Effect of Phytotoxins Produced by *Botryosphaeria obtusa*, the Cause of Black Rot of Apple Fruit and Frogeye Leaf Spot

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ABSTRACT

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Botryosphaeria obtusa, which causes black rot of apple fruit and frogeye leaf spot, produced phytotoxins in culture, infected fruit, and spore germination fluids. Mellein was the most abundant toxin isolated from the culture fluid. Other toxins isolated were tyrosol, 4-hydroxymellein, 5-hydroxymellein, and 4-hydroxybenzaldehyde. Seventeen apple cultivars and eight weed species were used in a leaf bioassay to determine phytotoxicity of the toxins. The apple cultivars, Supergold and Silverspur, were highly sensitive to all toxins. Only three apple cultivars showed moderate resistance to most toxins. There was no correlation between isolate pathogenicity and the amount of toxin production in culture. Among the weed species, prickly sida and morning glory were very sensitive. Extraction of fruit infected with *B. obtusa* yielded all toxins except 4-hydroxybenzaldehyde. When conidial germination fluids were extracted with solvent, mellein and 4-hydroxymellein could be detected by thin-layer chromatography.

Black rot of apple (*Malus domestica* Borkh.) and frogeye leaf spot are caused by *Botryosphaeria obtusa* (Schwein.) Shoemaker (= *Physalospora obtusa* (Schwein.) Cooke) and are important diseases in the eastern United States (23,24). Fruit and foliage infections can occur as early as silver tip. Hyphae penetrate lenticels and stomata directly; wounding is not a prerequisite for infection of fruit or leaves (24). Initial infections on leaves appear as reddish brown flecks that enlarge to circular brown lesions (4–8 mm diameter) and are often surrounded by a purple halo. Heavily infected leaves become chlorotic and abscise. Lesions on young fruit appear as small raised purplish pimples. Lesions on more mature fruit are dark brown to black and are irregular in shape. As fruit ripens, lesions rapidly enlarge, resulting in large, firm, brown lesions that may rot the entire fruit. Such symptoms suggest the possible involvement of pathogen-produced phytotoxins in the disease. However, there is no report of toxin production by *Botryosphaeria* spp. or *Physalospora* spp. Thus, the objective of this study was to determine if *B. obtusa* produces toxin(s), to identify any toxins found, and to investigate their possible role in infection and pathogenesis.

MATERIALS AND METHODS

Pathogen. Pure cultures of *B. obtusa* were isolated from infected fruit and leaves of apples from North Carolina (isolate 087) and Virginia (isolate VPI-46). Stock cultures were maintained on potato-dextrose agar (PDA) slants.

Plants. Seventeen different cultivars of apple (listed in Tables 1 and 2) and eight species of weeds: *Sida spinosa* L. (prickly sida); *Chenopodium album* L. (lamb's-quarters); *Ipomoea* sp. (morning glory); *Datura stramonium* L. (jimsonweed); *Sorghum bicolor* (L.) Moench (volunteer sorghum); *S. halepense* (L.) Pers. (johnson grass); *Nasturtium officinale* R. Br. (watercress), and *Cassia obtusifolia* L. (sicklepod) were used and maintained in a greenhouse. Fruit of the cultivar Smoothee were collected from an orchard at Central Crops Research Station, Clayton, NC, and used for inoculations.

Chromatographic materials and methods. E. Merck Silicagel 60 (230–400 mesh) (Merck & Co., Inc., West Point, PA) was used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out on E. Merck DC Alufolien, Kieselgel 60F-254 (0.2-mm thickness). The chromatograms were examined under 254 and 365 nm of UV light to detect fluorescent and quenching metabolites. Spots were visualized by dipping in a phosphomolybdic acid reagent (21). Preparative TLC was carried out on E. Merck precoated plates, Silicagel 60F-254 (20- × 20-cm, 0.5-mm thickness). Materials were detected by examining the plates under a UV lamp (254 nm).

Preparation, extraction, and chromatography of toxins. An agar block from a 7-day-old culture grown on PDA was transferred to each of a series of 0.5-L portions of potato-dextrose broth. Culture flasks were incubated in a shaker at 100 rpm at 26 C for 1 wk. The cell-free culture filtrate (10 L) was adjusted to pH 3 with 6 N HCl and extracted three times with 10 L of recycled ethyl acetate. The biologically inactive water layer was discarded. The layer of ethyl acetate was evaporated to dryness in a rotary evaporator under reduced pressure at 40 C. The toxic residue (2 g) from evaporation of ethyl acetate was chromatographed on a silica-gel column (4- × 52-cm) and eluted in six 200-ml steps with benzene-acetone containing 0, 5, 10, 25, 40, and 100% acetone.

