Cytochemical Responses of Pecan to *Cladosporium caryigenum*: Development of Specific Histological Indicators to Identify and Analyze In Situ Fungitoxic Phenols

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ABSTRACT

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The fungitoxic phenols, juglone, isoquercitrin, and condensed tannin, were purified from pecan leaves. We tested 10 phenolic-specific histological indicators to distinguish among these three compounds within the visible spectrum. The Hoepfner-Vorsatz stain was the best indicator for juglone because it shifted juglone's absorbance maximum from 423 to 533 nm, at which there was no interference from other phenols. The Hoepfner-Vorsatz reagent shifted the isoquercitrin absorbance maximum from 353 to 399 nm, but there was interference from minor flavonoids. Condensed tannins had a bathochromic shift from 300 to 551 nm with no interference from other phenolic compounds when heated to 90 C in *n*-butanol-HCl. Standard curves for each compound were generated with different light paths on a microspectrophotometer. Leaf sections 30 μm thick and nut sections 20 μm thick provided the best absorbancy range with the lowest coefficients of variation. All three phenolic compounds were quantified in situ. This system permits examination of specific sites of infection for location and quantity of juglone, isoquercitrin, and condensed tannins, and estimation of the response to infection for each of these compounds.

Additional keywords: *Carya illinoensis*, disease resistance, pecan scab.

Demonstrating that plant compounds are resistance factors (allelochemicals) is difficult because resistance often may be the result of multiple chemical components acting at specific sites (10,18). To fully understand host defense responses to pathogenic fungi and other pests, one must determine a plant's response to cellular invasion. Most studies on host resistance have focused on the presence of allelochemicals in homogenized whole organs and have not ascertained the mechanisms of defense at sites of infection. Susceptible and resistant host cultivars may have similar concentrations of the chemicals in whole plant analyses but different levels in specific tissues or sites (8). It is also necessary to demonstrate that a change in concentration of components thought to be involved in host plant resistance results in a corresponding change in resistance (18).

A number of specific indicators have been used to identify phenol structures in solutions. The two types of chemical assays for phenols include a general phenolic assay and a specific functional group assay (6). The number and position of hydroxyl groups can greatly influence the visible color and absorption spectra of phenolic compounds (20). Flavones and flavonols exhibit high intensity absorption from 320 to 380 nm (band I) and from 240 to 270 nm (band II) (12). Band I is chiefly associated with the B ring, whereas band II is influenced by the A ring. Introduction of a hydroxyl group into the B ring produces a considerable shift in band I. Introduction of a hydroxyl or methoxyl group into the A ring produces a bathochromic shift and an increase in the intensity of band II (12).

Comparing phenol extraction methods and direct analysis of plant tissue is problematic. Condensed tannins and other phenols are seldom completely extracted from a plant (1). In addition, extraction procedures eliminate the possibility of localizing and comparing phenols in specific areas (such as infection sites). Recently, Cork and Krockenburger (2) found that tissue drying conditions, extraction solvents, light, and temperature affect the success, stability, and extractability of phenols from eucalyptus leaves. Direct analysis procedures allow comparisons of chemical concentrations at specific sites rather than absolute concentrations from whole tissues.

Native pecan (*Carya illinoensis* (F. A. Wangenheim) K. Koch) populations exhibit a high incidence of scab disease caused by *Cladosporium caryigenum* (Ellis & Langl.) Gottwald, whereas native stands of other hickory species rarely have scab. These hickories may possess resistance factors not found in pecan or not found in concentrations or at critical locations needed to confer resistance. In addition, there is a low correlation between leaf and nut resistance of pecan to scab (4). Pecan extracts were screened for fungitoxic activity against *C. caryigenum* to discern host factors that may be associated with resistance (14). These efforts led to the identification of fungitoxic constituents in pecan and other hickories that include the phenolic juglones, condensed tannins, isoquercitrin, and the monoterpenoid, linalool (9,13,14). Juglone and its derivatives and precursors are the only quinones found in pecan (7,9). Further analysis revealed that isoquercitrin and condensed tannins were the only other major phenolic constituents in pecan, although two unidentified flavonoids were found in trace quantities (10).

