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Secondary Chemistry of the Leaf Surface of *Flourensia cernua*

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Key Word Index—*Flourensia cernua*; leaf surface chemistry; monoterpenoids; sesquiterpenoids; epicuticular waxes.

Abstract—Epicuticular wax accounted for 9.2% of the dry wt. of whole *Flourensia cernua* (tarbush) leaves. Leaf surface extracts of tarbush were analyzed for mono- and sesquiterpenoids with gas chromatography–mass spectrometry. Camphene, β-myrcene, 3-carene, limonene, 1,8-cineole, borneol, *cis*-jasmone, β-caryophyllene, caryophyllene oxide and globulol were consistently present on the leaf surface of tarbush. Concentrations of several unidentified compounds were estimated. A great deal of plant-to-plant variability was detected in leaf surface mono- and sesquiterpenoid concentration. Information obtained on surface chemistry might be exploited to enhance the use of tarbush as a forage for domestic herbivores.

Introduction

Tarbush (*Flourensia cernua*, Asteraceae) is a deciduous shrub endemic to the Chihuahuan Desert of the southwestern United States and northern Mexico. From 1915 to the present, tarbush has increased from 2 to 9% of the vegetation cover of the 78,325 ha Jornada Experimental Range of southern New Mexico. The transition from grassland to shrubland is typical of the Chihuahuan Desert and has occurred primarily within the more productive range sites. This transition (desertification) is viewed as a self-augmenting process, and may have negative implications for global climate (Schlesinger *et al.*, 1990). Current intervention technologies are not economical nor are they ecologically viable.

Prior tarbush research has been limited to a few studies, including brief assessments of nutritional value (Nelson *et al.*, 1970) and toxic attributes (Mathews, 1944; Dollahite and Allen, 1975). Kingston *et al.* (1975) evaluated sesquiterpenes in tarbush and Naidu and Rao (1980) studied an isolated hemiterpene. Rao *et al.* (1970) and Dillon *et al.* (1976) characterized flavonoids, and Bohlmann and Grenz (1977) described 4-hydroxyacetophenone derivatives in tarbush.

Secondary metabolites frequently serve as a defense against herbivory, particularly in resource-limited environments such as hot deserts (Freeland, 1991). Selective browsing favors increased abundance of more heavily defended species, and can alter composition of plant communities towards less palatable species (Bryant *et al.*, 1991). The stability of these shrublands may be related to the presence of secondary metabolites, especially those involved in biochemical defense against herbivory. The secondary chemical profile of the leaf surface may be important for assessment of plant–herbivore interactions. We are unaware of any existing profile of the phylloplane chemistry of tarbush or the variability associated with it. Information on secondary metabolites would aid our understanding of chemical defenses that may contribute to self-augmentation of shrub-dominated plant communities and subsequent desertification, and provide tools to enhance the use of shrubs by herbivores. Increased use of shrubs by herbivores would reduce both the ecological

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advantage and the need for expensive shrub control. Identification of pharmacologicals could also contribute to investigations of traditional biomedicines.

We are interested in the secondary metabolite profile of the leaf surface of tarbush and how this profile varies among plants. Our objectives were to quantify secondary metabolite profile and concentration on the leaf surface of tarbush and to evaluate intraspecific variation in secondary metabolites of tarbush.

Materials and Methods

The study site was located in a heavily infested tarbush area on the Jornada Experimental Range. Samples were collected during a companion study involving high-density forced use of tarbush by cattle, sheep and goats. Tarbush samples were collected from four 0.6 ha paddocks at two locations (approximately 1.6 km apart) during two collection periods approximately 2 weeks apart (mature leaf stage).

After livestock had been in paddocks for 4 days (August 23 and September 7, 1990), tarbush leaf samples were collected from 10 tarbush plants per site/period combination. Five plants each exhibiting either a high (>45%) or a low (<10%) degree of use (visual estimation) were sampled in each site/period combination. All remaining leaf material (approximately 50 g) was removed from these 40 plants and immediately placed on dry ice. Samples were stored at −10°C. Inclusion of both browsed and unbrowsed tarbush plants may have increased the chemical diversity of samples for this profile.

Dry matter was analyzed in duplicate on 10 whole leaves of each sample. Epicuticular wax (modification of Mayeaux *et al.*, 1981) was analyzed in duplicate on 10 whole leaves by extracting with 20 ml of chloroform for 20 s. Surface compounds were extracted from five whole thawed leaves from each plant sample in duplicate for 5 min at room tempreature with 5 ml of 95% ethanol and were then refrigerated. Leaves were handled with forceps and mature leaves of uniform size and appearance were used for all chemical analyses. Steam distillation of tarbush essential oil was not conducted, given our goal of removing leaf surface compounds. A low temperature extraction may also prevent dehydration of labile alcohols to hydrocarbons, and may allow extraction of less volatile compounds and nonvolatile di- and triterpenes (Witte, 1986).

