvestris*): field studies of emission rate variabilities. J. Geophys. Res. 107, 1—13.

Kost, C., Heil, M., 2006. Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. J. Ecol. 94, 619—628.

Kraus, T.E.C., Dalgren, R.A., Zasoki, R.J., 2003. Tannins in nutrient dynamics of forest ecosystems — a review. Plant Soil 256, 41—66.

Kuokkanen, K., Yan, S., Niemelä, P., 2003. Effects of elevated CO_2 and temperature on the leaf chemistry of birch *Betula pendula* (Roth) and the feeding behaviour of the weevil *Phyllobius maculicornis*. Agric. For. Entomol. 5, 209—217.

Lavola, A., Julkunen-Tiitto, R., Roininen, H., Aphalo, P., 1998. Host-plant preference of an insect herbivore mediated by UV-B and CO_2 in relation to plant secondary metabolites. Biochem. Syst. Ecol. 26, 1—12.

Llusià, J., Peñuelas, J., Alessio, G.A., Estiarte, M., 2006. Seasonal contrasting changes of foliar concentrations of terpenes and other volatile organic compound in four dominant species of a Mediterranean shrubland submitted to a field experimental drought and warming. Physiol. Plant. 127, 632—649.

Mata, R., Bye, R., Linares, E., Macías, M., Rivero-Cruz, I., Pérez, O., Timmermann, B.N., 2003. Phytotoxic compounds from *Flourensia cernua*. Phytochem 64, 285—291.

Nabeshima, E., Murakami, M., Hiura, T., 2001. Effects of herbivory and light conditions on induced defense in *Quercus crispula*. J. Plant Res. 114, 403—409.

Piechulla, B., Pott, M.B., 2003. Plant scents — mediators of inter- and intraorganismic communication. Planta 217, 687—689.

Powell, J.S., Raffa, K.F., 1999. Sources of variation in concentration and composition of foliar monoterpenes in tamarack (*Larix laricina*) seedlings: roles of nutrient availability, time of season, and plant architecture. J. Chem. Ecol. 25, 1771—1797.

Rinnan, R., Rinnan, A., Faubert, P., Tiiva, P., Holopainen, J.K., Michelsen, A., 2011. Few long-term effects of simulated climate change on volatile organic compound emissions and leaf chemistry of three subarctic dwarf shrubs. Environ. Exp. Bot. 72, 377—386.

Rousseaux, M.C., Julkunen-Tiitto, R., Searles, P.S., Scopel, A.L., Aphalo, P.J., Ballaré, C.L., 2004. Solar UV-B radiation affects leaf quality and insect herbivory in the southern beech tree *Nothofagus antarctica*. Oecologia 138, 505—512.

SAS, V9.4. SAS Institute Inc., Cary, North Carolina.

SCS, 1980. United States Department of Agriculture, Soil Conservation Service. Soil Survey of Doña Ana County Area, New Mexico, pp. 25—26. http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.

Semerdjieva, S.I., Sheffield, E., Phoenix, G.K., Gwynn-Jones, D., Callaghan, T.V., Johnson, G.N., 2003. Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. Plant Cell Environ. 26, 957—964.

Shelton, A.L., 2000. Variable chemical defences in plants and their effects on herbivore behaviour. Evol. Ecol. Res. 2, 231—249.

Tellez, M.R., Estell, R.E., Fredrickson, E.L., Havstad, K.M., 1997. Essential oil of *Flourensia cernua* DC. J. Essent. Oil Res. 9, 619—624.

Tellez, M.R., Estell, R.E., Fredrickson, E.L., Powell, J., Wedge, D.E., Schrader, K.K., Kobaisy, M., 2001. Extracts of *Flourensia cernua* (L): volatile constituents and antifungal, antialgal, and antitermite bioactivities. J. Chem. Ecol. 27, 2263—2273.

Thines, N.J., Shipley, L.A., Bassman, J.H., Fellman, J.K., Mattison, D.S., Slusser, J.R., Gao, W., 2007. Effects of enhanced UV-B radiation on plant chemistry: nutritional consequences for a specialist and generalist lagomorph. J. Chem. Ecol. 33, 1025—1039.

Vaughan, D., Ord, B.G., 1991. Extraction of potential allelochemicals and their effects on root morphology and nutrient contents. In: Atkinson, D. (Ed.), Plant Root Growth an Ecological Perspective. Blackwell Sci. Publ, London, England, pp. 399—421.

Xu, C., Sullivan, J.H., 2010. Reviewing the technical designs for experiments with ultraviolet-B radiation and impact on photosynthesis, DNA and secondary metabolism. J. Integr. Plant Biol. 52, 377—387.