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Distribution and concentration of total phenolics, condensed tannins, and nordihydroguaiaretic acid (NDGA) in creosotebush (*Larrea tridentata*)

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Abstract

This paper focuses on the presence and distribution of secondary phenolic compounds found within creosotebush [*Larrea tridentata* (Sess. & Moc. ex DC.) Cov.]. Total phenolics, condensed tannins and nordihydroguaiaretic acid (NDGA) were measured in nine categories of tissue within creosotebush. Total phenolic and condensed tannin concentrations were determined using colorimetric methods while NDGA content was determined with high performance liquid chromatography (HPLC). Phenolics were present throughout the plant with the highest concentrations in leaves (36.2 mg/g), green stems (40.8 mg/g) and roots (mean for all root categories=28.6 mg/g). Condensed tannins were found in all tissues with highest concentrations in flowers (1.7 mg/g), seeds (1.1 mg/g), and roots less than 5 mm in diameter (1.1 mg/g). Flowers, leaves, green stems and small woody stems (<5 mm in diameter) all contained NDGA with highest concentrations in leaves (38.3 mg/g) and green stems (32.5 mg/g).This is the first report we are aware of giving secondary chemical characteristics of creosotebush roots. Data reported here will be used to support further research into the dynamics of shrub replacement and dominance of arid grasslands.

Keywords: Allelopathy; Secondary compounds

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1. Introduction

Creosotebush [*Larrea tridentata* (Sess. & Moc. ex DC.) Cov.] is a ubiquitous shrub in the hot deserts of North America (Benson and Darrow, 1981). Arrival of creosotebush in the Chihuahuan Desert is believed to have occurred about 4000 years ago (Van Devender and Spaulding (1979). This shrub has apparently expanded and contracted its range several times since its first appearance (Van Devender (1995), with the most recent expansion occurring within the last 100 years (Buffington and Herbel, 1965). Hypotheses explaining the recent expansion offer a number of causes; competition for soil nutrients and/or water (Phillips and MacMahon, 1981; Cox et al., 1983; Briones et al., 1998), overgrazing, (Branscomb, 1958), increasingly arid climate (Branscomb, 1958), changes in precipitation regimes (Brown et al., 1997), recurring drought (Buffington and Herbel, 1965), lack of fire (Humphrey and Mehrhoff, 1958; McPherson, 1995), soil properties such as calcium content and depth (Hallmark and Allen, 1975; Stein and Ludwig, 1979), and allelopathy (Knipe and Herbel, 1966).

Plants employ two main mechanisms to limit competition, physical (exploitation) and chemical (interference) (Crawley, 1986). Physical methods include out-competing neighboring plants for above or below ground resources. Chemical methods make the immediate environment around the plant untenable for seed germination and/or establishment. Additionally, the presence of allelochemicals or phytotoxins may affect a neighboring plant's ability to compete or enhance the competitive ability of the source plant, i.e. protection from herbivory.

If plants typically produce 50 to 90% of their net primary production below ground (Anderson, 1987) then competitive mechanisms would be expected to be at least partly associated with the root system of the plant. Below ground herbivory is an important component of herbivory (Detling et al., 1980; Seastedt et al., 1988); thus, compounds associated with the root system may have anti-herbivory properties as well (Muller et al., 1989). We are unaware of other work that examines the chemical content of creosotebush roots. This study examines the distribution and concentration of total phenolics, condensed tannins, and NDGA in various tissues within creosotebush as a foundation for further work addressing the role of secondary compounds in the replacement of arid grasslands by shrubs.

2. Methods

The study site was in the northern Chihuahuan Desert on the USDA/ARS Jornada Experimental Range approximately 16 km north of Las Cruces, New Mexico. The site consists of an area of sandy loam soil on a 2° east-facing slope. Depth to the first calcic layer is approximately 0.9 m. Gravel and sand at this site was derived from andesites, monzonites, and rhyolites eroded from the nearby Doña Ana Mountains. Elevation at the site is 1322 m. Average annual long-term precipitation is 247 mm and the annual mean temperature is 15 °C (mean high and low is 26 °C in July and

6 °C in January, respectively) at the range headquarters approximately 13.5 km north of the study site (Gile et al., 1998).

