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Polyphenols in *Ceratocystis minor* Infected *Pinus taeda*: Fungal Metabolites, Phloem and Xylem Phenols

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Since *Ceratocystis minor* is central to the death of pines infested by southern pine beetles, changes in polyphenols of infected loblolly pine were examined with regard to accumulation of fungal metabolites and changes in concentrations of fungitoxic and fungistatic phloem and xylem constitutents. *C. minor* grown in liquid culture produced 6,8-dihydroxy-3-hydroxymethyl-1*H*-2-benzopyran-1-one (I), 6,8-dihydroxy-3-methyl-1*H*-2-benzopyran-1-one (II), and 3,6,8-trihydroxy-3,4-dihydro-1-(2*H*)-naphthalenone (III). Isocoumarin II appeared in both phloem and xylem of loblolly pine bolts within 7 to 10 days after inoculation with *C. minor*. Isocoumarin I was evident in both phloem and xylem after 14 to 17 days of incubation. The α-tetralone III was not detected in either phloem or xylem. The condensed tannins, catechin, and flavanonols were degraded by *C. minor*. The stilbenes pinosylvin, pinosylvin monomethyl ether, and a compound tentatively identified as resveratrol initially accumulated, but their concentrations also diminished in later periods of incubation.

The blue-stain fungus *Ceratocystis minor* (Hedgc.) Hunt is generally introduced into the phloem and xylem of southern pine trees during attack by the southern pine beetle *Dendroctonus frontalis* Zimmerman, and development of *C. minor* in the xylem is considered to be central to the death of beetle-attacked trees (Nelson, 1934; Basham, 1970). It is commonly accepted that death of these trees is caused by a reduction in stem water conductivity to such an extent that wilting occurs (Bramble and Holst, 1940; Basham, 1970). However, other mechanisms (e.g., phytotoxins) may also be important.

The symptoms of southern pine beetle infestation are similar to those of Dutch elm disease, where bark beetles introduce *Ceratocystis ulmi* (Buism.) C. Moreau (Banfield, 1968) and translocatable phytotoxic metabolites have been suggested by a number of studies (Zentmeyer, 1942; Dimond, 1947; Feldman et al., 1950; Salemink et al., 1965). Increases of respiration rate and electrolyte mobility of leaf cells before the development of high water stress have been observed in Dutch elm disease (Landis and Hart, 1972). Both high molecular weight carbohydrates (Feldman et al., 1950) and glycopeptides (Van Alfen and Turner, 1975) reduce stem water conductivity, but these metabolites have no effect on leaf physiology other than the development of high water stress. Alcohol-soluble metabolites of *C. ulmi* induce necrotic lesions of leaf veins (Feldman et al., 1950).

Attention has recently been focused on phenolic C-10 acid–dihydroisocoumarin tautomers which are produced in highest yields by the most virulent strains of *C. ulmi* (Claydon et al., 1974). Isocoumarins are known for their biological activity toward plant growth (Hardegger et al., 1966; Iwasaki et al., 1973; Kameda et al., 1973). The similarities in both the cause and symptoms of Dutch elm disease to destruction of pines by the southern pine beetle–microbial complex prompted us to examine the phenolic metabolites of *C. minor* and to determine if these compounds are formed in loblolly pine phloem and xylem at times when they could be translocated to the needles.

Another objective of this study was to examine changes in phloem and xylem polyphenol composition which were associated with infection by *C. minor*. Many of the polyphenols in southern pine phloem (Hergert, 1960; Porter, 1974; Hemingway and McGraw, 1976) and xylem (Lindstedt and Misiorny, 1951; Erdtman, 1956) exhibit fungitoxic or fungistatic properties (Rennerfelt and Nacht, 1955; Scheffer and Cowling, 1966). Additional phenols might be produced as a host response to infection by *C. minor*, as Shain has shown in *Fomes annosus* (Fr.) Karst. infection of loblolly pine (Shain, 1967) and Norway spruce (Shain and Hillis, 1971) and as Shrimpton (1973) found in lodgepole pine after attack by *Dendroctonus ponderosae* Hopkins. Accumulation of fungistatic or fungitoxic phloem or xylem polyphenols might be a basis for host resistance to the southern pine beetle.