Following changes of specific phenols during infection by a pathogen is of utmost importance in understanding the mechanisms of resistance and susceptibility. To localize and quantify phenolic compounds within specific tissues, we needed a histochemical procedure that detected differences in the visible absorbance spectra of juglone, isoquercitrin, and condensed tannins. The microspectrophotometer that was available only recorded in the visible range. The procedure had to maximize absorbance peaks of the desired phenols and minimize interference from other hickory phenols. The purpose of this study was to develop such a procedure by selecting appropriate specific indicators.

MATERIALS AND METHODS

Purification of juglone, isoquercitrin, and condensed tannins. Fresh pecan leaves from several pecan cultivars (Stuart, Schley, Odom, Success, Pabst) were collected in May 1987 from the orchard at Mississippi State University, placed on ice, and then frozen at −20 C. Leaves from all cultivars were combined into

a single sample for a total of 4.3 kg (fresh weight). The frozen leaves were divided into 800-g portions, and each portion was blended in 3 L of chloroform/methanol/water (2:1:1) for 2–3 min; solids were extracted further with 2 L of the same solvent mixture. The chloroform phase containing juglone and the aqueous phase containing isoquercitrin and condensed tannins were separated and extracted as described by Hedin (10) and Laird (13), with minor modifications.

The chloroform phase was solubilized in hexane/methylene chloride (1:1) and chromatographed on two successive BioRad Biosil-A (Melville, NY) columns (200–400 mesh; 5 × 15 cm; eluting with hexane/methylene chloride, 1:1, then methylene chloride only). Fractions were examined for the presence of juglone by silica gel thin-layer chromatography (TLC) developed in hexane/methylene chloride (1:1), and those fractions containing juglone were pooled and lyophilized. Commercial juglone (Sigma Chemical Co., St. Louis, MO) and pecan juglone previously isolated and identified (8,9) were run as TLC standards. Juglone is the only quinone found in pecan (7) and is very distinct on TLC; it appears as a visible orange band. Purity was estimated at 95%.

The aqueous phase was solubilized in 50% aqueous methanol and chromatographed on a 5- × 26-cm lipophilic Sephadex LH20 (Sigma Chemical Co.) column for batch separation. Fractions containing isoquercitrin were eluted in 50% aqueous methanol, and those containing condensed tannins were subsequently eluted in 70% aqueous acetone. All fractions were monitored by Polyamide 6 TLC (J. T. Baker Chemical Co., Phillipsburg, NJ) developed in 70% aqueous methanol; the spots were subsequently visualized with 1% diphenylboric acid 2-aminoethyl ester in methanol.

The isoquercitrin-rich fractions were further purified by chromatography on a 5- × 22-cm Sephadex LH20 column eluted with 50% aqueous methanol, 70% aqueous methanol, and 70% aqueous acetone in sequence. These were followed by chromatography on a 10- × 2.5-cm Polyamide 6 column eluted with a sequence of 50% aqueous methanol, 70% aqueous methanol, and 70% aqueous acetone. Isoquercitrin began to elute from the columns in 50% methanol but was mostly found in the 70% methanol fractions.

The condensed tannin fractions were chromatographed on a 5- × 22-cm Sephadex LH20 column and eluted with 70% aqueous methanol and 70% aqueous acetone. The presence of condensed tannin was identified by Polyamide 6 TLC visualized with either diphenylboric acid spray or vanillin-HCl spray and subsequent heating of the TLC plate (3). For isoquercitrin and condensed tannin, only fractions that appeared pure by TLC were pooled and lyophilized. Isoquercitrin and condensed tannin previously isolated and identified from pecan (13) were run as TLC standards. Isoquercitrin was further verified by UV-vis spectral shift reagents (NaOAc, $AlCl_3$, NaOMe), and purity was estimated at greater than 90%. The purity of condensed tannin fractions was further evaluated with *n*-butanol-HCl (95:5) and heat (100 C for 30 min). This results in the development of a red-pink chromophore, and purity was estimated at 95%. Unidentified phenolic compounds present as by-products were also collected and lyophilized for further examination.