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^{-1}; 240 s filament multiplier time; initial column temperature of 60°C, 3 min isothermal, 3°C min^{-1} ramp to final column temperature of 240°C; 5 min isothermal; 68 min total run time; 1 μl injection volume). Preliminary mono- and sesquiterpene identification involved comparison of spectral data to the internal spectral library of the instrument and retention times based on published information (Adams, 1989). Positive identification of leaf surface terpenes was based on acquisition of standard terpene compounds for comparison with unknown retention times. We were unable to locate purified compounds for several tentatively identified peaks (based on library spectra). Many other peaks provided spectra which did not closely match library spectra. Many of these unknown compounds appeared to be mono- and sesquiterpenes, but some of the unknowns were probably not terpenoids, while others may have been higher terpenes (probably diterpenes).

Because of the complexity of chromatograms, a limited number of unidentified peaks were examined. Peaks that could not be positively identified but that matched very closely the terpene library were examined, as were peaks that had consistently large areas. Concentrations of peaks at selected retention times were estimated from peak area using the standard curve of β-caryophyllene. Although estimates derived in this manner do not yield absolute concentrations, relative differences and an assessment of plant variation are valid.

Results and Discussion

Epicuticular wax was a major component of tarbush, accounting for 9.2% (SEM = 0.49) of the dry wt. of whole tarbush leaves. Epicuticular wax is deposited on the leaf cuticle surface of semiarid shrubs, and although it is not a chemical entity, embedded in this wax are a variety of compounds, including terpenoids.

During preliminary investigation of the chemical makeup of the leaf surface, 19 mono- and sesquiterpenes were tentatively identified and obtained for verification. Ten of these compounds (camphene, β-myrcene, 3-carene, limonene, 1,8-cineole, borneol, *cis*-jasmone, β-caryophyllene, caryophyllene oxide and globulol) were identified in tarbush consistently (Table 1). Nine others (tricyclene, α-pinene, sabinene, 2-carene, *cis*-verbenol, nerol, *trans*-jasmone, *cis*-nerolidol and *trans*-nerolidol) were either not present, present below the detection threshold, or present only erratically. Concentrations of the 10 identified compounds and estimated concentrations of 29 unidentified peaks appear in Table 1, with tentative identifications

TABLE 1. MONO- AND SESQUITERPENE CONCENTRATIONS ON THE SURFACE OF TARBUSH LEAVES

Compound	RT*	Mean†	SEM*	MaxV*	MinV*	*n*	TentID*
Camphene	467	333	27.5	785	0	40	
β-Myrcene	565	107	5.5	190	42	40	
3-Carene	614	178	15.4	511	0	40	
Limonene	671	76	14.9	479	0	40	
1,8-Cineole	679	88	12.2	284	0	40	
Borneol	1081	753	63.7	2051	0	40	
cis-Jasmone	1466	15	6.4	209	0	40	
β-Caryophyllene	1482	196	13.5	490	105	40	
Caryophyllene oxide	1561	13	6.0	170	0	40	
Globulol	1565	455	140.9	4404	0	40	
Unknown **1**	585	65	11.1	374	0	40	
Unknown **2**	660	13	1.8	40	0	40	*o*- or *p*-cymene
Unknown **3**	788	25	5.0	130	0	40	
Unknown **4**	816	477	70.9	1665	0	40	artemisia alcohol
Unknown **5**	1006	6	1.0	25	0	40	camphor
Unknown **6**	1056	96	33.5	830	0	40	trans-verbenol
Unknown **7**	1430	9	1.1	34	1	40	α-copaene
Unknown **8**	1452	17	2.3	73	2	40	α-copaene
Unknown **9**	1459	15	3.2	114	0	40	β-bourbonene
Unknown **10**	1461	15	1.8	59	3	39	β-cubebene
Unknown **11**	1502	10	1.8	42	0	34	α-humulene
Unknown **12**	1512	23	2.3	59	0	40	γ-cadinene
Unknown **13**	1516	45	6.8	227	0	40	γ-cadinene
Unknown **14**	1519	30	3.6	106	0	39	viridiflorene or valencene
Unknown **15**	1523	32	7.0	174	0	39	
Unknown **16**	1527	29	3.5	95	6	40	
Unknown **17**	1556	84	12.7	333	0	40	ledol
Unknown **18**	1569	106	13.8	354	0	39	
Unknown **19**	1589	239	28.2	975	12	40	β-eudesmol or viridiflorol
Unknown **20**	1620	612	199.2	7515	0	39	
Unknown **21**	1647	229	31.2	836	2	40	
Unknown **22**	1668	6758	718	20 929	0	40	
Unknown **23**	1734	652	69.0	2141	65	40	
Unknown **24**	1746	1119	114	2615	0	40	
Unknown **25**	1873	830	225	4629	0	39	
Unknown **26**	1888	602	148.3	4094	0	38	
Unknown **27**	1904	2892	333.6	8777	36	39	
Unknown **28**	1975	343	43.4	1110	0	39	
Unknown **29**	1984	224	51.1	1255	0	39	

*RT = retention time (s); SEM = standard error of mean; MaxV = maximum value; MinV = minimum value; TentID = tentative identification of unknown compounds based on instrument spectral library.