EXPERIMENTAL SECTION

Liquid Cultures. Isolates of *C. minor* and *C. ulmi* were grown in liquid shake culture on malt extract and on a defined medium containing glucose, 20 g; *l*-asparagine monohydrate, 2 g; KH_2PO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 20 mg; $FeCl_3$, 10 mg; $ZnSO_4 \cdot 7H_2O$, 20 mg; pyridoxin, 1 mg; and thiamin, 1 mg/L. Development of the fungus was followed by measuring pH and residual reducing sugar yields. When the pH had increased from 3.5 to 5.5 and reducing sugar concentrations were comparatively low (normally 3 to 4 weeks on malt extract) (Claydon et al., 1974), the cultures were harvested; the filtrates were saturated with NaCl and were extracted with chloroform, followed by ethyl acetate. Ethyl acetate extracts were concentrated and examined by TLC with diisopropyl ether–formic acid–water (90:7:3) or chloroform–acetone (9:1) and by PC with 1-butanol–acetic acid–water (6:1:2) and/or 6% acetic acid. Chromatograms were viewed under UV light and sprayed with either $FeCl_3$:$K_3Fe(CN)_6$ or diazotized sulfanilic acid, followed by K_2CO_3. Three major metabolites obtained from *C. minor* were identified by their spectral properties (McGraw and Hemingway, 1977). Phenolic metabolites of *C. ulmi* were identified by comparison of TLC R_f values, color reactions, and spectral properties with published data (Claydon et al., 1974).

***C. minor* Inoculated Loblolly Pine.** Loblolly pine trees were felled on Aug 12 and 19, 1975, from the Kisatchie National Forest in central Louisiana, and the stems were cut into bolts 1 m long (12 to 22 cm DIB). Six bolts from tree A (Aug 12) were inoculated with *C. minor* at 18 sites—six sites around the circumference at each of three positions along the length of each bolt. One control bolt was aseptically wounded with a 1.5-cm punch in a similar

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Table I. Chromatographic Properties of Compounds

	Ref[a]	R_f values: Paper BAW	Paper 6 HOAc	Thin layer	Thin layer DFW	Color reactions, on PC: UV[b]	UV + NH_4OH	Sulfanilic acid
6,8-Dihydroxy-3-methyl-isocoumarin	A			0.75	0.95		Ab	yw-or after base
6,8-Dihydroxy-3-hydroxymethyl-isocoumarin	A	0.78	0.47	0.18	0.70		Ab	yw-or after base
3,6,8-Trihydroxy-α-tetralone	A	0.77	0.63		0.57	yw	yw	or after base
		P. taeda Polyphenols						
Catechin	A	0.55	0.49			Ab	Ab	yw before base
Proanthocyanidin a	Porter, 1974	0.20	0.55			Ab	Ab	yw before base
Proanthocyanidin b	Porter, 1974	0.22	0.45			Ab	Ab	yw before base
Proanthocyanidin c	Porter, 1974	0.33	0.38			Ab	Ab	yw before base
Dihydroquercetin glucoside	Hergert, 1960	0.45	0.56			Ab	Ab	yw-or after base
Dihydroquercetin	A	0.71	0.37			Ab	Ab	wh-yw after base
Dihydromyricetin	Hergert, 1960	0.55	0.32			Ab	Ab	wh-yw after base
trans-Ferulic acid	A	0.76	0.31			bl	bl	pr after base
cis-Ferulic acid	A	0.76	0.61			bl	bl	pr after base
Pinosylvin	A	0.79	0.13	0.39		bl	bl	yw-or after base
Pinosylvin monomethyl ether	A	0.85	0.10	0.80		bl	wh	yw-or after base
Resveratrol	A	0.72	0.00	0.21		bl	bl	or-rd after base

[a] A, authentic compound used for comparison. [b] Ab, absorbs ultraviolet light; bl, blue; yw, yellow; or, orange; wh, white; pr, purple; rd, red.

pattern. The second tree B (Aug 19) was cut into 12 bolts; six bolts were inoculated with *C. minor*, and six bolts were aseptically wounded in the pattern described above.

