

Phenolic Compounds in Understory Species of Alder, Conifer, and Mixed Alder-Conifer Stands of Coastal Oregon

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ABSTRACT.—Phenolic compounds in hydrolysates and neutral extracts of leaves of understory vegetation in stands of red alder (*Alnus rubra* Bong.), conifer, and mixed alder-conifers near coastal Oregon were determined by two dimensional thin-layer chromatography coupled with different spraying reagents. *p*-Coumaric, *p*-hydroxybenzoic, and vanillic acids occurred in all species except *Galium triflorum*, which lacked *p*-hydroxybenzoic acid. Ferulic acid occurred in about two-thirds of the species. Protocatechuic and caffeic acids occurred sporadically among the species. Gentisic and syringic acids occurred in *Stachys mexicana*, *Menziesia ferruginea*, and *Ceanothus velutinus*. *Acer circinatum* contained syringic acid. *C. velutinus* leaves also contained phloroglucinol. Root nodules of alder and roots of *Ceanothus* were additionally extracted. The belowground parts of these species more resembled each other in content of phenolic compounds than they resembled leaves of their own respective species.

Decomposition of dying plant tissues and dead residues in soil produces phenolic and other substances potentially deterrent to soil-borne pathogens. Such substances, as identified from soil, include vanillic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, caffeic, phenylacetic, benzoic, syringic, ferulic, sinapic, and 3,5-dihydroxybenzoic acids (1-5). At a 2.0 mM concentration level, benzoic acid, ferulic acid, and phenylacetic acid completely inhibited *in vitro* growth of *Poria weirii* (Murr.) Murr., a serious root pathogen of coniferous tree species in the Pacific Northwest (6). Caffeic acid, syringic acid, and vanillic acid were weakly inhibitory; *p*-coumaric acid, *p*-hydroxybenzoic acid, and protocatechuic acid were not effective or stimulatory. The combination of *p*-coumaric acid, syringic acid, and ferulic acid, as found in roots of the *Poria*-resistant red alder (*Alnus rubra* Bong.) inhibited growth of *P. weirii* (7).

Plant species can be expected to differ as sources of pathogen-inhibiting substances. The study reported here was designed to compare the understory vegetation as sources of pathogen-inhibiting phenolic compounds in three forest types, as represented at the Cascade Head Experimental Forest of coastal Oregon: conifers, red alder (*Alnus rubra* Bong.), and mixed alder-conifer. The understory vegetation of these stands was described by Franklin and Pechanec (8). In addition, *Ceanothus velutinus* Dougl. ex Hook, collected in H. J. Andrews Experimental Forest near Blue River, Oregon, was included in the study because of its wide distribution in the West and its nitrogen-fixing root nodules.

MATERIALS AND METHODS

Fresh leaves were collected from selected species of understory plants on plots in each of the three forest types. The frequency and understory cover provided by these species are shown in table 1. As an adjunct to the main study, roots of *Ceanothus* were compared to nodules of *Alnus rubra* for phenolic compounds.

Each sample was lyophilized, ground to pass through a 40-mesh screen, and then 6g of plant material were exhaustively extracted with warm methanol (55°). The methanol was removed in a flash evaporator at 40°, and the residue was taken up in 60% methanol. Chlorophyll and lipid-soluble material were removed by extraction into *n*-hexane by the procedure of Steck and Bailey (9). The methanol was removed and the residue taken up with 150 ml of boiling water. The aqueous solution was divided into three equal aliquots. The first aliquot was acidified and refluxed with 5 ml of 12N HCl on a steam bath for 1 hour, after which it was cooled and extracted with diethyl ether in a liquid-liquid extractor for 12 hours to provide an acid-hydrolyzable fraction. A second aliquot was hydrolyzed with 4g of NaOH overnight at room temperature in a nitrogen atmosphere. Afterward, it was acidified to pH 2.0 with hydrochloric acid and extracted

with diethyl ether as above to provide an alkali-hydrolyzable fraction. The third aliquot was continuously extracted with diethyl ether for 12 hours without pretreatment to provide a neutral fraction.

