

SOIL FATTY ACIDS UNDER ALDER, CONIFER, AND MIXED ALDER- CONIFER STANDS OF COASTAL OREGON

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ABSTRACT

Total soil lipid contents among alder, conifer, and mixed alder-conifer stands near the Oregon coast did not differ significantly. The lipids contained a number of fatty acids ranging in carbon-chain length from 12 (lauric acid) to 24 (lignoceric acid). Concentration levels of several fatty acids differed significantly among stands: lignoceric acid was highest in conifer soil; palmitic acid, in alder soil; and lauric acid, in mixed alder-conifer soil.

INTRODUCTION

Certain naturally occurring fatty acids possess antifungal and bactericidal properties (Fay and Farias 1975; Kabara et al. 1972; and Rigler and Greathouse 1940). Plant species differ as sources of fatty acids in soils (Wang, Hwang, and Chen 1971). The study reported here was designed to compare fatty acids among forest types as a basis for studies of the effects of fatty acids on *Phellinus weirii* (Murr.) Gilb., a destructive pathogen of conifer roots in the northwestern United States. The three forest types are represented in experimental plots at the Cascade Head Experimental Forest of coastal Oregon: red alder (*alnus rubra* Bong.), conifer, and mixed alder-conifer. The chemical and microbial properties of soil under these stands have been described by Franklin and Pechanec (1968) and Lu, Chen, and Bollen (1968).

MATERIALS AND METHODS

The upper 30 cm of soil, excluding litter, was collected from five randomly selected sites around each of three plots, 60 cm away from the randomly selected stems, in each of the three forest types. A sample was composited from the five subsamples of each plot and immediately sieved through a 10-mesh screen.

Two hundred grams of soil was shaken with 300 ml chloroform:methanol (2:1, v/v) in a nitrogen atmosphere for 1 h and then filtered. The procedure was repeated, and the combined filtrate was evaporated to dryness in a rotary

evaporator under vacuum at 50°C. The total lipid was then washed into a centrifuge tube with 30 ml chloroform:methanol (2:1, v/v). The mixture was washed with water and centrifuged by the procedure of Folch, Lees, and Sloane-Stanley (1957).

Fatty acid methyl esters were prepared by refluxing total lipids with 100 ml methanol:benzene:conc. sulfuric acid (17:2:1, v/v/v) for 1 h in a nitrogen atmosphere. Water was added to stop the reaction. The mixture was then neutralized with aqueous sodium hydroxide. The esters were extracted into hexane and purified by thin-layer chromatography on silica gel G with hexane:ethyl ether (19:1) as the developing solvent. The plates were sprayed with 0.2 percent solution of 2', 7'-dichlorofluorescein in 95 percent ethanol. The esters, appearing as a yellow band under ultraviolet light (366 nm), were collected from the plates and eluted with ether. The ether was removed under nitrogen, and the residue was dissolved in 0.5 ml hexane.

Esters were analyzed with a Microtek GC 2000-R gas chromatograph equipped with hydrogen flame detectors.¹ A stainless steel column, 1.8-m × 2.1-mm (ID), packed with 15 percent stabilized diethylene glycol succinate on 80- to 100-mesh Chromosorb W was operated isothermally at 200°C with a helium carrier gas flow rate of 40 ml/min. Inlet and detector

¹ The use of trade names in this publication does not constitute an official endorsement by the U.S. Department of Agriculture.

temperatures were adjusted to 225°C. Columns of different polarity, 10 percent SP-2300, 10 percent SP-2330, and 10 percent SP-2340 on the same support, were also operated at the same conditions in order to obtain further evidence of unsaturated fatty acid esters.

Fatty acids were identified by comparison of the relative retention time of the standards, and by a plot of the logarithm of retention time against carbon number. Standard compounds were also added to each unknown for peak enhancement studies. The quantity of each fatty acid was determined from its proportion of the total sample on the basis of plot area, calculated as the product of peak height and retention time.

Data from three plots within each of these three stands were subjected to analyses of variance. The mean levels of fatty acids for the three stands were further pairwise compared with Scheffé tests.

RESULTS AND DISCUSSION

Total soil lipid content did not differ significantly among alder, conifer, and mixed alder-conifer stands (Table 1). The fatty acids compositions of soil lipids under these stands were qualitatively similar; concentration levels of several fatty acids did differ significantly among stands: lignoceric acid was highest under conifers; unknown 1 and palmitic acid were highest under alder; and lauric acid was highest under mixed alder-conifer. Oleic acid was the most abundant fatty acid, constituting over 21 percent of the total fatty acid in all stands.

In addition to effects of vegetation of these forest types on soil lipid composition, soil microorganisms may have played a role in degrading soil lipids from dying plant tissue and dead residue to produce new lipids, which themselves differ in fatty acid composition. Thus, the differences in soil lipid composition among the tree stands are probably related to differences in the composition of the vascular vegetation as they affect the species composition of soil microflora (Wicklow, Bollen, and Denison 1974).

The effects of fatty acids on *P. weirii* have been reported. Puritch and Etheridge (1975) reported the inhibition of *P. weirii* by capric

TABLE 1
Total lipids and fatty acid composition of soils under conifer, red alder, and mixed alder-conifer stands^a

	Alder soil	Conifer soil	Mixed alder-conifer soil
Total lipids, % dry weight ^b	0.3a	0.2a	0.3a
Composition of fatty acids, relative % ^b			
Lauric	0.8b	0.6b	1.0a
Myristic	3.9a	2.3b	4.6a
Myristoleic	5.4a	3.9a	5.0a
Unknown 1	2.6a	Trace b ^c	Trace b
Palmitic	19.0a	9.3c	13.0b
Palmitoleic	8.4a	7.8a	8.7a
Heptadecanoic	2.1a	1.6a	2.3a
Stearic	5.2a	2.9b	3.9ab
Oleic	21.0a	21.4a	23.4a
Linoleic	4.3a	4.9a	5.9a
Unknown 2	7.2a	6.6a	7.3a
Arachidic	4.9a	7.7a	6.3a
Behenic	8.9a	14.2a	10.4a
Unknown 3	1.6a	1.4a	1.2a
Lignoceric	4.7b	15.4a	7.0b

^a Average of three replicates.

^b Means within rows not sharing a common postscript differ significantly at 5% confidence level with the Scheffé test.

^c Fatty acid less than 0.1%.

acid at 0.15 percent concentration level. Li et al. (1970) also reported that linoleic acid, present in *Phellinus*-resistant red alder, inhibited this fungus at 0.15 percent concentration. The concentration levels in soils under the three forest types, however, are not likely inhibitory to *Phellinus*, because linoleic acid, occurring in soils at 0.05×10^{-3} percent– 0.3×10^{-3} percent as soil weight basis, was 100 times less than inhibition concentrations. The effects of other fatty acids and of their interactions on *P. weirii* are yet to be studied.

Soil microorganisms, capable of producing effective concentrations of pathogen-inhibiting lipid metabolites, could be introduced into soil to create an environment hostile to *P. weirii*. Norén and Odham (1973) demonstrated that 13-methyl tetradecanoic acid, produced in soil by *Myxococcus xanthus*, inhibited germination of *Fusarium* conidia. Antagonistic soil microorganisms, stimulated by fatty acids, could also be introduced into soil to impair survival of *P. weirii*. The stimulatory effect of some fatty acids on soil microorganisms has been reported by several workers (Glasare 1970; Lindeberg and Lindeberg 1974; and Mukherjee 1952).

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