The *C. minor* inoculated and control wound sites were sampled periodically over a 24-day incubation period by peeling the bark off at the xylem cambium in a zone 3 to 5 cm wide and 6 to 18 cm long around the treatment site. The extent of different colored reaction zones of the phloem (normal white phloem, a pale-yellow zone ahead of a dark-brown zone, and the black tissue where the perithecia were abundant) was measured, and phloem of each of these reaction zones was dissected with a scalpel. To test the purity of the inoculation, phloem tissues were cultivated. Excellent growth of *C. minor* was obtained from isolates of both brown and black phloem. *C. minor* was obtained occasionally from the yellow zone, but no other fungus was present. Sufficient treatment sites were sampled to obtain 0.5 to 2.0 g dry weight of each color reaction zone. Care was taken to avoid inclusion of any outer bark in the phloem samples. The xylem directly below the treatment site was sampled by drilling a 2.5-cm hole toward the pith; the shavings were collected, and the depth of penetration of the blue stain was measured. The control bolts were drilled to a comparable depth.

Phloem samples were dried under vacuum at room temperature and ground with a mortar and pestle. The ground phloem was extracted with 30 to 60 °C petroleum and then methanol by soaking for at least 24 h in 50 mL of each solvent. Extracts were recovered by filtration, dried under vacuum, and weighed. The methanol-soluble extract was redissolved in methanol (4 mL/g of phloem extracted), and 1 mL of this solution was applied to a 0.4-g Polyamide-6 column to remove the condensed tannins. The lower molecular weight polyphenols were eluted with 10 mL of methanol; the eluate was evaporated to dryness and redissolved in 1 mL of methanol.

Wood shavings (10 to 20 g) were dried under vacuum at room temperature and ground with a Wiley mill to pass a 1-mm screen. The ground wood was extracted with 30 to 60 °C petroleum and then methanol by soaking the wood for at least 24 h in 150 to 200 mL of solvent. The petroleum- and methanol-soluble materials were recovered by filtration, dried under vacuum, and weighed. The methanol extract was redissolved in methanol (1 mL/7 g of wood).

Whatman No. 1 papers were spotted with 40 μL of the methanol extracts from phloem and xylem, and the chromatograms were developed with 1-butanol–acetic acid–water (6:1:2) and in the second dimension with 6% acetic acid. The dried sheets were examined under UV light before and after fuming with ammonia and then were sprayed with diazotized sulfanilic acid, followed by K_2CO_3. Changes in concentrations of compounds were evaluated by visual comparision of spot intensity. Polyphenols of the phloem or xylem were identified by comparison of their chromatographic and spectral properties with either authentic compounds or published literature. The major phenolic metabolite of *C. minor*, 6,8-dihydroxy-3-hydroxymethylisocoumarin (I), was isolated from both phloem and xylem in sufficient quantities to crystallize. Melting points and spectral properties were identical with those of I, obtained from liquid culture.

RESULTS AND DISCUSSION

Phenolic Metabolites in Liquid Cultures. Culture filtrates from *C. minor* grown on both media contained three major phenols. Chromatographic and UV spectral properties of these compounds are summarized in Tables I and II, and detailed arguments for the proof of their structure have been reported (McGraw and Hemingway, 1977). The most abundant compound was 6,8-dihydroxy-3-hydroxymethyl-1*H*-2-benzopyran-1-one (I) (Figure 1). This isocoumarin has not previously been isolated as a fungal metabolite. A second isocoumarin was present in liquid cultures in much lower concentrations. It was identified primarily through its high-resolution mass spectrum as 6,8-dihydroxy-3-methyl-1*H*-2-benzopyran-1-one (II). Isocoumarin II was previously reported as a metabolite of *Ceratocystis fimbriata* Ell. and Halst. (Curtis, 1968), where it was found in small quantities along with larger proportions of the 6-methoxy analogue IV (Stoessel, 1969). The closely related dihydroisocoumarin V is a metabolite of *C. ulmi* (Claydon et al., 1974). The third compound isolated from liquid culture of *C. minor* was the α-tetralone 3,6,8-trihydroxy-3,4-dihydro-1(2*H*)-naphthalenone (III), which was previously identified as a

Table II. Ultraviolet Spectral Properties of Compounds

Compound	λ_{max}, nm		
	MeOH		MeOH + NaOH
	C. minor Metabolites		
6,8-Dihydroxy-3-methyl isocoumarin	236, 245, 277, 290, 329	244, 254, 275 sh, 309 335	244, 254, 306, 335, 354
6,8-Dihydroxy-3-hydroxymethyl isocoumarin	236, 245, 277, 290, 329	244, 254, 275 sh, 309 334	244, 254, 306, 335, 354,
3,6,8-Trihydroxy-α-tetralone	221, 232, 282, 315	240, 255, 285 sh, 331	214 250, 331
	P. taeda Polyphenols		
Catechin	279	279	decomp
Proanthocyanidin a	279-280	279-280	decomp
Proanthocyanidin b	279-280	279-280	decomp
Proanthocyanidin c	279-280	279-280	decomp
Dihydroquercetin glucoside	287, 325 sh	287 sh, 325	246, 325
Dihydroquercetin	287, 325 sh	287 sh, 325	246, 325
Ferulic acid	290, 319	285, 309	301, 347
Pinosylvin	298, 310 sh	298, 310 sh	315
Pinosylvin monomethyl ether	299, 310 sh	299, 310 sh	302, 311 sh
Resveratrol	307, 323	308, 323	322 sh, 341