Each diethyl ether extract was evaporated to dryness, taken up in 4 ml of warm methanol and applied as a spot on the corner of a thin-layer plate (20x20 cm) coated with 250 μ of microcrystalline cellulose (Sigma Cell, type 19, Sigma Chemical Co., St. Louis, Mo.). The plate was developed with 2% formic acid, air dried, rotated 90 degrees, and developed with benzene-acetic acid-water (7:6:3). Authentic compounds, alone and in mixtures, or co-spotted with plant

TABLE 1. Frequency and canopy cover of selected understory species in adjacent red alder, conifer, and mixed alder-conifer stands.^a

Understory plant species	Alder		Mixed		Conifer	
	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)
<i>Stokesiella oregona</i> (Sull.) Robins.	56	8	98	54	100	55
<i>Polystichum munitum</i> (Kaulf.) Presl.	26	14	24	14	20	11
<i>Carex deweyana</i> Schw.	4	2	2	< .5		+
<i>Maianthemum dilatatum</i> (Wood) Nels. & Macbr.	98	24	70	38	82	19
<i>Montia sibirica</i> (L.) Howell	94	37	82	42		+
<i>Rubus parviflorus</i> Nutt.	10	4				
<i>Rubus spectabilis</i> Pursh.	80	58	4	2		
<i>Acer circinatum</i> Pursh.	10	9		+		+
<i>Stachys mexicana</i> Benth.	28	6				
<i>Galium triflorum</i> Michx.		+	2	< .5	4	1
<i>Menziesia ferruginea</i> Smith		+				+
<i>Vaccinium parvifolium</i> Smith in Rees.		+		+		+
<i>Sambucus racemosa</i> L.	52	43	2	< .5		

^aJ. F. Franklin and Anna A. Pechanec (8); + indicates that a species was present in the stand but not observed on a plot.

extracts were run in the same procedure. After air drying, the chromatograms were examined under ultraviolet light (366 and 254 μ). The plates were sprayed with one of the following reagents: (a) diazotized *p*-nitroaniline followed by 2N NaOH, (b) diazotized sulfanilic acid, or (c) 2% ferric chloride solution. Identification of compounds was based on the position of the spot, appearance under ultraviolet light, and the color reactions to the spraying reagents. The combination of these tests has been found to be a reliable identification procedure for the substituted benzoic and cinnamic acids; no other biochemicals are known to produce identical results (10).

RESULTS AND DISCUSSION

A variety of phenolic compounds is known to occur in plants either in the form of glycosides and esters or as free acids. It is important to perform alkaline hydrolysis to hydrolyze esters and acid hydrolysis to hydrolyze glycosides in order to identify all the phenolics in given plant material.

In most species the neutral extracts contained relatively few identifiable phenolic compounds (tables 2 and 3). On the other hand, the acid and alkali hydrolysates generally contained several known phenolics. Both fractions provide clues to the nature of the bound form in which most of the phenolic compounds exist in the living plants. In most cases, a larger number of phenolic acids was liberated by alkaline hydrolysis than by acid hydrolysis; the former proceeds faster than the latter. This may also indicate that only very small amounts of phenolic acids, if any, exist as free acids or as glycosides in plants. Cinnamic acid derivatives may have broken down to some extent by acid treatment at high temperature; therefore, the failure to detect some phenolic acids may have been due to low concentration and decomposition by the acid treat-

TABLE 2. Phenolic compounds in leaves of selected understory species in adjacent red alder, conifer, and mixed alder-conifer stands.

Plant taxa	Caffeic acid	p-Coumaric acid	Ferulic acid	Gentisic acid	p-Hydroxybenzoic acid	Phloroglucinol	Protocatechuic acid	Syringic acid	Vanillic acid
Fractions in which compounds were detected ^a									
MOSSES									
Hypnaceae									
<i>Stokesiella oregona</i>	—	BN	B	—	ABN	—	—	—	ABN
FERNS									
Polypodiaceae									
<i>Polystichum munitum</i>	B	aB	—	—	ab	—	—	—	Abn
GYMNOSPERMS									
Pinaceae									
<i>Pseudotsuga menziesii</i>	—	B	—	—	aBN	—	—	—	bn
ANGIOSPERMS									
Cyperaceae									
<i>Carex deweyana</i>	B	aBN	aB	—	ABn	—	—	AB	AB
Liliaceae									
<i>Maianthemum dilatatum</i>	—	AB	—	—	AB	—	A	—	ABN
Betulaceae									
<i>Alnus rubra</i>	BN	Bn	—	—	abn	—	BN	—	aBN
Portulacaceae									
<i>Montia sibirica</i>	—	AB	aB	—	Ab	—	A	—	Ab
Rosaceae									
<i>Rubus parviflorus</i>	B	B	AB	—	AB	—	AB	—	AB
<i>Rubus spectabilis</i>	AB	b	AB	—	Ab	—	A	—	abN
Aceraceae									
<i>Acer circinatum</i>	AB	AB	—	A	B	—	A	—	AB
Rhamnaceae									
<i>Ceanothus velutinus</i>	—	AB	B	A	AB	B	—	A	A
Labiatae									
<i>Stachys mexicana</i>	AB	AB	AB	A	AB	—	—	AB	AB
Rubiaceae									
<i>Galium triflorum</i>	—	aB	—	—	—	—	—	—	An
Ericaceae									
<i>Menziesia ferruginea</i>	AB	AB	B	A	Ab	—	AB	AB	ABN
<i>Vaccinium parvifolium</i>	B	ABn	B	—	ABN	—	AB	—	ABn
Caprifoliaceae									
<i>Sambucus racemosa</i>	—	b	B	—	abn	—	—	b	b