Creosotebush is the dominant shrub on this site, with tarbush, mesquite, yucca (*Yucca elata* Engelm.) and Mormon tea (*Ephedra trifurca* Torrey, *E. torryana* Wats.) present as minor components. Grasses present include burrograss (*Scleropogon brevifolius* Phil.), fluffgrass (*Dasyochloa pulchella* (Kunth) Steudel), black grama, (*Bouteloua eriopoda* Torrey) and bush muhly (*Muhlenbergia porteri* Scribn.), (plant nomenclature from Allred (1997).

Five plants were harvested in October 1997 using a backhoe and pressurized water spray to gather as much of the below ground portion of the plant as possible. Canopy volumes were calculated using the inverted cone formula from Ludwig et al. (1975). Categories were developed for the different tissues based on obvious divisions (flowers, seeds, leaves, green stems, woody stems, and roots). Woody stems and roots were subdivided into classes based on diameter (less than 5 mm and 5 to 12 mm for stems and roots, and greater than 12 mm for roots only). Samples (approximately 10 g) of each category were collected, placed on dry ice, transported to the lab, and stored at −10 °C. If tissue present on the plant was less than 10 g, then all tissue was collected. Preparation of the samples involved grinding in liquid nitrogen to a size that passed through a number 18 standard testing sieve (1 mm mesh) using a mortar and pestle. Dry matter factors were calculated according to standardized methods (AOAC, 1990).

Samples were extracted in methanol (50 ml for total phenolics and 5 ml for condensed tannins and NDGA). Sample weights for analysis were: total phenolics (0.5 g for all woody stems and roots, 0.25 g for flowers, seeds, leaves, and green stems); condensed tannins (0.5 g for all woody stem and roots, 0.25 to 0.15 g, for flowers, seeds, leaves, and green stems due to limited sample size); NDGA (0.25 g for all samples). Total phenolics and condensed tannins were analyzed colorimetrically (Milton Roy 401 Spectronic® spectrophotometer) using modified Folin-Dennis procedures (AOAC, 1990) and vanillin-HCL procedures (Price et al., 1978), respectively. Modifications to the total phenolic protocol consisted of changes in sample weight (0.5 g or 0.25 g for most depending on tissue availability), time of extraction (4 h) and volumes of reagents decreased proportionately. For condensed tannins, the protocol consisted of reducing sample weight and, consequently, volumes of reagents.

Nordihydroguaiaretic acid content was analyzed using High Performance Liquid Chromatography (HPLC). A Waters® system containing the following components was used: 600 E controller, 700 Satellite WISP autosampler, and a 486 tunable absorbance detector set at 283 nm. The method used was modified from Gonzalez-Coloma et al. (1988). Modifications consisted of using methanol instead of acetone as the extraction solvent and eluting isocratically with a 60/40 mixture of 99% H_2O-1% H_3PO_4 and 85% acetonitrile-14% H_2O-1% H_3PO_4. A Nova Pak® C-18 column was used with a flow rate of 1ml/min. NDGA (minimum 90% purity, Sigma Chemical Co., St. Louis, MO) was used as a standard and was injected periodically at 1 mg/ml to verify calibration. Sample injection volume was 10 μl.

In all cases, tests were performed to verify that modifications did not affect results.

Statistical analysis failed to detect any significant differences in results from tests of modifications vs methods as detailed in the original protocols. Kolmogorov–Smirnov tests to determine normality of distributions were conducted. T-tests and Analysis of Variance (ANOVA) or the non-parametric equivalent were used along with appropriate pair wise multiple comparison tests (Tukey's and Dunn's for equal and unequal sample size, respectively). Statistical analyses were performed using SigmaStat® for Windows, version 2.03 (SPSS, Inc., Chicago, IL). A voucher specimen was deposited in the New Mexico State University, Range Science Herbarium (NMCR).