metabolite of a *Scytalidium* sp. fungus (Findlay and Kwan, 1973a,b). α-Tetralones have not previously been reported as metabolites of any *Ceratocystis* sp. fungus. Both isocoumarins and α-tetralones are produced by *Pyricularium oryzae* Cavara, where compounds VI, VII, and VIII are produced along with the dihydroisocoumarin IX (Iwasaki et al., 1973).

The phenolic metabolites of a *C. ulmi* isolate were examined to provide a comparison with those produced by *C. minor*. TLC of ethyl acetate extracts from *C. ulmi* culture filtrates showed three blue fluorescent compounds (R_f 0.51, 0.67, and 0.79), which gave a blue coloration with $FeCl_3$–$K_3Fe(CN)_6$. The compound at R_f 0.51 had spectral properties indicative of the dihydroisocoumarin X, and the compound at R_f 0.79 had spectra indicative of the diketone XI. A third compound had chromatographic and spectral properties suggesting the ketone V (Claydon et al., 1974). None of these compounds was found in *C. minor* culture filtrates so the *C. ulmi* cultures were not studied further.

Since related dihydroisocoumarins and isocoumarins are known for their biological activity, it is possible that these phenolic metabolites of *C. minor* may alter the physiology of southern pine beetle infested trees. Some of these compounds (XII and XIII) exhibit antifungal activity (Condon and Kuč, 1960, 1962; Aldridge et al., 1966). Others (V, IX, XIV, and XV) stimulate the growth of seedlings at low (5 to 60 ppm) concentrations (Sassa et al., 1968; Kameda et al., 1973; Iwasaki et al., 1973). At higher concentrations (500 ppm), compounds V and IX inhibited the growth of rice seedlings, and V was phytotoxic to pear trees, causing necrotic lesions on the leaves (Kameda et al., 1973). Dihydroisocoumarins (V, X, XI) were implicated as possible phytotoxins in the Dutch elm disease (Claydon et al., 1974). The isocoumarin XVI is a major metabolite of *Endothia parasitica* (Murr.) Anderson and Anderson, a pathogenic fungus causing wilting of chestnut trees (Hardegger et al., 1966). There is less evidence for α-tetralones as possible phytotoxins. 3,6,8-Trihydroxy-α-tetralone (III) did not exhibit fungitoxic properties (Findlay and Kwan, 1973b), and compounds VII and VIII did not influence radical elongation of seedlings unless they were present in high concentrations (Iwasaki et al., 1973).

Although *C. minor* produced isocoumarins and α-tetralones in liquid culture, it was necessary to determine if they also were produced in infected loblolly pine phloem and xylem. The development of *C. minor* in loblolly pine was therefore studied in some detail.

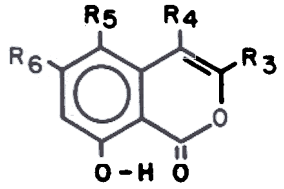
Table III. Petroleum Solubility of *P. taeda* Phloem and Xylem Infected with *C. minor*

Tissue	Percent of dry weight						
	Incubation period, days						
	3	7	10	14	17	21	24
Phloem							
White (*C. minor*)	3.2	2.5	1.9	1.8	1.9	1.6	0.94
(aseptic)	3.7	2.4	2.6	2.0	1.1	0.82	0.96
Yellow (*C. minor*)	3.7	2.3	3.5	2.3	3.6	2.0	0.94
(aseptic)	3.9		9.5		7.4		
Brown (*C. minor*)	3.7	3.3	4.7	5.6	3.8	2.7	3.4
(aseptic)	5.2		3.3		2.3		
Black			12.0	4.5	5.1	4.0	5.1
Xylem							
Normal		0.91	0.82	0.86	1.1	0.78	0.91
Blue stain		0.72	0.83	1.2	1.1	1.1	1.6

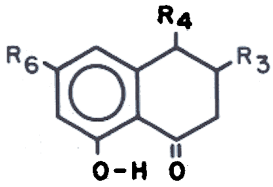
Development of *C. minor* in Loblolly Pine. Wound sites on control bolts were surrounded by narrow, brown rings with narrow, pale-yellow zones outside the rings. These color reactions did not change in appearance or extent throughout the experiment. No blue-stained xylem was observed in the control wounds. In the last days of the experiment, some of the control wounds were infected by unidentified fungi.