Spectral analysis of specific indicators for the three purified phenols. Wavelength scans of juglone, isoquercitrin, and condensed tannin fractions were made with a Spectronic 1201 spectrophotometer (Milton-Roy, Rochester, NY) by testing nine different phenolic-tannin indicators. Each scan consisted of 2 ml of 0.005% extracted pecan phenolic standard in 75% aqueous methanol plus indicator, with methanol plus indicator as a blank. Different concentrations of each indicator were tested for the maximum shift of band I toward the visible range and maximum peak height. Optimal concentrations of each indicator for each 2 ml of sample were the following: two drops of 1.35% methanolic ferric chloride (3,6,16); one drop of 2.5% ethanolic phosphomolybdic acid (17); one drop of 1% methanolic diphenylboric acid 2-aminoethyl ester (8); one drop of 3% ethanolic *p*-toluenesulfonic acid plus 40 C (3); one drop of 0.125% ethanolic vanillin in 0.03% sulfuric acid (6,8); one drop of 0.1% sodium nitrite/0.2% urea/0.1% acetic acid, 1:1:1, followed after 2 min by one drop of 0.5 N sodium hydroxide (Hoepfner-Vorsatz) (17); one drop of 2,6-dichloroquinone-4-chloroimine in borate buffer, pH 9.4, followed in 3.5 min with one drop of 5% ammonium hydroxide (16,20); one drop of 1.75% sodium ethoxide in absolute ethanol (12); and three drops of 5% aluminum chloride in absolute ethanol plus 10 min (12). Condensed tannins were further tested by dissolving purified pecan standards in *n*-butanol-HCl (95:5) and heating at 100 C for 30 min (6). Wavelength scans were also made on these samples. We tested the Hoepfner-Vorsatz and *n*-butanol-HCl reagents on the unidentified phenolic fractions and commercial quercetin (Sigma Chemical Co.) to determine if there was overlap with the three principal phenols.

Optimization of standards on a microspectrophotometer. A computer-controlled Nanometrics (Sunnyvale, CA) Docuspec II microspectrophotometer equipped with a holographic grating monochrometer, Olympus BHT (BF/DF) microscope (Lake Success, NY) and quartz-halogen lamp was used to measure transmittance spectra at wavelengths between 380 and 764 nm. The fixed aperture measurement area was 18 × 126 μm, and samples were measured at ×300 magnification. A series of concentrations of each purified pecan phenol standard was tested against a series of varying Hoepfner-Vorsatz and *n*-butanol-HCl concentrations. The Hoepfner-Vorsatz reagent concentrations (per 2 ml of standard) ranged from one drop (0.113 mg) of the Hoepfner-Vorsatz reagent mixture (0.1% sodium nitrite/0.2% urea/0.1% acetic acid, 1:1:1) plus one drop (0.56 mg) of 0.5 N sodium hydroxide to two drops (2.26 mg) of the Hoepfner-Vorsatz reagent mixture plus four drops (8.96 mg) of 2 N sodium hydroxide. Condensed tannin standards (200 μl in 1.8 ml of *n*-butanol-HCl) were heated in boiling water for 30 min. Indicator plus phenolic standard concentrations that gave an optimal peak height and shift but did not record above the microspectrophotometer upper limit were selected. Standard curves were generated with light paths of 0.020, 0.133, 0.171, 0.449, and 0.649 mm in length.

Modification of histochemical stains to tissues. Leaf material was collected in June 1988, and nut husk material was collected in August 1988 from several Stuart trees located on the Mississippi State University campus. Plant materials were collected on ice and transported to the laboratory where individual samples were frozen at −20 C. Samples were selected at random from the pooled frozen material, but care was taken to avoid any damaged or wounded tissue. Both leaf and nut husk samples were embedded in ice and sectioned with an IEC cryostat microtome (Model CTI; International Equipment Co., Needham Heights, MA) to 20, 30, 40, or 50 μm thickness cut with IEC cryostat knives. Alternate sections were fixed over 40% formaldehyde vapor for 48 h for leaves and 96 h for nuts (5). These sections were analyzed for juglone and isoquercitrin by staining with the Hoepfner-Vorsatz reagent (17), rinsing the stain away with water, and recording the absorbances with the microspectrophotometer. Juglone and isoquercitrin were measured at 515 and 408 nm, respectively. All absorbances were recorded in the palisade parenchyma tissue of leaf sections, avoiding vascular tissues, and in the nut husk tissue immediately below the epidermis.