3. Results

Canopy volumes for plants 1–5 were, 2.2, 1.3, 1.0, 0.8, and 0.6 m^3, respectively. Correlation coefficients of chemical concentration of the nine tissue categories on canopy volume resulted in no significant relationships. Phenolics were present throughout the entire plant with concentrations highest in green stems and leaves followed by roots (Fig. 1A). One-way ANOVA showed differences among tissue categories (n=4 for flowers, 5 for all others, $P<0.001$). Tukey's pairwise multiple comparisons test showed no difference between the phenolic content of green stems and leaves (P=0.900), or among the three root categories (roots 5–12 mm vs roots <5 mm, P=0.583; roots >12 mm vs roots <5 mm, P=0.742; and roots 5–12 mm vs roots >12 mm, P=1.00).

Kruskal-Wallis one-way ANOVA on ranks indicated differences between tissue categories of condensed tannins (n=4 for flowers, 5 for all others, P=0.003). Condensed tannins were found throughout creosotebush in low concentrations with the highest levels in flowers, seeds, and roots less than 3 mm in diameter (Fig. 1B). Numerically, concentrations in roots were inversely proportional to root diameter to at least 3 mm in diameter and statistically different (P=0.001). Tukey's pairwise multiple comparison procedure showed roots with diameters less that 5 mm to be different from all other roots ($P<0.001$) while roots from 5–12 mm did not differ from roots >12 mm (P=0.051).

NDGA was found in photosynthetic tissue (leaves and green stems) and associated tissues (flowers and small woody stems less than 5 mm in diameter) (Fig. 1C). Kruskal-Wallis One-way ANOVA on ranks revealed a difference ($P<0.001$) in concentration among tissue categories. Concentrations were highest in leaves and green stems. Tukey's test showed differences ($P<0.05$) between leaves vs. flowers and leaves vs woody stems <5 mm; as well as green stems vs woody stems < 5 mm and green stems vs flowers.

4. Discussion

We found total phenolics throughout the plant (Fig 1A). Highest concentrations were in leaves and green stems (photosynthetic tissue) and roots. Lowest concentrations were in large woody stems. Green leaves had a mean concentration of 32.5

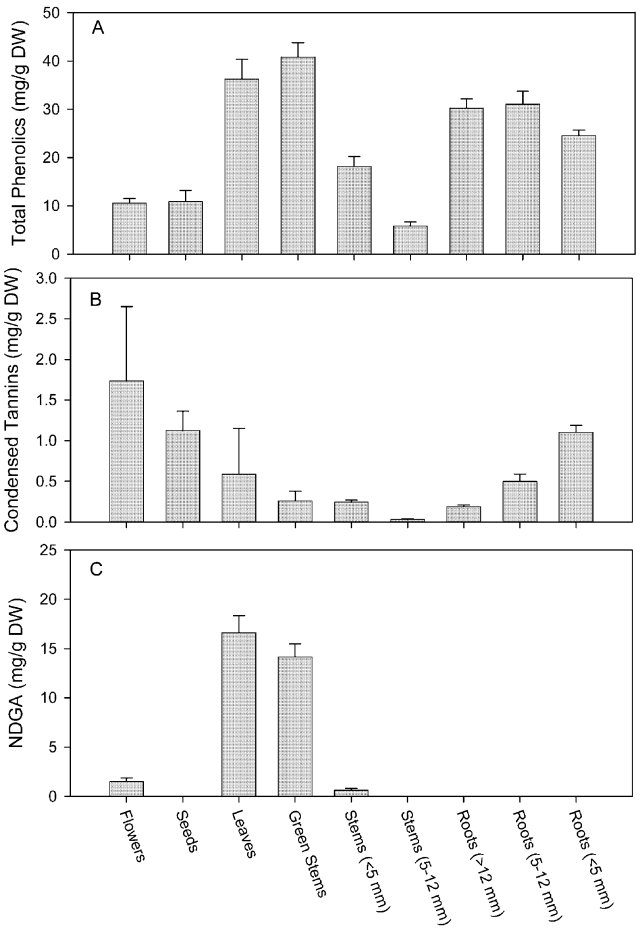

Fig. 1. Concentrations of phenolics (mg/g, DW) in various tissues in creosotebush (Bars are 1 SEM). A=total phenolics. B=condensed tannins. C=NDGA.

mg/g DW. In a subsequent analysis of a second collection of leaves we found total phenolic concentration to be 45.5 mg/g OM in air-dried leaves (Hyder et al. in prep.). Holechek et al. (1990) report 98.6 mg/g OM from air-dried leaves. Our collections were taken on the Jornada Experimental Range in October and December, respectively. Holechek et al. report their collection times as July to October, and location as " near Las Cruces". Time and location of collection may have a bearing on differences in reported concentrations. Mabry et al. (1977) report phenolic compounds from dry leaf material as 14.99% of the compounds in the extract. They use a diethyl ether extraction with dried whole leaves, as opposed to our methanol extraction using crushed leaf material, so a comparison of the two is inappropriate. We are unaware of other reports concerning the total phenolic concentration in creosotebush tissues.