When inoculated with *C. minor*, phloem tissue revealed the yellow, brown, and black color reactions typical of this fungus. In the early stages of incubation, the yellow reaction zone was readily detected as a discrete zone, but it became difficult to measure after 14 days of incubation. The brown reaction zone increased in length steadily until the 17th day of incubation, after which the zones from different inoculation sites began to coalesce. The black reaction zone, indicative of perithecia development, was evident after the 10th day of incubation, and it spread until it covered most of the brown reaction zone by the 24th day. The blue stain of xylem was evident after 7 days, and it developed in a long, narrow zone toward the pith, reaching the center of the bolts after 21 days of incubation. A light-brown reaction zone was apparent ahead of the blue-stained xylem.

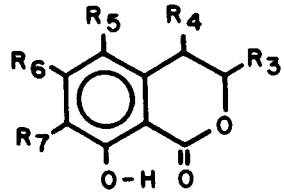
Extractive Content of Phloem and Xylem. *Petroleum-Soluble Extractives.* The petroleum-soluble extracts from the normal white phloem were pale-yellow oils, suggesting a predominantly fatty composition. The



	R_3	R_4	R_5	R_6
I	CH_2OH	H	H	OH
II	CH_3	H	H	OH
IV	CH_3	H	H	OCH_3
XIII	H	$OCCH_3$	CH_3	OH
XVI	$CH_2CHOHCH_3$	H	H	OCH_3



	R_3	R_4	R_6
III	OH,H	H_2	OH
VI	OH,H	OH,H	H
VII	OH,H	OH,H	OH
VIII	H_2	OH,H	OH



	R_3	R_4	R_5	R_6	R_7
V	CH_3,OH	H_2	H	OH	H
IX	CH_3,H	H_2	H	OH	H
X	CH_3,OH	OH,H	H	OH	H
XI	CH_3,OH	= O	H	OH	H
XII	CH_3,H	H_2	H	OCH_3	H
XIV	CH_3,OH	CH_3,H	CH_3	OH	CH_3
XV	CH_3,OH	H_2	CH_3	OH	CH_3

Figure 1. Phenolic metabolites of fungi.

amounts of petroleum-soluble material in the normal white phloem decreased during the incubation period in both the *C. minor* inoculated and aseptically wounded bolts, so the decrease was not related to infection by *C. minor* (Table III). The petroleum solubility of the brown and black reaction zones of the *C. minor* infected phloem was usually higher than that of the white phloem. The more solid and tacky properties of the extract suggested a higher proportion of resin acids in these reaction zones than in normal phloem. In a concurrent study, Barras (1976) found that the proportion of neutral lipids in the phloem decreased after 4 weeks' growth of *C. minor*. Different inoculation sites varied considerably in the extent of resinosus. The fungus appeared to develop well in sites where resinosus was readily apparent, so resinosus did not occur sufficiently to retard the growth of *C. minor*. The amounts of petroleum-soluble materials in normal and blue-stained xylem did not differ, and the only change during the incubation period was a slight upward trend in the petroleum solubility of the blue-stained xylem of tree A.

Methanol-Soluble Extractives. The methanol solubility of the normal white phloem decreased during the experiment, and, as with the petroleum solubility, this decrease in concentration of methanol-soluble materials was not dependent upon infection by *C. minor* (Table IV). The yellow and particularly the brown reaction zones of the aseptically wounded sites contained less methanol-soluble material than did the normal white phloem. In the early stages of incubation of the *C. minor* inoculated sites, the yellow reaction zone contained much less methanol-soluble material than did the normal white phloem. The difference between these two tissue types became less pronounced later in the incubation period. Since the yellow reaction zone became more difficult to detect as a discrete zone, the convergence in the methanol solubility of these two tissue types may reflect difficulties encountered in sampling.

