Great Basin Naturalist

Volume 48 | Number 2 Article 11

4-30-1988

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MONOTERPENE CONCENTRATIONS IN LITTER AND SOIL OF SINGLELEAF PINYON WOODLANDS OF THE WESTERN GREAT BASIN

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ABSTRACT.—Mean monoterpene content of singleleaf pinyon (*Pinus monophylla* [Torr.] Frem.) litter from stands growing on two soil series was 340 ± 310 ug/g air dry weight (adw). Individual monoterpene hydrocarbons suggested as potential allelopathic compounds occur in extremely small amounts, 0.5–110 ug/g adw. Mineral soils contained 50 times less total terpene, 6.6 ug/g ± 4.8 adw, than the litter immediately above. Results suggest that allelopathic effects would more likely occur in litter than mineral soil. These findings are substantiated by previous reports of decreased emergence and growth of herbaceous species in pinyon litter but not mineral soil.

Terpene hydrocarbons are potentially involved in the inhibition of germination and growth of herbaceous plant species under the pinyon-juniper canopy (Everett 1981, Jameson 1980). Terpenes have long been suspected as allelopathic substances causing germination inhibition or growth regulation (Rice 1974, 1984, Muller 1966, 1968, 1971). The possibility of such interactions has also been suggested by various field and laboratory observations (Rice 1974, 1984, Elmore 1985, Groves and Anderson 1981, Putnam 1985).

The terpene content of wood and gum turpentines from living pinyon has been established. Zavarin and Snajberk (1980) reported the presence of monoterpenes α-pinene, β-pinene, sabinene, camphene, 3-carene, myrcene, limonene, γ-terpinene, terpinolene, p-cymene, and β-phellandrene in 11 species of pinyon (Fig. 1). Also reported was the inconsistent occurrence of cis-ocimene, tricylene, α-phellandrene, α-thujene, and α-terpinene. These compounds are characteristic of the three conifer families Cupressaceae, Pinaceae, and Taxodiaceae (Zavarin 1971) and have been suggested as having allelopathic properties (Rice 1984, Mandava 1985).

Data are not available on the transport and fate of these compounds in litter and soils. It has been suggested that soils act as a repository for the sorption of volatile terpene hydrocarbons (Muller 1971), but studies have not specifically revealed where or at what concentrations terpene compounds accumulate on the forest floor.

The purpose of this study was to document the presence and amounts of monoterpenes found in singleleaf pinyon litter and the surface mineral soils immediately below. Monoterpene levels contained in mineral soil or pinyon litter suggest probable concentrations at which allelopathic effects are naturally exhibited. Defined in situ monoterpene concentrations will be used to test for allelopathic effects on herbaceous species in future research.

FIELD AND LABORATORY METHODS

Samples of singleleaf pinyon litter and surface mineral soils below the litter were taken from two sites 113 km apart on the western edge of the Great Basin. Site 1 was located on an east slope of the Virginia Range on the Duco soil series (Lithic Argixeroll; Soil Survey Staff 1975). Site 2 was on an east slope of the Wellington Hills on the Roloc soil series (Aridic Argixeroll). Litter samples taken 50 cm from the tree trunk were comprised of decomposing needle, twig, cone, and bark tissue. Mineral soil samples were taken from the upper 2.5 cm of soils immediately below the litter layer.

Paired litter and soil samples were collected adjacent to 13 randomly chosen trees at each site. Samples were analyzed for monoterpenes α-pinene, β-pinene, sabinene, camphene, 3-carene, myrcene, limonene, p-cymene, and γ-terpinene.

Samples of pinyon tree litter and mineral

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TABLE 1. Monoterpene concentrations in tree litter.