Condensed tannins generally inhibit herbivory through reduction of plant digesti-

bility, leading to a reduction in nutritive value, although metabolic responses of certain herbivores to condensed tannins may limit the effectiveness of this defense (Hagerman et al., 1992; Owen-Smith et al., 1993). Condensed tannins found in creosotebush flowers and seeds suggest the use of secondary compounds to discourage herbivory of reproductive structures and influence seed dissemination. In green leaves we found condensed tannins to be present in a mean concentration of 0.59 mg/g DW. Holechek et al. (1990) found tannins at a concentration of 5.8 CE/100 mg OM. Relatively high levels of condensed tannins in roots (Fig. 1B) suggest that root herbivory is sufficiently important that an advantage is gained by plants that allocate resources in a way that maintains useful levels of anti-herbivore compounds in the root system. The inverse relationship between concentrations of condensed tannins and diameter in roots was interesting (Fig 1B). Increasing concentrations of condensed tannins, with decreasing root diameter, may be an indicator of the importance of small roots in resource acquisition or an increasing probability of herbivory on small, relatively active roots. We are unaware of any work on secondary compounds in creosotebush roots.

The presence of NDGA in photosynthetic tissue and the distal portions of branches suggest anti-herbivore and ultra-violet screening functions (Fig. 1C). NDGA and associated compounds in resin from creosotebush leaves complexes with proteins in a manner similar to tannins (Rhoades, 1977). The presence of NDGA in low concentrations in small woody stems may be due to contamination during collection, or movement of resins containing NDGA from leaves to stems or low levels of production during transition from photosynthetic to woody tissue. Additionally, NDGA levels in flowers may be a result of photosynthetic tissue in flower petioles.

Previous studies have reported a wide range of NDGA content in leaves; 0–6.5% dry weight (dw) (Gizvold, 1948), 7–12% dw (Botkin and Duisberg, 1949), and 5–15% dw (Mabry et al., 1977). Our work showed a mean of 3.8% dw of NDGA in leaves collected in October. Other studies have shown that concentrations of NDGA differ by location within a plant (Botkin and Duisberg, 1949), geographically (Downum et al., 1988), and temporally (Downum et al., 1988; Gonzalez-Coloma et al., 1994).

Phenolic secondary compounds are found throughout creosotebush (Fig. 1). The presence of these compounds in the above ground portion of creosotebush, with the highest concentrations in green stems and leaves, may be primarily related to limiting above ground herbivory, with protection of photosynthetic tissue from damage by ultra-violet radiation being a secondary function (Gonzalez-Coloma et al., 1988). The below ground content of phenolic compounds may be targeted toward limiting herbivory by fossorial organisms and inhibition of root growth into the rhizosphere by neighboring plants (Anderson, 1987; Mahall and Callaway, 1992). An additional function of below ground phenolic compounds may be the inhibition, or enhancement, of fungal/bacterial communities that interact with nutrient uptake capabilities of the root system (Vaughan et al., 1983; Blum and Shafer, 1988). Further research is needed to address the chemistry of roots less than 3 mm in diameter and their exudates. The roots and root tips at this scale are likely to be of interest. Creosotebush roots extend horizontally up to 4 m from the base of the plant (Gile et al., 1998) so

they can influence a large area in a plant community. The relatively high level of phenolics in the root system (mean=28.6 mg/g vs a mean of 12.0 mg/g for woody stems) could contribute to below ground chemical defense of the plant. Understanding the chemical properties of creosotebush, and the interactions influenced by these chemicals, may provide additional clues to the dynamics of grassland/shrub interactions and the recent success of creosotebush in these systems.

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