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TRANSPORT OF PHENOLIC COMPOUNDS FROM LEAF SURFACE OF CREOSOTEBUSH AND TARBUSH TO SOIL SURFACE BY PRECIPITATION[1]

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Abstract—During the last 100 years, many desert grasslands have been replaced by shrublands. One possible mechanism by which shrubs outcompete grasses is through the release of compounds that interfere with neighboring plants. Our objective was to examine the movement of secondary compounds from the leaf surface of creosotebush and tarbush to surrounding soil surfaces via precipitation. Units consisting of a funnel and bottle were used to collect stemflow, throughfall, and interspace precipitation samples from 20 creosotebush (two morphotypes) and 10 tarbush plants during three summer rainfall events in 1998. Precipitation samples were analyzed for total phenolics (both species) and nordihydroguaiaretic acid (creosotebush only). Phenolics were detected in throughfall and stemflow of both species with stemflow containing greater concentrations than throughfall (0.088 and 0.086 mg/ml for stemflow and 0.022 and 0.014 mg/ml for throughfall in creosotebush morphotypes U and V, respectively; 0.044 and 0.006 mg/ml for tarbush stemflow and throughfall, respectively). Nordihydroguaiaretic acid was not found in any precipitation collections. The results show that phenolic compounds produced by creosotebush and tarbush can be transported to the soil surface by precipitation, but whether concentrations are ecologically significant is uncertain. Nordihydroguaiaretic acid was not present in the runoff from creosotebush.

Key Words—*Flourensia cernua*, *Larrea tridentata*, nordihydroguaiaretic acid, total phenolics.

[1]Mention of a trade name, proprietary product or vendor does not constitute a warranty of the product by the USDA or imply its approval to the exclusion of other products or vendors that may also be suitable.

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INTRODUCTION

Replacement of arid grasslands by shrubs in the southwestern United States has been ongoing since the late 1800s (Buffington and Herbel, 1965; Archer, 1994). Two shrubs that have increased during this transition are creosotebush [*Larrea tridentata* (Sess. & Moc. ex DC.) Cov.] and tarbush (*Flourensia cernua* DC.). Both of these species have extensive root systems (depths of 5 m or more, radial spreads of 1.5–5 m, and surface roots within 10 cm of the soil surface) allowing efficient harvest of water and nutrients compared to grasses associated with these species whose roots rarely exceed 1.5 m in depth (Gibbens and Lenz, 2001). These two species are also well defended against herbivory. The resin on the leaf surface of creosotebush ranges from approximately 8 to 26% of the dry matter, depending on leaf age (Mabry et al., 1977; Rhoades, 1977). The phenolic fraction typically constitutes 80–90% of the resin (Mabry et al., 1977), and this fraction contains mainly phenolic aglycones, with nordihydroguaiaretic acid (NDGA) being the predominant component (Rhoades, 1977). Approximately 5–10% of the dry weight of creosotebush is NDGA (Mabry et al., 1977). Total phenolics in creosotebush ranged from 9.9% of organic matter in leaves collected between July and October (Holechek et al., 1990) to 3.6% in leaves collected in late fall (Hyder et al., 2002). Tarbush also has a resinous leaf surface (8–12% of dry matter) (Estell et al., 1994), with total phenolics ranging from 6.5 to 8.1% for leaves collected in four growth stages over a 3-year period (Estell et al., 1996).

Shmida and Whittaker (1981) concluded that allelopathic effects did not appear to exist for creosotebush based on spatial associations between herbaceous species and creosotebush. Knipe and Herbel (1966) demonstrated that an aqueous extract of creosotebush inhibited germination of black grama [*Bouteloua eriopoda* (Torr.) Torr.] seeds, but did not affect either bush muhly (*Muhlenbergia porteri* Scribn.) or creosotebush seeds. Bush muhly normally grows in close association with creosotebush, occasionally even to the detriment of the creosotebush (Welsh and Beck, 1976). Cox et al. (1983) observed greater leaf lengths and shoot production on grasses grown in soil collected from under creosotebush than in grasses grown in soil from interspaces. Elakovich and Stevens (1985) showed that NDGA inhibited root and hypocotyl growth in eight plant species. We are not aware of any studies regarding the allelopathic potential of compounds from tarbush. However, many phenolic compounds (individually or in mixtures) have been shown to exhibit allelopathic activity toward a number of plant species based on a variety of criteria (Einhellig, 1987; Li et al., 1993; Inderjit, 1996).