The second group of alternate sections was stained for condensed tannins with *n*-butanol-HCl (95:5) plus heat without fixation. Sections were submersed in *n*-butanol-HCl and sealed with a coverslip. Of several methods tested, heating in steam within an enclosed water bath at 90 C for 40 min produced the most consistent results. Absorbances were measured at 550 nm with the microspectrophotometer. Phenolic concentrations within the tissues were estimated from standard curves of juglone, isoquercitrin, and condensed tannin generated on the microspectrophotometer. Differences in light path lengths between standards and tissue samples were adjusted with Beer's law (21).

RESULTS AND DISCUSSION

Hoepfner-Vorsatz and *n*-butanol-HCl with heat distinguished and quantified spectrometrically the three subject phenols.

Additionally, these two indicators are specific for phenolic compounds, thus, no other plant constituents should interfere with these assays (6).

Aluminum chloride caused the largest bathochromic shift of isoquercitrin (from 380 to 422 nm; Table 1), but separation from juglone was not adequate. Sodium ethoxide shifted isoquercitrin to 402 nm (Table 1) with some small decrease in intensity by reacting with the free hydroxyl and the 4′ hydroxyl groups. Isoquercitrin is stable in sodium ethoxide because the 3-hydroxyl is glycosylated. Diphenylboric acid (395 nm), dichloroquinone (382 nm), and Hoepfner-Vorsatz (399 nm) all caused bathochromic shifts by reacting with the free hydroxyl groups located on the A and B rings (Table 1). In addition to isoquercitrin, these indicators shifted a few other unidentified flavonoids into the visible range. Unfortunately, the peaks of these other flavonoids overlapped with isoquercitrin, and these phenolics could not be adequately separated from each other. Because these flavonoids are found within tissues at much lower concentrations than isoquercitrin, it would be more accurate to refer to this peak as a predominantly isoquercitrin peak.

The Hoepfner-Vorsatz reagent can distinguish juglone from all other major phenolic compounds found in pecan. With Hoepfner-Vorsatz, the juglone absorbance maximum is shifted from 423 to 533 nm (Table 1), and there is no overlap with the isoquercitrin maximum at 399 nm. Juglone had a slightly larger bathochromic shift to 538 nm with sodium ethoxide; however, juglone's instability in this reagent would make it difficult to use this indicator for generating standard curves. Because isoquercitrin and juglone are stable in the Hoepfner-Vorsatz reagent, this would be the best indicator for distinguishing between them.

n-Butanol-HCl was the only indicator that shifted the absorptivity of condensed tannins into the visible range (551 nm) (Table 1) by conversion to anthocyanidins. Even though the condensed tannins contain free hydroxyl groups on the B ring, they did not react like flavonoids with the various indicators. Neither juglone nor isoquercitrin overlapped into the condensed tannin range when treated with this reagent. Several unidentified phenolic fractions (probably flavonoids) found in trace quantities overlapped with isoquercitrin treated with either Hoepfner-Vorsatz or *n*-butanol-HCl. None of the fractions overlapped with juglone or the condensed tannins.

Juglone concentrations that could be detected microspectrophotometrically ranged from 0.25 to 4.2 $\mu g/\mu l$. Juglone solubility determined the highest concentration tested for that standard curve. Isoquercitrin concentrations ranged from 0.375 to 6.0 $\mu g/\mu l$, and condensed tannins ranged from 0.037 to 0.6 $\mu g/\mu l$. The upper concentration limit was restricted to that concentration that gave an optical density reading below 3. For condensed tannins, optical density ranged only from 0.001 to 0.027 with the 0.02-mm light path. With the 0.133- and 0.171-mm light paths, optical densities ranged from 0.046 to 0.922 for condensed tannins, 0.055 to 1.057 for isoquercitrin, and 0.016 to 0.38 for juglone. The broadest range for juglone was with the 0.449-mm path at which absorbances ranged from 0.043 to 0.829. In considering the standard curves generated from the different light paths, 0.133, 0.171, and 0.449 mm seem to cover the broadest absorbance and concentration ranges. The absorbances generated with the 0.02-mm path were too low, particularly at the low concentrations, to produce a reliable and reproducible standard curve.