Spectral analysis. UV spectra were obtained on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer (Perkin-Elmer Corp., Ridgefield, CT), IR spectra were recorded on a Mattson Instruments Sirius 100 FT-IR controlled by a Mattson Instruments Starlab computer (Mattson Instruments, Madison, WI). High resolution mass spectra were determined on an AEI MS-902 mass spectrometer (Allied Engineers Inc., San Ramon, CA). Low-resolution chemical ionization and electron-impact ionization mass spectra were obtained with a Hewlett-Packard 5985-B mass spectrometer (Hewlett-Packard Co., Avondale, PA). Proton NMR spectra were determined with a Bruker WM-250 spectrometer (Bruker Instruments, Inc., Billerica, MA). ^{13}C-NMR spectra were obtained with either a Bruker WM-250 or an IBM-NR 100AS spectrometer.

Toxin extraction from the infected apple fruit. Surface-sterilized ripe apple fruit were inoculated with two mycelial plugs (5 mm

diameter) of *B. obtusa* isolate VPI-46 and incubated in sealed plastic boxes at 26 C for 7 days. Uninoculated fruits incubated under similar conditions served as controls. After 7 days, fruits were completely rotted. Rotted fruit (1 kg wet weight) as well as control fruit (300 g) were ground separately in a Waring blender with the addition of 50 ml of methanol in each case. Each slurry was extracted with equal volume of methanol for 24 hr. The filtered methanol fractions were concentrated and mixed with three volumes of distilled water. The aqueous alcoholic extracts were adjusted to pH 3.0 and extracted three times with ethyl acetate. Ethyl acetate was evaporated in vacuo. The residues were chromatographed on thin-layer silica-gel plates and columns as described previously.

Release of toxins from germinating conidia. Isolate VPI-46 was grown on cellulose film placed on top of oatmeal agar medium (Difco Laboratories, Detroit, MI) for 14 days under continuous fluorescent illumination. Conidia were obtained by scraping the mycelium containing pycnidia into a blender that contained sterile water. After 20 sec of blending, conidia were strained twice through two layers of cheese cloth and the conidia concentration was standardized with the aid of a hemacytometer. The conidia were washed with distilled water four times by a mild centrifugation at 350 *g* for 5 min to remove potentially inhibitory materials. The washed conidial suspension (100,000 conidia per milliliter) was spread on six layers of cheesecloth and incubated at 22 C for 48 hr.

Leaf bioassay for toxicity. Phytotoxicity was determined on apple cultivars (listed in Tables 1 and 2) and weed species by a detached leaf assay. The freshly detached leaves were cut into 2- × 4-cm pieces, and the center of each piece was slightly injured by gently scratching it with a cutoff (bristles) artist's brush. The cell-free culture filtrate was assayed by placing 20 μl of filtrate on the injured area of the leaf. To test purified toxins of less water solubility, the toxin was first dissolved in ethyl acetate. A measured volume of the solution was adsorbed onto a 0.5- × 0.5-cm square of silica gel on a glass plate, and the solvent was allowed to evaporate. The impregnated silica gel was then placed on the leaf piece. The silica gel was wet with 20 μl of sterile distilled water. The leaf disks were placed in a petri plate on moistened filter paper and incubated at 26 C for 48–72 hr. Compounds that produced a necrotic reaction were considered to be phytotoxins. The necrosis was measured by using transparent graph paper to trace the necrotic area. Distilled water checks were maintained in each test. Four leaf pieces were used in each test and the tests were done twice.