The brown and black reaction zones contained far less methanol-soluble material than did the white or yellow tissue. To determine how much of this response may have resulted from fungal utilization of low molecular weight carbohydrates, the amounts of reducing sugars present in the methanol extracts were determined after acid hydrolysis. Results shown in Table V confirmed our suspicion that some of this reduction in methanol solubility was related to the carbohydrates. A similar effect was reported by Barras and Hodges (1969). However, the major cause of the lower methanol solubility in the black and brown reaction zones than in normal white phloem was a reduction in the amounts of tannins extractable with methanol.

The methanol solubility of the blue-stained wood was consistently lower than that of the normal wood, but no substantial changes were observed with an increase in the incubation period.

***C. minor* Metabolites in Phloem and Xylem.** The three major phenolic metabolites of *C. minor* grown in liquid culture were not detected in the white or yellow reaction zone phloem at any stage of incubation. Isocoumarin II was detected in the brown reaction zone phloem 7 to 10 days after incubation, and isocoumarin I was evident in the brown reaction zone after 14 days. Concentrations of both of these metabolites increased in the black reaction zone with increasing length of incubation. However, the concentration of II remained at relatively low levels, but I reached very high concentrations. After 24 days of incubation, when the perithecia had covered the entire reaction zone, I was the dominant phenol in the methanol extracts. The α-tetralone III was not detected in the phloem after any stage in development of the fungus.

The xylem of control bolts and the normal white xylem of *C. minor* inoculated bolts contained none of the three phenolic metabolites of *C. minor*. In the blue-stained xylem, II appeared after 7 days of incubation, and I was evident after 14 days. As was evident in the phloem samples, the concentration of II remained at relatively low levels, while the concentration of I continually increased until it became the dominant phenol of the blue-stained xylem after 17 days. Because of interference by other xylem constituents at the R_f values of III, it was impossible to determine if small amounts of the α-tetralone were present in the blue-stained xylem.

Table IV. Methanol Solubility of *P. taeda* Phloem and Xylem Infected with *C. minor*

	Percent of dry weight						
	Incubation period, days						
Tissue	3	7	10	14	17	21	24
Phloem							
White (*C. minor*)	29	30	27	25	25	22	23
(aseptic)	30	32	27	29	23	20	21
Yellow (*C. minor*)	20	21	20	22	20	22	20
(aseptic)	23		22		19		
Brown (*C. minor*)	14	13	9.0	6.3	7.9	6.4	6.0
(aseptic)	17		16		12		
Black (*C. minor*)			7.0	4.4	4.8	3.8	4.2
Xylem							
Normal		0.75	0.83	0.80	0.82	0.91	0.88
Blue stain		0.57	0.70	0.62	0.70	0.70	0.67

Table V. Changes in Carbohydrates and Polyphenols in Methanol Extracts of Phloem Infested with *C. minor*[a]

Phloem type	H_2O insoluble	
White	155	31
Yellow	137	31
Brown	45.2	6.9
Black	53.6	3.8

[a] Samples from tree A after 14 days of incubation.

Changes in Phloem and Xylem Polyphenols. *Polyphenols in Aseptic Phloem.* The major polyphenol present in the normal white phloem of both trees was catechin (Table I). Large amounts of proanthocyanidins were also present. The phloem of tree A differed from that of tree B because of the presence of relatively large amounts of a glucoside of dihydroquercetin in tree A. Flavanonol and flavonol aglycones were not detected in the white phloem tissue of either tree. Only subtle changes in the polyphenol composition of the white phloem were observed as incubation progressed. In tree A the concentration of the dihydroquercetin glucoside decreased, but no corresponding increase in dihydroquercetin concentration was detected. In the normal white phloem tissues of both trees, the distribution of the proanthocyanidins shifted to compounds of lower R_f values in the 6% HOAc dimension and higher R_f values in the 1-BuOH–HOAc–H_2O dimension. In the yellow and brown reaction zones of the aseptically wounded sites, the amounts of proanthocyanidins and catechin were much lower than in the normal white tissue. Small amounts of ferulic acid were also detected in the brown reaction zone.

