

## Occurrence of Juglone in Various Tissues of Pecan and Related Species

A. Borazjani, C. H. Graves, Jr., and P. A. Hedin

Former graduate assistant and professor of Plant Pathology, Department of Plant Pathology and Weed Science, and research chemist, Boll Weevil Research Laboratory, USDA-ARS, respectively, Mississippi State University, Mississippi State 39762.

Mississippi Agricultural and Forestry Experiment Station Journal Article 5832.

Accepted for publication 27 June 1985.

## ABSTRACT

Borazjani, A., Graves, C. H., Jr., and Hedin, P. A. 1985. Occurrence of juglone in various tissues of pecan and related species. *Phytopathology* 75:1419-1421.

Measurements of the seasonal distribution of juglone (5-hydroxy-1,4-naphthoquinone) among various tissues of pecan (*Carya illinoensis* 'Van Deman') revealed that the highest concentrations occurred in the leaflets in June but in the nuts in September. The leaf rachis, twigs, twig bark, trunk, root, and root bark had lower concentrations at both dates. There were no changes in juglone content of twigs sampled at monthly intervals or differences among twigs of five pecan cultivars at any date. Very little juglone was detected within pollen of several hickories (including pecan).

*Additional key words:* *Cladosporium caryigenum*, disease resistance, Juglandaceae, pecan scab.

The concentration of juglone in husk, kernel, and leaflet of black walnut (*Juglans nigra*), shagbark hickory (*Carya ovata*), and four pecan cultivars were: in husks, walnut > hickory and pecan; in leaflets, walnut > pecan and hickory; and in kernels, walnut > hickory > pecan. Seedlings grown in greenhouse pots and girdled to the cambium had a higher concentration of juglone in the leaves than did nongirdled seedlings, suggesting that juglone was synthesized in the leaf area and translocated by the phloem.

Juglone (5-hydroxy-1,4-naphthoquinone) was shown to be a host factor that may be associated with resistance (3,5-7) of pecan (*Carya illinoensis* Koch.) and other hickory species to scab caused by *Cladosporium caryigenum* (Ell. et Lang) Gottwald. Juglone and hydrojuglone glucoside have been correlated with resistance of juvenile leaves of black walnut (*Juglans nigra* L.) to anthracnose which is caused by *Gnomonia leptostyla* L. (3). The complete biochemical nature of resistance is not fully understood. Scab is the most serious disease of pecan.

Previous studies of seasonal variations of juglone content indicated that in leaves the level was higher in June and decreased during the season. This decrease in the leaves was accompanied by an increase of juglone concentration in nuts (5-7). These studies did not include an assessment of seasonal juglone variations within twig tissues, where superficial lesions form a major source of primary inoculum of *C. caryigenum*, or in various other tissues of the tree. Leaf and nut samples of walnut and various other hickories were compared to pecan (5,7), but these studies did not include a dissection of the nut tissues to determine levels in the various tissues.

Tissue culture procedures have been adapted as potential tools for new approaches to plant breeding, including the development of dihaploids through pollen culture techniques, colchiploidy through regenerating systems, and screening for disease resistance. Quinones and phenolics are often troublesome in tissue culture procedures (4,9-13). Thus, an understanding of the occurrence of juglone among various tissues of hickories could be important to an understanding of explant responses in tissue culture studies.

The purpose of this study was to determine levels of juglone among various tissues of pecan and related species to provide a better understanding of the possible role of this host constituent in disease resistance and in explant responses in tissue culture.

## MATERIALS AND METHODS

**Samples from mature trees.** Samples of plant tissues were collected from mature trees of several species at appropriate times

(Table 1). All samples were transported in ice chests to the laboratory and frozen until juglone analyses were made.

*Comparison of leaves, nuts, twigs, trunks, and roots of pecan.* Samples were collected in June and September from trees of cultivar Van Deman, since the highest level of juglone occurs in leaves and nuts, respectively, at these times (5-7). At each sampling date, two samples (no less than 10 g per sample) from each of three trees were taken of leaves, leaf rachises, nuts, twigs, twig bark, the trunk at 2.5-, 10-, and 30-cm depth (average tree trunk diameter of 65 cm), root and root bark, and soil immediately surrounding the roots. Trunk samples were taken with a 0.42-cm-diameter increment borer. Root and root bark samples were from 2.5-cm-diameter roots on two sides of the trees. Soil samples were obtained by washing the soil from roots in the area where root samples were taken. Leaf, nut, and twig samples were taken from widely spaced random locations from each tree.

*Seasonal comparison of twig tissues from five pecan cultivars.* Three twig samples of no less than 10 g per sample were taken from cultivars Desirable, Lewis, Moore, Stevens, and Stuart in May, June, July, August, and September.