Comparison of isolate pathogenicity. Seedlings of open pollinated apple cultivar, Delicious, having 8–12 leaves, were inoculated with conidial suspensions (50,000 conidia per milliliter) of either isolate, 087 or VPI-46, by spraying the lower leaf surface to runoff with an artist's airbrush. Isolate 087 was selected for comparison because it was highly virulent in a previous study (2). Seedlings immediately were sealed in a polyethylene bag containing a moistened paper towel and placed in a controlled temperature chamber at 24 C for 48 hr. Six plants, inoculated with conidia of each isolate, were placed into each of three chambers. After removal from controlled temperature chambers, trees were unbagged and placed on a greenhouse bench (approximately 20–25 C). The three most severely affected leaves on each plant were evaluated after 14 days for lesion development according to a modified Horsfall and Barratt (16) scale, in which 1 = 0–3% of leaf surface with lesions, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, and 5 = 25–50%. The three most severely affected leaves were chosen for evaluation because of the variation within plants due to leaf age. Data were subjected to an analysis of variance. The experiment was repeated.

Investigations of the role of toxins in pathogenesis. Detached leaf and seedling tests were performed to investigate the possible role of mellein in infection of apple leaves by *B. obtusa* (087). For each test, a solution of mellein (10 or 20 μg/ml) was prepared by first dissolving the mellein in 1 ml of methanol and then adding an appropriate amount of distilled water. For the detached leaf test, leaves of similar size (the youngest expanded leaf from open pollinated Delicious seedlings) were sprayed on the adaxial surface, allowed to dry for 5 min, and sprayed with a conidial suspension (50,000 conidia per milliliter) of isolate 087. Inoculated leaves and leaves treated with methanol served as controls. (Preliminary experiments had shown no phytotoxicity associated with the methanol solvent.) Leaves were placed on moistened laboratory towels in sealed plastic boxes and incubated for 5 days at either 20 or 26 C. Five leaf pieces were used for each mellein treatment, were paired in each box with inoculated leaves, and were treated with methanol. Data were taken by counting the number of lesions per leaf and were subjected to an analysis of variance.

Similar treatments were used in the seedling tests. Seedlings were sprayed with a solution of mellein, allowed to dry for 30 min, inoculated with 50,000 conidia per milliliter, and then maintained as described for the pathogenicity study, except that all seedlings were kept in one controlled temperature chamber. Three seedlings were used for each treatment combination. The number of lesions was recorded on the three most severely affected

TABLE 1. Phytotoxicity of *Botryosphaeria obtusa* (VPI-46) cell-free culture filtrates[a] on different apple cultivars

Cultivar	Reaction of leaf tissue to dilutions[b]		
	Undiluted	1:1	1:4
Super Gold	+++	++	+
Classic Red	+++	++	+
Ida Red	+++	+	−
Oregon II	++	++	+
Red Chief	++	++	+
Gala	++	+	+
Law Rome	++	+	+
McIntosh	++	+	+
Firm Gold	++	++	−
Golden Delicious	++	+	−
Red Delicious	++	+	−
Empire	+	−	−
Jonathan	+	−	−
Smoothee	+	−	−
Paula Red	−	−	−
Silverspur	−	−	−
Stayman	−	−	−

[a]Culture filtrates were concentrated 10-fold and subjected to further dilution as indicated.
[b]Reactions were evaluated as necrotic areas: +++ = 0.6–1 cm^2; ++ = 0.25–0.5 cm^2; + = 0.1–0.24 cm^2; − = no reaction.

TABLE 2. Phytotoxicity of *Botryosphaeria obtusa* (VPI-46) on different apple cultivars

Cultivar	Necrotic leaf area (mm^2)[a]				
	Mellein	Tyrosol	4-HM	4-HB	5-HM
Silverspur	98	83	95	46	72
Super Gold	85	116	100	30	48
Classic Red	44	4	41	NT[b]	NT
Ida Red	31	113	28	NT	NT
Law Rome	31	25	28	41	64
Gala	10	23	12	NT	NT
Red Chief	7	13	4	NT	NT
Jonathan	5	7	2	NT	NT
Red Delicious	5	3	10	18	8
Smoothee	3	10	3	NT	NT
Oregon II	3	8	2	NT	NT
Firm Gold	3	4	2	NT	NT
Golden Delicious	2	34	4	NT	NT
Paula Red	2	35	14	NT	NT
Stayman	2	12	13	NT	NT
McIntosh	1	29	1	NT	NT
Empire	0	27	0	NT	NT

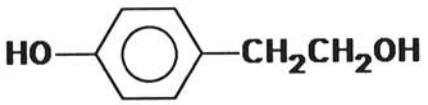
[a]Toxins were applied at 10 μg. Abbreviations: 4-HM = 4-hydroxymellein, 4-HB = 4-hydroxybenzaldehyde, and 5-HM = 5-hydroxymellein.
[b]NT = Not tested.

leaves per treatment. The data were subjected to an analysis of variance. The experiment was repeated.