Wavelength scans of juglone, isoquercitrin, and condensed tannin in Hoepfner-Vorsatz reagent and in *n*-butanol-HCl produced on the microspectrophotometer are presented in Figures 1 and 2. The absorbance scans of the standard phenols, measured in nanometers (spectrophotometrically and microspectrophotometrically), were similar. The absorption maxima were 533 and 515 nm for juglone, 399 and 401 nm for isoquercitrin, and 551 and 550 nm for condensed tannins. Differences in juglone peaks were probably caused by the slight differences in Hoepfner-Vorsatz reagent proportions used. In particular, changes in sodium hydroxide concentrations can influence the absorbance maximum. Because the microspectrophotometer only records every 7.1 nm, the reading nearest to the peak was chosen.

Estimations of juglone, isoquercitrin, and condensed tannin concentrations in leaf tissues of different thicknesses are given in Table 2. For juglone and condensed tannins, the standard deviation increased with tissue thickness above 30 μm. Juglone estimations were significantly less in 20-μm leaf sections than for other thicknesses. The leaf sections used in subsequent

TABLE 1. Absorbance maxima (nm) for juglone, isoquercitrin, and condensed tannins in response to various phenolic-specific indicators tested to distinguish between the three compounds in the visible spectrum

Indicator[a]	Juglone	Isoquercitrin	Condensed tannins
Control	423	353	300
FC	422	402	300
PM	417	353	300
NP	416	395	313
TS	418	352	300
VAN	418	352	300
HV	533	399	320
DCQ	522,435	382	300
AC	430	422	300
SE	538	402	326
BUT	412	373	551

[a] FC = ferric chloride; PM = phosphomolybdic acid; NP = diphenylboric acid 2-aminoethyl ester; TS = *p*-toluenesulfonic acid; VAN = vanillin-H_2SO_4; HV = Hoepfner-Vorsatz; DCQ = dichloroquinone 4-chloroimine-ammonium hydroxide; AC = aluminum chloride; SE = sodium ethoxide; BUT = *n*-butanol-HCl.

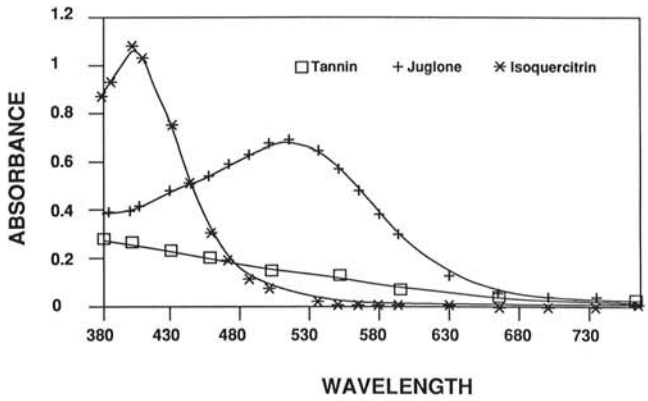

Fig. 1. Wavelength scans (nm) of juglone, isoquercitrin, and condensed tannins stained with Hoepfner-Vorsatz and measured on the microspectrophotometer. There is little overlap of isoquercitrin or condensed tannin with the juglone peak at 515 nm, but there is some overlap of juglone and condensed tannin with the isoquercitrin peak at 401 nm.