	Terpene ug/g sample air dry weight					
	Site 1			Site 2		
Compound	Mean	SD	Range	Mean	SD	Range
α-pinene	14.0	25.0	0.6–110	5.4	7.3	0.5–23
sabinene	7.1	4.1	2.4–15	3.5	1.6	0.9–7.3
β-pinene/ myrcene	3.0	3.6	0.7–15	7.6	7.8	0.8–23
camphene	5.3	1.2	4.1–6.5	ND	ND	ND
3-carene	2.1	1.4	0.5–9	1.2	0.6	0.7–2.7
p-cymene	3.1	3.1	0.7–9	1.0	0.6	0.5–2.4
limonene	4.0	7.4	0.6–3.1	4.0	7.6	0.6–2.5
γ-terpinene	3.1	3.2	0.8–1.5	1.6	1.3	0.6–4.3
	Total terpene ug/g sample air dry weight					
	Litter			Soil		
	Mean	SD	Range	Mean	SD	Range
Site 1	340	300	130–1400	7.3	4.5	2.3–15.4
Site 2	340	320	23–960	5.8	5.0	0.5–19.9

ND not detected.
SD standard deviation.

soil were prepared for analysis by pulverizing the material with mortar and pestle. Ten-gram aliquots of either soil or litter were placed in 25-ml test tubes and extracted with 10 ml of petroleum ether. Samples were subjected to 60 seconds of vigorous shaking on a Pulser Vortex Test Tube Mixer (Kraft Apparatus, Inc., Mineola, N.Y.), and then solvent and sample were allowed to equilibrate for 48 hours at room temperature. The extracts were then filtered through Whatman #1 and washed with 100 ml pet ether. The resulting extracts were concentrated to 0.5 ml under nitrogen in calibrated centrifuge tubes. Each sample was then analyzed by injection of 2 ul aliquots onto a Varian Aerograph series 1700 gas chromatograph using a 0.75 mm i.d. × 60 m Supelco methylsilicone widebore glass capillary column. The oven was temperature programmed from 70 to 270 C at a rate of 10 C/min. Individual compounds were integrated using a Hewlett-Packard 18850A GC terminal.

Quantitation of monoterpenes present in the extracts was accomplished using external standards. Standards of monoterpenes were prepared at a concentration of 1 ug/ul. β-phellandrene and terpinolene were not available as standards at the time of analysis. Monoterpenes in the extracts were identified by cochromatography and GC-MS (gas chromatograph–mass spectrometer) analysis. The quantity of individual monoterpenes identified in the extracts was determined from the integrated values obtained from each

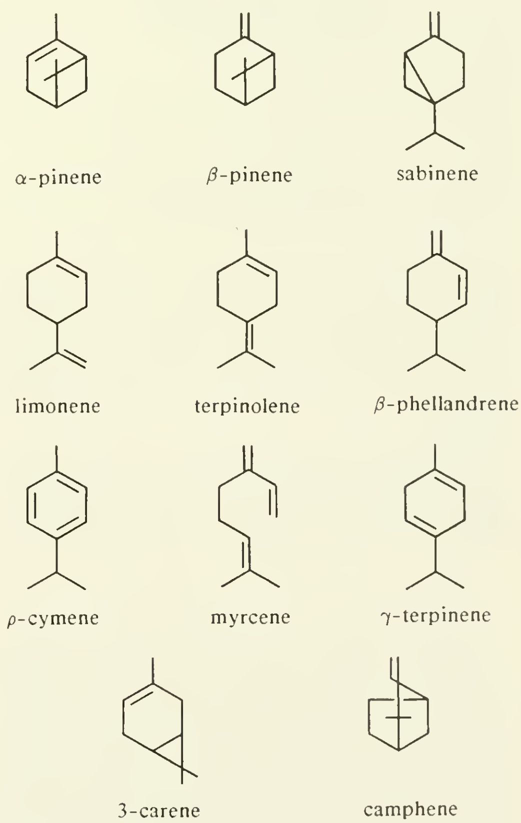

Fig. 1. Monoterpenes from Coniferales.