†μg g^{-1} of dry matter. Estimated concentration of unknowns obtained using standard curve of β-caryophyllene.

when appropriate. Retention times of compounds with our specific instrument conditions appear in Table 1. The data revealed much plant-to-plant variation in many of the compounds examined (both knowns and unknowns), as evidenced by standard errors and minimum and maximum values (Table 1).

Unidentified peaks with early retention times generally had spectra characteristic of monoterpenes, followed by oxygenated monoterpenes, sesquiterpenes and compounds of larger molecular weight. One unidentified compound (unknown compound **4**; probably an oxygenated monoterpene, tentatively identified as artemisia alcohol) made a substantial contribution to the chemical makeup of the leaf surface of tarbush. Other unidentified compounds with relatively large contributions to the surface chemistry profile were those with very late retention times. Some of these compounds with later (1700+ s) retention times (and larger molecular weights) were probably not terpenoids, while others may have been higher terpenes (probably

diterpenes). Most of the peaks with the largest areas and greatest estimated concentrations were those with later retention times. These compounds may be present on the leaf surface in greatest amounts or may have greater recovery because they are less volatile than compounds with early retention times. Higher terpenes were less completely represented in the instrument spectral library, which may explain why we could not identify the later peaks.

Kingston *et al.* (1975) described two sesquiterpenes (flourensic acid and flourensadiol) in tarbush. Flourensic acid is a bicyclic eremophylane sesquiterpene ($C_{15}H_{22}O_3$). Flourensadiol is a tricyclic aromadendrane sesquiterpene ($C_{15}H_{26}O_2$) similar to viridiflorol. The latter compound was frequently offered as a possible identification during the internal library search (unknown compound **19**). No mass spectral data are available for these two compounds. However, the study cited above extracted whole flowering plant heads collected in March, and these compounds may not be present on the leaf surface of actively growing tarbush. More likely, these compounds represent two of the unidentified compounds present in tarbush.

Several of the terpenes identified in tarbush (Table 1) exhibit antimicrobial activity in ruminants. Monoterpene alcohols were strong inhibitors of *in vitro* digestibility with ruminal fluid inoculum from both sheep and deer (Oh *et al.*, 1967). Schwartz *et al.* (1980) reported borneol was inhibitory to cellulose digestion *in vitro*, camphor was intermediate, and limonene and β-pinene were least inhibitive of cellulose digestion.

The leaf surface is a recently recognized plant compartment and is the site of deposition of numerous compounds (Zobel *et al.*, 1991). These workers suggested phenolic compounds may be localized on the plant surface of certain species as a mechanism to communicate with and protect itself from its environment. Secondary products stored in and secreted by epidermal glands have exudates that can serve as a contact poison or volatile inhibitor (Levin, 1976). Volatile compounds are effective deterrents because they repel herbivores prior to defoliation (Levin, 1976). Volatile aromatic compounds may be involved in olfaction, whereas leaf surface compounds and plant components may affect taste and/or pain receptors that influence preference. Volatile terpenoids have been implicated in the selection process of grazing herbivores (Longhurst *et al.*, 1968; Elliot and Loudon, 1987; Morrison *et al.*, 1987). We suggest that describing the secondary metabolite profile of the leaf surface of tarbush can provide insight into mechanisms to increase the use of this shrub by herbivores.

Conclusions

Borneol and globulol were present in highest concentrations of compounds identified on the tarbush phylloplane. Unknown compound **4** (tentatively identified as artemisia alcohol) was also an important constituent. Other unknown compounds contributing greatly to the overall surface compound makeup nearly all had very late retention times. A large amount of intraspecies variability was noted in the concentration of many of the volatile compounds on the external surface of tarbush leaves. The variability might be advantageous for developing mechanisms to overcome herbivore aversion to tarbush. The relationships of these compounds to environmental factors should be examined as well. Components of the epicuticular wax which were not examined (terpenoids of larger molecular weight, alkanes, etc.) as well as internal compounds (alkaloids, phenolics, tannins, flavonoids, etc.) may respond to environmental cues, and should be evaluated. We recognize that the assumption that leaf surface terpenoids are of plant origin may be equivocal, and microbial, fungal and insect contributions to the chemical profile of the leaf surface need to be explored.

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