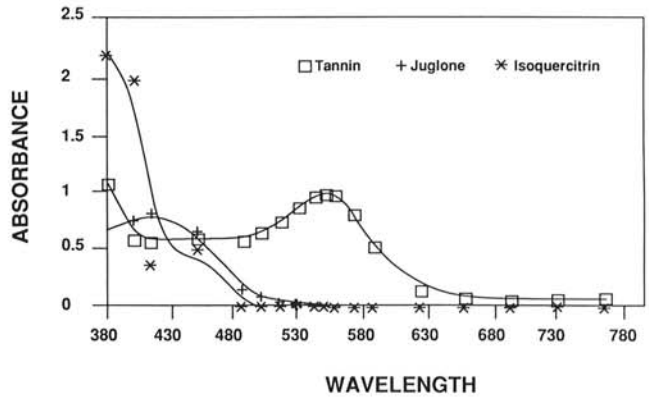

Fig. 2. Wavelength scans (nm) of juglone, isoquercitrin, and condensed tannins stained with *n*-butanol-HCl and measured on the microspectrophotometer. There is no overlap between the condensed tannin peak at 550 nm and the peaks of the other two phenols.

TABLE 2. Estimation of concentration ($\mu g/\mu l$) of juglone, isoquercitrin, and condensed tannin on the microspectrophotometer in pecan leaf tissues of various thicknesses[a]

Compound	Leaf section thickness (μm)			
	20	30	40	50
Juglone	15.4 ± 4.3[b]	26.2 ± 2.5	28.1 ± 7.7	38.8 ± 10.7
	0.28[c]	0.10	0.27	0.28
Isoquercitrin	9.4 ± 2.4	12.0 ± 1.8	11.2 ± 2.8	11.3 ± 1.0
	0.26	0.15	0.25	0.09
Condensed tannin	41.5 ± 3.6	42.0 ± 3.1	39.0 ± 6.1	63.0 ± 8.5
	0.09	0.07	0.16	0.14

[a]Standard curves of each phenolic standard were also measured on the microspectrophotometer.
[b]Data given are means ± standard deviation ($n = 5$).
[c]Coefficients of variation.

experiments were 30 μm, because values consistently fit within the limits of the standard curve and had lower coefficients of variation compared to the other section thicknesses. The best absorbancy range for nut husks was obtained with 20-μm sections, because the thicker sections often yielded values above the absorbance limit of the standard curve.

n-Butanol-HCl reagent plus steam heat converted condensed tannins to anthocyanidins. Autoclaving of samples or heating on a hot plate or in a drying oven severely desiccated the tissues. Heating on a slide warmer did not produce sufficient heat. Heating over boiling water or in steam within an enclosed water bath produced sufficient heat without complete tissue desiccation. Use of an enclosed water bath set at 90 C for 40 min consistently produced the desired results and was used in subsequent condensed tannin assays.

This histochemical technique cannot measure phenolic concentrations within individual pecan cells, with the exception of some of the larger spongy parenchyma cells and large glandular trichomes. We can, however, measure specific tissues, including the upper or lower epidermis, palisade parenchyma, and the xylem, phloem, or bundle sheath layers of the midrib.

This study exclusively looked at phenolic compounds found in *Carya* species. Other plant species will have very different phenolic profiles. If the phenolic profile of a given plant species is known, it may be possible to use these same indicators to distinguish some of the more chemically unique phenolic compounds such as quinones or condensed tannins. However, flavanoids appear to be much more difficult to separate.

Phenolic compounds such as juglone, isoquercitrin, and condensed tannins are pre-existing protectants. These compounds must be released from sites where they are sequestered, before they can be effective against an invader. Other studies have shown not only release of phenols on infection, but also an increase in phenolic concentration (11,19,22). Levin (15) stated that resistance due to phenols may be quantitative as well as qualitative in nature. Thus, the percentage increase of one phenolic compound on infection may not be the same as that of another phenolic compound. A single phenolic compound may be more important to resistance to a given pathogen than the available phenolic pool. The techniques presented in this paper will allow for a quantitative and qualitative study of juglone, isoquercitrin, and condensed tannins at the infection sites of *C. caryigenum* within pecan tissues.