^aA = acid fraction, B = basic fraction, N = neutral fraction, present in substantial amount; the same letters in lower case indicate only a trace detected; — = absent from all fractions.

ment (11). All these could in part account for some inconsistencies in the results.

p-Coumaric, p-hydroxybenzoic, and vanillic acids were encountered in leaves of all species except *Galium triflorum*, which lacked p-hydroxybenzoic acid (table 2). Gentisic acid was detected only in acid fractions of *Stachys*, *Acer*, *Menziesia*, and *Ceanothus*. Caffeic and protocatechuic acids occurred sporadically among the species; ferulic acid occurred in more than half of those examined. Syringic acid was found only in *Carex*, *Ceanothus*, *Stachys*, *Menziesia*, and *Sambucus*. Only *Ceanothus* leaves contained phloroglucinol.

Although *Alnus* and *Ceanothus* differed considerably in the phenolic compounds detected in leaves (table 2), their below-ground parts were strikingly similar (table 3). Ferulic acid was found in nodules of *Alnus* but not in roots of *Ceanothus*. Otherwise, the compounds and the fractions in which they were detected were essentially the same for each. Indeed, the difference was greater between below-ground parts and leaves within a species. Caffeic and protocatechuic acids were found in *Alnus* leaves but not in nodules; ferulic, gentisic, and syringic acids were in nodules but not in leaves. For *Ceanothus*, ferulic

TABLE 3. Phenolic compounds^a in root nodules of *Alnus rubra* and in roots of *Ceanothus velutinus*.

Species and plant parts	Caffeic acid	<i>p</i> -Coumaric acid	Ferulic acid	Gentisic acid	<i>p</i> -Hydroxybenzoic acid	Phloroglucinol	Protocatechuic acid	Syringic acid	Vanillic acid
<i>Alnus rubra</i> Bong., root nodules.....	—	aB	aB	A	aB	—	—	AB	AB
<i>Ceanothus velutinus</i> Dougl. ex Hook, roots..	—	B	—	A	AB	—	—	AB	ABN

^aA = acid fraction, B = basic fraction, N = neutral fraction, present in substantial amount; the same letters in lower case indicate only a trace detected; — = absent from all fractions.

acid and phloroglucinol were detected in leaves but not in roots. The results verify the presence of gentisic acid and the absence of ferulic acid in *Acer circinatum* as previously reported by Bate-Smith (12) and Tomaszewski (13), and add vanillic and protocatechuic acids to the list of phenolic compounds found in this species.

These results evidence the wide distribution of certain phenolic compounds in understory species native to Pacific Northwest coastal forests and support the hypothesis that pathogen-inhibiting or stimulating compounds in soil can be derived from decomposition or leaching from plant tissues. Species coverage and frequency, determined on fifty 20- by 50-cm plots systematically placed at one meter intervals along the inside boundaries of the center 5- by 25-m segment (8), differ markedly between the three forest types sampled (table 1). The understory is more lush under pure alder and mixed alder-conifer stands than under the pure conifer stand. Only the alder stand has significant coverage by *Rubus spectabilis*, *Sambucus racemosa*, and *Stachys mexicana*. These three species contained substances previously determined to inhibit *P. weirii* *in vitro* — ferulic acid and other, less strongly inhibiting phenolics (6). *p*-Coumaric acid, a compound stimulatory to *P. weirii* (7), also occurred in these plants, but it can be transformed into the weakly inhibitory caffeic acid by alder tissues (unpublished results by author). Only *R. spectabilis* contained protocatechuic acid, which is stimulatory to the pathogen.

A. circinatum and *Polystichum munitum* are common in conifer stands in the Pacific Northwest, including sites infected with *P. weirii*. They lacked the *Poria*-inhibiting ferulic acid and had few other inhibitory compounds, they both contained the *Poria*-stimulating *p*-coumaric acid, and *Acer* had protocatechuic acid as well. *Vaccinium parvifolium* is also common in coastal conifer stands. It contained both *Poria*-inhibiting and stimulating compounds.

In addition to the direct effects of *Alnus rubra* on soils in relation to inhibition of *P. weirii* (14), alder appears to be associated with understory species that are potential sources of *Poria*-inhibiting substances. The higher nitrogen content of soil under alder (15) promotes strong development of these understory species, thereby fostering a soil environment hostile to *Poria* or other pathogens of conifer roots.

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