de Soyza et al. (1997) described two growth forms of Chihuahuan Desert creosotebush: hemispherical and inverted cone morphotypes (referred to as U and V morphotypes, respectively). These authors suggested the inverted cone shape, which is found among young plants and those in water-limited environments, enhances deep soil water storage through root channeling, while the hemispherical

shape, which is found in established older plants and plants in environments where water stress is less important, enhances soil nutrient development.

Our objectives were to determine if phenolic compounds from creosotebush and tarbush leaves can be transported to the soil surface via precipitation and if concentrations of secondary compounds in runoff differ between creosotebush morphotypes.

METHODS AND MATERIALS

The study site was in the northern Chihuahuan Desert on the USDA/Agricultural Research Service/Jornada Experimental Range approximately 16 km north of Las Cruces, New Mexico, USA. 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 were derived from andesites, monzonites, and rhyolites eroded from the nearby Doña Ana Mountains. Elevation at the site is 1322 m. Long-term (1915–1999) mean annual and growing season (July, August, and September) precipitation for the area is 246 and 133 mm, respectively. Long-term mean monthly temperatures for the coldest (January) and warmest (July) months are 6° and 26°C, respectively. Creosotebush is the dominant shrub on this site, with tarbush, mesquite (*Prosopis glandulosa* Torrey), yucca (*Yucca elata* Engelm.), and Mormon tea (*Ephedra trifurca* Torrey) present as minor components. Grass species include burrograss (*Scleropogon brevifolius* Phil.), fluffgrass (*Dasyochloa pulchella* (Kunth) Steudel), black grama, and bush muhly.

During June 1998, 20 creosotebush [10 of each morphotype, as described by de Soyza et al. (1997)] and 10 tarbush were randomly selected by using a point-quarter method. Each plant was equipped with three precipitation collection devices by using methods described by Martinez-Meza and Whitford (1996). One container collected water intercepted by the shrub canopy and channeled down the stems (stemflow). Stems used for collecting stemflow were selected with a leaf cluster unobstructed from above by other branches. The stem collection unit consisted of a polyethylene funnel (11 cm upper diameter with an area of approximately 95 cm^2) glued to the base of a branch with silicone glue. A hole was cut in the funnel and placed such that the funnel completely surrounded the branch. A Nalgene tube attached to the funnel was inserted into a 500-ml Nalgene collection container through a hole drilled in the lid to minimize evaporation and contamination. The tube fit through the hole loosely to allow pressure equalization as the container filled. A second unit collected water passing through the canopy without stem channeling (throughfall), and a third unit (control) collected rain that had not physically encountered a plant (interspaces). Throughfall and interspace collectors consisted of 500-ml collection containers with funnels inserted directly into the container lids. A throughfall collection unit was placed beneath the canopy of each

plant midway between the base and outer edge of the plant, and an interspace unit for each plant was placed an equal distance between it and the nearest neighboring plant. The collection containers were buried half way in the soil to provide stability and minimize exposure to light. Precipitation events occurred on July 22, 25, and 26, 1998, and samples were collected within 30 min of the end of each event. Occasionally, collection units were destroyed by animals or wind, resulting in fewer than 30 samples for a given runoff source/plant species (or morphotype) combination.