Polyphenols in C. minor Infected Phloem. Although catechin and proanthocyanidin concentrations in the yellow reaction zones of *C. minor* infected tissues were lower than those in the normal white phloem, these compounds remained the major constitutents of the yellow phloem throughout the experiment. In tree A the glucoside of dihydroquercetin remained a prominent constitutent through 14 days of incubation, but thereafter its concentration decreased more rapidly than was observed in the normal white phloem. Both dihydroquercetin and dihydromyricetin were detectable after 3 days of incubation, but the concentration of these compounds decreased so that only a trace of dihydroquercetin and no dihydromyricetin was detected after 17 days of incubation. Neither of these compounds was observed in the yellow reaction zones of tree B. Quercetin and myricetin were not detected in the yellow reaction zones of either tree. Small amounts of pinosylvin, pinosylvin monomethyl ether, and resveratrol were detected in yellow reaction zone phloem after 7 to 10 days of incubation, but their concentrations remained low. Resveratrol has not previously been reported in normal (Lindstedt and Misiorny, 1951; Erdtman, 1956) or fungal-infected pines (Shain, 1967; Shrimpton, 1973). Although PC and TLC R_f values, color reactions, and UV spectra were identical with those of an authentic sample and similar to published literature (Hillis and Ishikura, 1968), identification of this compound should be considered tentative until sufficient amounts of crystalline materials are obtained.

The concentrations of catechin and proanthocyanidins were much lower in the brown reaction zone than in the normal white phloem of both trees. In the later stages of incubation, only traces of catechin and one of the proanthocyanidins could be detected. As suggested by the low methanol solubility and low proportion of methanol-soluble tannins, these compounds were apparently polymerized to such an extent that they were no longer extractable with methanol. Dihydroquercetin and dihydromyricetin concentrations were relatively high in the brown reaction zone through the first 10 days of incubation, but by 24 days only traces of dihydroquercetin remained. Ferulic acid was also present in substantial proportions after 7 days of incubation, but its concentration decreased thereafter. Neither quercetin nor myricetin was observed in the brown reaction zone of tree A or tree B. Small amounts of pinosylvin, pinosylvin monomethyl ether, and resveratrol were present in the brown reaction zone phloem, but their concentrations appeared to decrease with increasing incubation period. None of the polyphenols normally present in the phloem and none of the stilbenes which were found in infected phloem during early stages of fungal development could be detected in the black reaction zone after 24 days of incubation.

Polyphenols in Normal White Xylem. In the first week of incubation, the wood of aseptically wounded bolts contained only traces or undetectable quantities of flavonoids and stilbenes. However, the amounts of catechin and proanthocyanidins in the sapwood increased dramatically in later incubation periods, and these compounds were major phenolic constituents after 17 to 20 days of incubation. Proanthocyanidin b was the major compound in the sapwood; proanthocyanidin a dominated this class of compounds in the phloem (Table I). The stilbenes pinosylvin, pinosylvin monomethyl ether, and resveratrol were detectable in small proportions after the first week of incubation, but their concentrations remained low throughout the experiment. The glucoside of dihydroquercetin was not detected in the sapwood, although it was commonly found in sapwood of other conifers by Hergert and Goldschmid (1958).

Polyphenols in C. minor Infected Xylem. As was observed in the control bolts, the amounts of catechin and

proanthocyanidins initially increased in the blue-stained xylem of the *C. minor* inoculated bolts. The concentrations of these compounds reached a maximum after 14 days and thereafter decreased, as was observed in the phloem which was infected with *C. minor*. Concentrations of pinosylvin, pinosylvin monomethyl ether, and particularly resveratrol were much higher in the blue-stained wood than in the uninfected sapwood. In the later periods of incubation, the stilbenes were present in lower concentrations than in the 7 to 14 days after inoculation.

CONCLUSIONS

Both isocoumarins I and II, which were produced by *C. minor* in liquid culture, were also found in the phloem and xylem of infected loblolly pine bolts. The α-tetralone III, which was found in liquid culture filtrates, was not found in infected loblolly pine bolts. The concentration of II remained at relatively low levels, but I accumulated to high concentrations in both xylem and phloem after 24 days of incubation. Since compounds of the isocoumarin class are noted for their biological activity, I and II may alter the physiology of southern pine trees, other microorganisms associated with the southern pine beetle, or certain life-stages of the beetle itself. In addition, it should be emphasized that these changes occurred in bolts rather than intact trees. Difficulties encountered in artificially inoculating southern pine trees with *C. minor* suggest that analysis of southern pine beetle infested trees for these changes in polyphenol composition should be attempted next. These questions deserve further study.

C. minor was so effective in degrading flavonoids and stilbenes, which are generally known for their fungistatic or fungitoxic properties, that apparently these compounds would not contribute to host resistance to *C. minor*.

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