LITERATURE CITED

1. Bate-Smith, E. C. 1975. Phytochemistry of proanthocyanidins. Phytochemistry 14:1107-1113.
2. Cork, S. J., and Krockenberger, A. K. 1991. Methods and pitfalls of extracting condensed tannins and other phenolics from plants: Insights from investigations on Eucalyptus leaves. J. Chem. Ecol. 17:123-134.
3. Freudenberg, K., and Weinges, K. 1962. Catechins and flavonoid tannins. Pages 197-216 in: The Chemistry of Flavonoid Compounds. T. A. Geissman, ed. Pergamon Press, New York.
4. Graves, C. H., Hedin, P. A., and Langhans, V. E. 1979. A survey of juglone levels among pecan, hickory, and walnuts. Proc. S.E. Pecan Growers Assoc. 72:103-112.
5. Graves, C. H., Jr., MacGown, M., Hedin, P. A., and Filer, T. H. 1986. Histochemical localization of juglone and related constituents in pecan. Phytopathology 76:205-208.
6. Hagerman, A. E., and Butler, L. G. 1989. Choosing appropriate methods and standards for assaying tannin. J. Chem. Ecol. 15:1795-1810.
7. Hedin, P. A., Collum, D. H., Langhans, V. E., and Graves, C. H. 1980. Distribution of juglone and related compounds in pecan and their effect on *Fusicladium effusum*. J. Agric. Food Chem. 28:340-342.
8. Hedin, P. A., Davis, F. M., Williams, W. P., McCarty, J. C., Shepard, R. L., and Porath, A. B. 1985. Screening to identify chemical markers of plant resistance to pests and plant stress. Ind. Eng. Chem. Prod. Res. Dev. 24:126-129.
9. Hedin, P. A., Langhans, V. E., and Graves, C. H., Jr. 1979. Identification of juglone in pecan as a possible factor in resistance to *Fusicladium effusum*. J. Agric. Food Chem. 27:92-94.
10. Hedin, P. A., Neel, W. W., Burks, M. L., and Grimley, E. 1985. Evaluation of plant constituents associated with pecan phylloxera gall formation. J. Chem. Ecol. 11:473-484.
11. Jersch, S., Scherer, C., Huth, G., and Schlosser, E. 1989. Proanthocyanidins as basis for quiescence of *Botrytis cinerea* in immature strawberry fruits. Z. Pflanzenkr. Pflanzenschutz 96:365-378.
12. Jurd, L. 1962. Spectral properties of flavonoid compounds. Pages 107-155 in: The Chemistry of Flavonoid Compounds. T. A. Geissman, ed. Pergamon Press, New York.
13. Laird, D. W., Graves, C. H., Jr., and Hedin, P. A. 1990. In vitro reactions of *Cladosporium caryigenum* with pecan condensed tannins and isoquercitrin. Phytopathology 80:147-150.
14. Langhans, V. E., Hedin, P. A., and Graves, C. H. 1978. Fungitoxic chemicals in pecan tissue. Plant Dis. Rep. 62:894-898.
15. Levin, D. A. 1971. Plant phenolics: An ecological perspective. Am. Nat. 105:157-181.
16. Mace, M. E. 1963. Histochemical localization of phenols in healthy and diseased banana roots. Physiol. Plant. 16:915-925.
17. Reeve, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Tech. 26:91-96.
18. Schonbeck, F., and Schlosser, E. W. 1976. Preformed substances as potential protectants. Pages 653-678 in: Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, New York.
19. Treutter, D., and Feucht, W. 1990. Accumulation of flavan-3-ols in fungus-infected leaves of Rosaceae. Z. Pflanzenkr. Pflanzenschutz 97:634-641.
20. Vankataraman, K. 1962. Methods for determining the structure of flavonoid compounds. Pages 70-106 in: The Chemistry of Flavonoid Compounds. T. A. Geissman, ed. Pergamon Press, New York.
21. Willard, H. H., Merritt, L. L., Jr., and Dean, J. A. 1974. Instrumental Methods of Analysis. Van Nostrand, New York. 860 pp.
22. Wisniewski, M., Bogle, A. L., Shortle, W. C., and Wilson, C. L. 1984. Interaction between *Cytospora leucostoma* and host-phenolic compounds in dormant peach trees. J. Am. Soc. Hortic. Sci. 109:563-566.