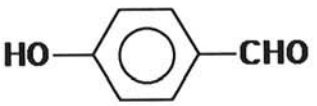
RESULTS

Purification of toxins from culture filtrates. The crude ethyl-acetate extracted toxins were fractionated on a silica-gel column by elution with benzene and acetone. The first four fractions showed phytotoxicity when bioassayed on detached apple leaves. The pooled column fractions 1 and 2 yielded a yellow crystalline material on concentration. The crystals were recrystallized from acetone-chloroform to give 255 mg of colorless crystals that were identified as mellein (Fig. 1) by comparing melting point, circular dichroism, NMR, UV, and mass spectra to an authentic standard of mellein isolated from *Septoria nodorum* in this laboratory (12).

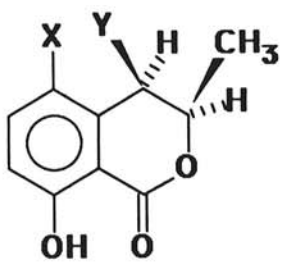
Both enantiomers of mellein have been isolated from fungi previously. (*R*)-(-)-Mellein is the better known fungal enantiomer (25). However, (*S*)-(+)-mellein has been isolated from *Cercospora taiwanensis* (4), *Fusarium larvarum* (15), and *Gyrostroma missouriense* (19). Therefore, it was necessary to establish the chirality of mellein isolated in this study. The circular dichroism spectrum of mellein isolated from *B. obtusa* had a negative trough at 257 nm, a positive peak at 239 nm, and a negative trough at 216 nm; this identified the compound as (*R*)-(-)-mellein (Fig. 1) (1).



Tyrosol



p-Hydroxybenzaldehyde



Mellein: X = H, Y = H

4-Hydroxymellein: X = H, Y = OH

5-Hydroxymellein: X = OH, Y = H

Fig. 1. Structures of phytotoxins isolated from cell-free culture filtrates of *Botryosphaeria obtusa*.

Solvent was evaporated from pooled column fractions 1 and 2 after removal of the crystals of mellein. The residual toxic syrup contained two major components that were resolved by preparative TLC in ethyl ether/benzene, 60:40. The 4.5 mg of the more polar compound recovered from preparative TLC was active in the leaf-spot assay. It was identified as 4-hydroxybenzaldehyde by comparison of its UV, NMR, and mass spectra to the spectra of an authentic standard. The 4.7 mg of the less polar metabolite isolated from the preparative thin-layer chromatogram had an ultraviolet spectrum similar to that of mellein, but its mass spectral molecular ion (m/z 194) occurred 16 mass units higher than that of mellein, indicating that this metabolite is a hydroxylated mellein. The NMR spectrum of this metabolite differed from that of mellein in that it possessed two aromatic vicinal protons (7.04 ppm doublet and 7.25 ppm doublet in acetone-d_6) in place of three vicinal aromatic protons in the spectrum of mellein. The only hydroxylated derivative of mellein with two vicinal aromatic protons is 5-hydroxymellein. This metabolite is designated as (*R*)-(-)-5-hydroxymellein based on the similarity of its circular dichroism (negative extrema at 257 and 236 nm) to that of (*R*)-(-)-mellein.

Thin-layer chromatography of column fractions 3 and 4 showed the presence of two major compounds in both fractions. The residue from evaporation of solvent from pooled fractions 3 and 4 was chromatographed on a preparative TLC plate (petroleum ether/acetone, 80:20) to purify the two major components. Elution of the fluorescence-quenching, low-mobility zone, and concentration of the eluting solvent produced 30 mg of colorless crystals of tyrosol (Fig. 1), identified by NMR and UV spectroscopy and by comparison to an authentic standard. Elution of the fluorescent, high R_f zone gave, after solvent evaporation, 25 mg of a colorless syrup with an ultraviolet spectrum similar to mellein. This metabolite had a NMR spectrum similar to that of mellein, but its mass spectral molecular ion occurred at m/z 194, indicating the presence of one more oxygen than in mellein. The NMR spectrum had the same aromatic proton multiplet pattern as mellein, indicating that the hydroxyl group was not in the aromatic ring. This hydroxylated mellein was identifed as *cis*-4-hydroxymellein (Fig. 1) by comparing its NMR spectrum to published spectra for this isomer (12). The 4-hydroxymellein had circular dichroism spectrum (negative extrema at 256 and 216 nm and positive extremum at 236 nm) almost identical to that of (*R*)-(-)-mellein isolated from *B. obtusa*; therefore, it is (3*R*,4*R*)-*cis*-4-hydroxymellein.