Precipitation samples were analyzed for total phenolics and NDGA. Samples were filtered through 0.2-μm PTFE syringe filters (Millex-FG, Millipore Corp., Bedford, Massachusetts, USA). A Waters 2690 separations module coupled to a Waters 486 UV detector (283 nm) equipped with a Symmetry C-18 column (2.1 $\times$ 50 mm, 3.5-μm particle diameter; Waters Corp., Millford, Massachusetts, USA) was used to analyze filtrate for NDGA (100-μl injection size, 65% water–35% HPLC grade acetonitrile mobile phase, flow rate of 0.3 ml/min). Concentrations were quantified with an external standard curve, and either a blank or an NDGA (minimum 90% purity, Sigma Chemical Co., St. Louis, Missouri, USA) standard (ranging from 1 to 100 ng) was injected after every 10 samples to verify calibration. Total phenolics concentration was analyzed using AOAC (1990) procedures (Folin-Denis method, tannic acid as standard). Samples (2-ml aliquots) were analyzed in duplicate on a Milton Roy Spectronic 401 spectrophotometer (760 nm). Statistical analyses were performed using SigmaStat® for Windows, version 2.03 (SPSS, 1997). Statistical procedures used were Kruskal-Wallis one-way ANOVA on ranks with Dunn's method to test all pairwise multiple comparisons.

RESULTS

Total phenolics were detected in both stemflow and throughfall (Figure 1), with greater concentrations in stemflow than throughfall for both creosotebush morphotypes and tarbush ($P < 0.001$). Total phenolic concentrations were higher in stemflow of creosotebush than tarbush, with no difference between the two morphotypes (Kruskal-Wallis one-way ANOVA on ranks, $Q = 0.175$, $P > 0.05$). Mean Stemflow concentrations were (mean $\pm$ SD) 0.088 ± 0.044 ($N = 26$) and 0.086 ± 0.033 ($N = 29$) mg/ml for the U and V morphotypes, respectively. Total phenolic concentrations in throughfall differed between creosotebush morphotypes (Kruskal-Wallis one way ANOVA on ranks, $Q = 1.088$, $P < 0.05$). Throughfall concentrations were (mean $\pm$ SD) 0.022 ± 0.016 ($N = 26$) and 0.014 ± 0.017 ($N = 26$) mg/ml for the U and V morphotypes, respectively. Tarbush exhibited a similar pattern (Figure 1) of greater total phenolics in stemflow than throughfall. Mean concentrations were 0.044 ± 0.022 ($N = 20$) and 0.006 ± 0.009 ($N = 23$) mg/ml for stemflow and throughfall, respectively. Nordihydroguaiaretic acid was not detected in the creosotebush runoff samples, and does not occur in tarbush.

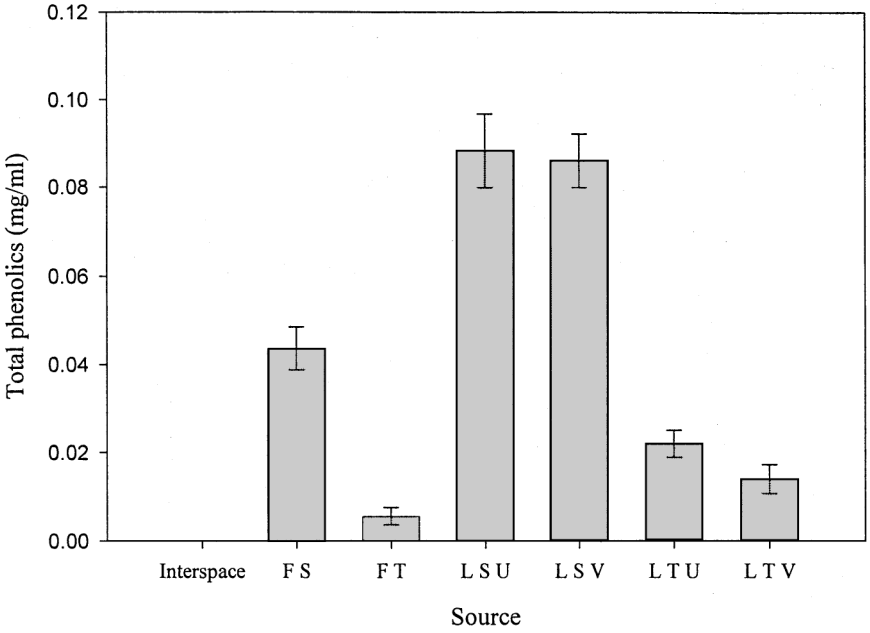

FIG. 1. Total phenolic concentrations (± SEM) in runoff from stemflow (S), throughfall (T), and interspace (I) of tarbush (F) and inverted cone (V) and hemispherical (U) morphotypes of creosotebush (L) during 1998, $N = 5$, 20, 23, 26, 29, and 26, respectively.