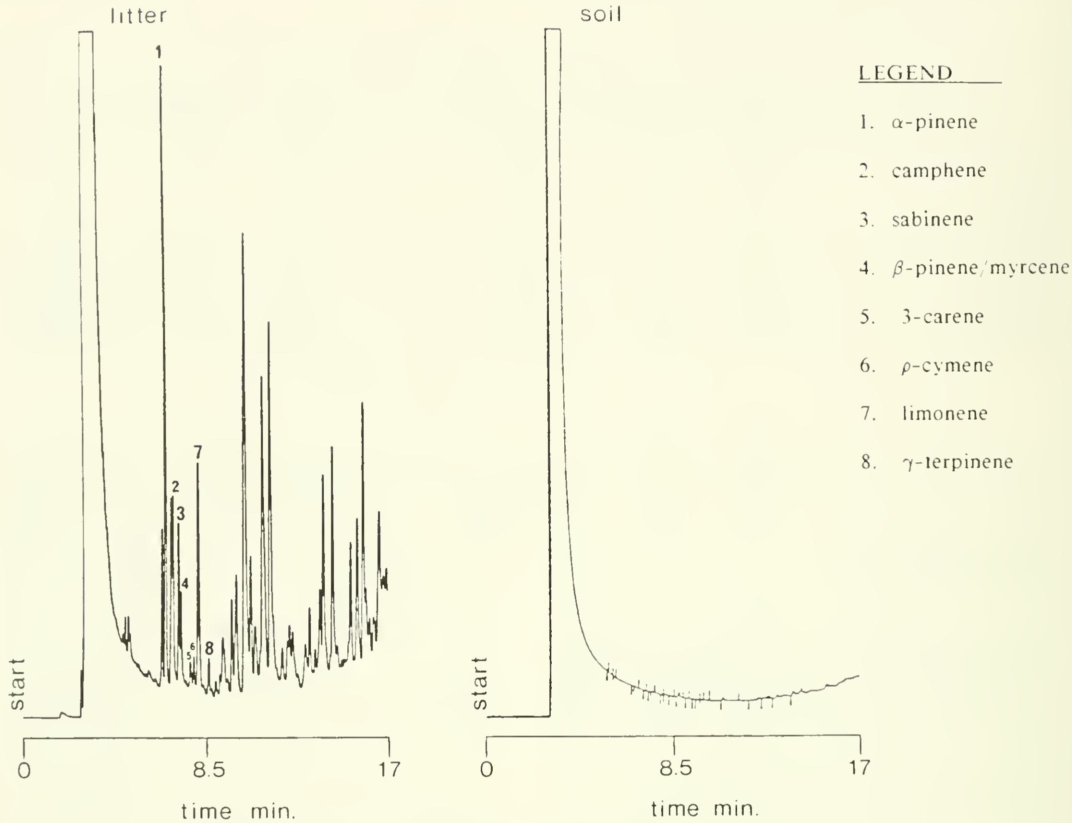

Fig. 2. Gas chromatograms of litter and soil extracts of sample number 4 from Site 1.

sample and expressed as mg/g air dry weight. Total monoterpene content was estimated by summing integrated areas of each compound from 6 minutes retention time to 17 minutes and is also expressed as an air dry weight basis.

Compounds eluting after 17 minutes were not included in the estimation of the monoterpene content since they were presumably higher molecular weight hydrocarbons.

Results and Discussion

The monoterpenes present in litter extracts (Table 1) were consistent with those reported for oleoresins of pinyon (Zavarin and Snajberk 1980, Fig. 1). Several other 10-carbon compounds were present at comparable concentrations but were not identified. Other constituents were evident in the extracts and probably included higher carbon number terpenes. A large variety of sesquiterpenes (C–15) and oxygenated monoterpenes were found present by Zavarin and Snajberk (1980) in both wood and gum turpentines. Gum and wood rosins were reported to be mainly composed of pimaric with smaller amounts of abietic acids. Low amounts of free fatty acids were found mainly in sapwood, along with trace amounts of unidentified ether-soluble compounds in wood. Our analysis of pinyon litter extracts by GC-MS confirmed the presence of higher molecular weight constituents, although they were not identified.

No attempt was made to identify individual monoterpenes from the mineral soil extracts because of their very low levels of occurrence. These soils have not previously been reported as allelopathic (Everett 1987). However, any integrated areas for the mineral soil samples were assumed to be terpene hydrocarbons. β-pinene coeluted with myrcene; therefore, these monoterpene concentrations are expressed as combinations. Camphene was detected in only two samples, both from Site 1.

The concentration of monoterpenes in the litter samples exhibited substantial variation, probably corresponding with the actual amount of woody material in the samples and the degree of weathering of the litter. The total terpene content of some individual Site 2 samples was much lower than those of Site 1, although the average values for both sites were equal.

Soil samples at both sites showed very low levels of monoterpene, which, on the average, were 50 times less than in the litter samples (Fig. 2). Litter results indicate a range of concentrations appropriate for testing allelopathic effects on understory species. Further study is suggested to identify and quantify the remaining components of these litter extracts and evaluate their involvement in seed germination inhibition and/or growth regulation.

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