The crystalline residue left after evaporation of solvent from the last, most polar column fractions 5 and 6 was not phytotoxic when assayed in buffered water. Recrystallization of this nontoxic

TABLE 3. Phytotoxicity of *Botryosphaeria obtusa* (VPI-46) cell-free culture filtrate and toxins on different weed species

Weed species	Necrotic leaf area (mm^2)[a]					
	Cf	Mellein	Tyrosol	4-HM	4-HB	5-HM
Prickly sida	12	82	32	92	132	28
Lamb's-quarters	15	77	31	24	72	20
Morning glory	17	65	20	25	54	16
Jimson weed	10	24	8	17	39	20
Sorghum	16	31	12	65	21	8
Johnson grass	19	48	17	34	27	8
Watercress	15	16	4	24	15	2
Sicklepod	17	5	2	9	42	2

[a] Cell-free culture filtrate (Cf) was concentrated 10-fold, and toxins were applied at 10 μg. Abbreviations: 4-HM = 4-hydroxymellein, 4-HB = 4-hydroxybenzaldehyde, and 5-HM = 5-hydroxymellein.

component from methanol-hexane gave 125 mg of succinic acid (mp 182).

Leaf bioassay for toxicity. Characteristic brown necrotic lesions occured within 48 hr of incubation on apple cultivars as well as on weed species. The culture filtrate caused necrosis on all apple cultivars except on Stayman, Paula Red, and Silverspur. Super Gold, Ida Red, and Classic Red were highly susceptible (Table 1). On the other hand, the culture filtrate induced necrosis on the leaves of all eight weed species (Table 3); Johnson grass, sicklepod, and morning glory were very sensitive among the weed species.

Mellein, the major toxin found in culture filtrate, caused necrosis at 10 μg on all apple cultivars except Empire. Silverspur and Super Gold were highly sensitive to mellein. On Empire, McIntosh, and Stayman cultivars, necrosis appeared when mellein was applied at 50 μg or higher levels. Mellein was more active on leaves of weed species. Prickly sida and lamb's-quarters were the most sensitive, whereas watercress and sicklepod were least sensitive. Tyrosol, the second major compound isolated, showed activity on all apple cultivars screened. However, the toxin was more active on Super Gold and Ida Red. Red Delicious, Classic Red, and Firm Gold were less sensitive to tyrosol (Table 2). Mellein and 4-hydroxymellein had similar toxicity to the apple cultivars tested, but they differed in the toxicity to weed species. Mellein was more toxic than 4-hydroxymellein to lamb's-quarters, morning glory, and johnson grass, and 4-hydroxymellein was more toxic to prickly sida and sorghum (Table 3). 4-Hydroxybenzaldehyde and 5-hydroxymellein were phytotoxic to all four apple cultivars bioassayed (Table 3). Phytotoxicity of 4-hydroxybenzaldehyde was greater on the weed leaves than apple. Prickly sida was highly sensitive to 4-hydroxybenzaldehyde, whereas watercress showed moderate resistance.

Toxin extraction from the infected apple fruit. When column fractions were purified, mellein (12 mg), tyrosol (18 mg), 4-hydroxymellein (4 mg), 5-hydroxymellein (2.5 mg), and succinic acid (110 mg) were recovered. There was no trace of 4-hydroxybenzaldehyde. No toxins were evident in the healthy fruit extract. The toxins isolated had similar phytotoxic profiles on apple cultivars and weed species as those from culture filtrate. No such activities were observed when the corresponding extract fractions from healthy fruit were bioassayed.

Release of toxins from the germinating conidia. Approximately 40% of the conidia germinated within 48 hr. The fluid was collected, filtered, and concentrated under reduced pressure at 25 C to one-tenth. Concentrated fluid (20 μl) obtained from the conidia and applied to apple leaves caused necrosis within 48 hr. A crude ethyl acetate fraction also induced similar necrotic reactions when applied to apple and weed leaves. Mellein and 4-hydroxymellein were identified in the crude ethyl acetate extract by TLC cochromatography of standards in three solvent systems.