DISCUSSION

Creosotebush and tarbush produce phytochemicals with the potential to impact their immediate environment. Phenolic compounds were present in runoff from plant surfaces of both plants, and total phenolic concentrations varied for plant species and source of runoff. Concentration of total phenolics in runoff was greater for creosotebush than tarbush for both sources (approximately two fold for stemflow and three- to four fold for throughfall). Stemflow concentrations were approximately four- to five fold greater than throughfall for both creosotebush morphotypes and nearly an order of magnitude greater than throughfall for tarbush. Concentration of total phenolics in stemflow did not vary between creosotebush morphotypes, but did vary for throughfall (nearly two fold more in U- than V-shaped creosotebush). The hemispherical shape may provide more leaf surface area for precipitation to encounter compared to the inverted cone shape.

Nordihydroguaiaretic acid was not detected in precipitation samples. Potential allelopathic effects of creosotebush are often ascribed to this compound (Knipe and Herbel, 1966; Elakovich and Stevens, 1985). If so, NDGA must reach the soil by

some other presently unidentified route (e.g., leaf litter, root exudates), assuming our results are typical of creosotebush over its geographical and phenological range. Conversely, other phenolic compounds may be responsible for the effects observed in some research, given the wealth of data linking phenolics to allelopathy (Einhellig, 1987; Li et al., 1993; Inderjit, 1996). Lack of movement of NDGA may be due to its hydrophobic nature; this compound is only slightly soluble in water (Botkin and Duisberg, 1949). In prior tests of the effect of creosotebush extracts on seeds of various species, the extracts were obtained by soaking leaves and other tissues in water or Hoagland's solution for extended periods (Dalton, 1962; Knipe and Herbel, 1966; Barbour, 1967). Aqueous extracts (24 hr) of creosotebush contained as much as 150 mg/ml of NDGA (E. L. Fredrickson, unpublished data). Thus, extracts containing high concentrations of NDGA may not be biologically relevant, given the lack of NDGA in runoff observed in this study.

Nevertheless, some phenolic compounds reach the soil surface during precipitation events. Whether they are delivered in quantities sufficient to impact the surrounding environment is unknown. Concentrations of individual phenolics as low as 0.01 mM (Li et al., 1993) and 0.05 mM (Gallet, 1994) have been reported to exhibit allelopathic effects. Total phenolics concentration in our runoff samples would be approximately 0.2–0.4 mM (assuming a mean MW of 200), but this estimate represents a mixture of compounds.

Factors such as intensity, frequency, and duration of rainfall, plant phenology, etc., could affect the amount of secondary compounds delivered to the soil, their distribution (both depth and distance from source), and potential impact on surroundings. For example, Tromble (1988) demonstrated that for creosotebush and tarbush, precipitation events less than 3.6 and 3.0 mm, respectively, would be captured by the canopy and evaporate. Biotic factors may also affect concentrations and persistence of phenolics in the soil. Blum (1998) demonstrated that phenolic acids can have extremely transient lifetimes in soil due to microbial utilization, although Friedman (1987) noted desert ecosystems have properties (low rates of leaching and periodically reduced soil microfauna activity) that may favor persistence of allelopathic compounds.

In conclusion, movement of phenolic compounds from creosotebush and tarbush leaves to the soil surface occurs during precipitation events. Stemflow and throughfall samples from both species contained phenolics, with greatest concentrations in stemflow samples. However, NDGA was not found in any precipitation samples. Actual concentrations of phenolics in soil beneath creosotebush and tarbush are unknown and should be addressed in future work.

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