Investigations of the role of toxins in pathogenesis. Isolate 087 was more virulent on apple leaves than VPI-46 (mean ratings for isolates 087 and VPI-46 were 2.27 and 0.69 ($P = 0.01$) and 2.86 and 1.53 ($P = 0.05$) for tests 1 and 2, respectively. Treatment of either detached leaves or apple seedlings with mellein at 10 or 20 μg did not result in an increase in the number of lesions per leaf. Mean lesions per leaf for *B. obtusa* alone were 31.5, 37.8, and 37.4 for the detached leaf test and greenhouse seedling tests 1 and 2, respectively. Mean lesions per leaf for 10 or 20 μg mellein plus *B. obtusa* were 32.2, 18.3, 16.4, and 17.3, and 11.3 and 11.2 for the detached leaf test and greenhouse tests 1 and 2, respectively. Differences between mellein treatments and the control were not significant due to the large variation among leaves.

DISCUSSION

The present study identified five phytotoxins in the culture filtrate of the apple pathogen, *B. obtusa*. These are the first phytotoxins identified from the genus. Three, mellein, 4-hydroxymellein, and 5-hydroxymellein, are acetogenins; and two, tyrosol and 4-hydroxybenzaldehyde, are products of the shikimate pathway. Four of the toxins were found in infected apple fruit, and two were detected in conidial germination fluids. All toxins caused necrotic lesions that resembled those resulting from infection by *B. obtusa* on apple leaves. All five phytotoxins of *B. obtusa* are nonspecific. Necrosis was induced on several weed species' leaves and susceptible host plants at a concentration as low as 1 μg. With the exception of sicklepod, watercress, and jimsonweed, all other weed species were more sensitive than susceptible apple cultivars.

Foster (14) demonstrated that wounding was not necessary for infection of apple leaves by *B. obtusa* and concluded that infection occurred chiefly through stomata. Our studies provide no evidence for the role of mellein, the most abundantly produced toxin, in the infection process. In two greenhouse tests, treatment of leaves with mellein did not result in an increase in the number of lesions per leaf. Furthermore, there was no correlation between the ability of two isolates to produce toxins and their pathogenicity on apple foliage. Inoculations with conidia of a highly virulent isolate (isolate 087) produced significantly more lesions per leaf than VPI-46, but isolate VPI-46 produced a greater amount of toxin in culture. In addition, there was no correlation between the size of the necrotic area produced on detached leaves of various cultivars by the toxins and the susceptibility of cultivars to *B. obtusa*. For example, Law Rome is very susceptible to foliar infection by *B. obtusa* (L. F. Aruaz, *unpublished*), and large necrotic areas were produced by all five toxins when applied to the leaves. Ida Red, Silverspur, and Super Gold are somewhat resistant to foliar infection, yet application of the toxins to leaves resulted in large necrotic areas. Nevertheless, the presence of mellein and 4-hydroxymellein in the conidial germination fluids and the presence of four toxins in infected apple fruit suggests the possible involvement of these toxins in some other aspects of pathogenesis.

This is the first report of the isolation of 5-hydroxymellein as a fungal metabolite. The only previous isolation of this metabolite was from an Amazonian wood sample of *Virola venosa* (10). Because 5-hydroxymellein was not found in a freshly cut sample of *V. venosa*, it appeared possible that this metabolite might be a product of a fungal contaminant. However no attempt was made to demonstrate the presence of a fungus in the wood. Our isolation of 5-hydroxymellein from *B. obtusa* culture filtrate lends credence to the suggestion that this metabolite was a fungal artifact in *V. venosa* wood. Although 4-hydroxymellein has been reported from several plant pathogenic fungi (6,9,12,22), no data on phytotoxicity have been reported previously.

Tyrosol has been identified as a phytotoxin in culture filtrates of several plant pathogens (5,8,11,17). Fungally produced tyrosol also has been implicated in resistance of elm infected with *Ceratocystis* to bark beetle (8) and in resistance of diseased Timothy containing the endophyte, *Epichloe typhina*, to secondary fungal invasion (18). Several plant pathogenic fungi are known to produce 4-hydroxybenzaldehyde (3,5). Some insects (7,13) and plants, notably sorghum (20,26), use hydroxybenzaldehyde as a defensive metabolite, but it has not heretofore been associated with fungal phytotoxicity.

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