*Comparison of pollens of various sources.* Pollens were collected from shagbark, red, and nutmeg hickories, McCallister hican, and

TABLE 1. Identification and location of trees sampled for juglone analyses during the 1979, 1980, and 1981 growing seasons

Species	Common and cultivar names	Location of trees
<i>Carya illinoensis</i>	Pecan (cultivars Frotscher, Lewis, Moore, Stevens, Stuart, and Desirable) Van Deman	MS State University Brookville, MS
<i>C. illinoensis</i> × <i>Carya</i> sp. <sup>a</sup>	Hican (cultivar McCallister)	Yazoo City, MS
<i>C. ovata</i>	Shagbark hickory	Starkville, MS
<i>C. ovatis</i>	Red hickory	Starkville, MS
<i>C. myristicaeformis</i>	Nutmeg hickory	Starkville, MS
<i>Juglans nigra</i>	Black walnut	MS State University

<sup>a</sup> Interspecific hybrids of unknown parentage.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1985.

from Frotscher, Desirable, Lewis, Moore, Stevens, and Stuart pecan. Mature catkins were spread over aluminum foil in the laboratory. After 24 hr, expelled pollen was collected, sifted through two layers of cheesecloth to remove large pieces of trash, and placed in glass vials.

*Comparison of husk and kernel of the nut and leaves of walnut, hickory, and pecan.* Nut samples for husk and kernel determinations were taken in September since juglone reaches its peak in whole nuts near the end of the season (5-7). Three samples each were collected from black walnut, shagbark hickory, and pecan cultivars Desirable, Lewis, Moore, and Stevens. Leaf samples were taken on the same date. Kernels and husks were separated before the samples were frozen.

**Samples from juvenile trees.** Juglone levels were compared in various tissues of small seedling plants on three sampling dates. Twelve uniform 2-yr-old greenhouse seedlings were used. Samples from three seedlings were taken of stems, primary roots, fibrous roots, root bark, and soil surrounding the roots before bud-break in April. In July, three seedlings were girdled by removing a 2-cm band of bark and phloem. Two weeks later, samples were taken from leaflets, stems, roots, and soils surrounding the roots of three girdled and three nongirdled seedlings. In September, leaflet, stem, root, and soil samples were taken from the three remaining seedlings. Samples were frozen until analyzed.

**Quantitative analysis of juglone.** Juglone analyses were conducted by using a technique described by Hedin et al (6). Split-plot design was employed to analyze for seasonal fluctuation of juglone in twig tissues of five pecan cultivars. Results of other experiments were analyzed as a randomized complete block design. Duncan's multiple range test was used to compare mean differences ( $P=0.05$ ).

## RESULTS AND DISCUSSION

Data for tissues of mature cultivar Van Deman pecan trees are given in Fig. 1. At the June sampling date, the highest level of

juglone was noted in leaflets, followed by that of the small developing nuts. All other plant parts and the soil surrounding roots exhibited considerably lower levels. The September readings revealed a notably lower level in the leaflets and a less striking reduction in the leaf rachises, twigs, and twig bark. There was an increase at this time in the juglone level of the nuts, but this increase was not dramatic. This finding was consistent with prior reports for cultivar Van Deman (5,7), and in this regard, Van Deman differs from most cultivars which have shown dramatic seasonal increases. The juglone level was also significantly higher in the roots and the root bark. This is perhaps significant in that there was a decrease in juglone levels from June to September in twigs and twig bark, possibly suggesting a translocation pattern.

No significant change in the juglone level was observed in twigs of the five pecan cultivars during the entire growing season (Table 2), and there were no significant differences among cultivars. The very low level of juglone in twigs throughout the growing season may be significant in that overwintering stomata survive on twigs.

TABLE 2. Within-season variations of juglone concentrations in twig tissues of five pecan cultivars and their seasonal variations from May to September in 1979

Pecan cultivars	Juglone concentration (mg/g of fresh tissue)					
	May	June	July	Aug.	Sept.	Mean
Moore	0.027 <sup>a</sup>	0.007	0.005	0.012	0.035	0.017
Stuart	0.020	0.015	0.035	0.016	0.008	0.019
Desirable	0.018	0.027	0.012	0.009	0.012	0.015
Lewis	0.007	0.025	0.040	0.016	0.004	0.018
Stevens	0.020	0.020	0.015	0.015	0.015	0.017
Average	0.018	0.019	0.021	0.014	0.015	0.017

<sup>a</sup>No significant difference in juglone contents was observed among cultivars or months according to Duncan's multiple range test ( $P=0.05$ ). Each figure represents an average of three samples.

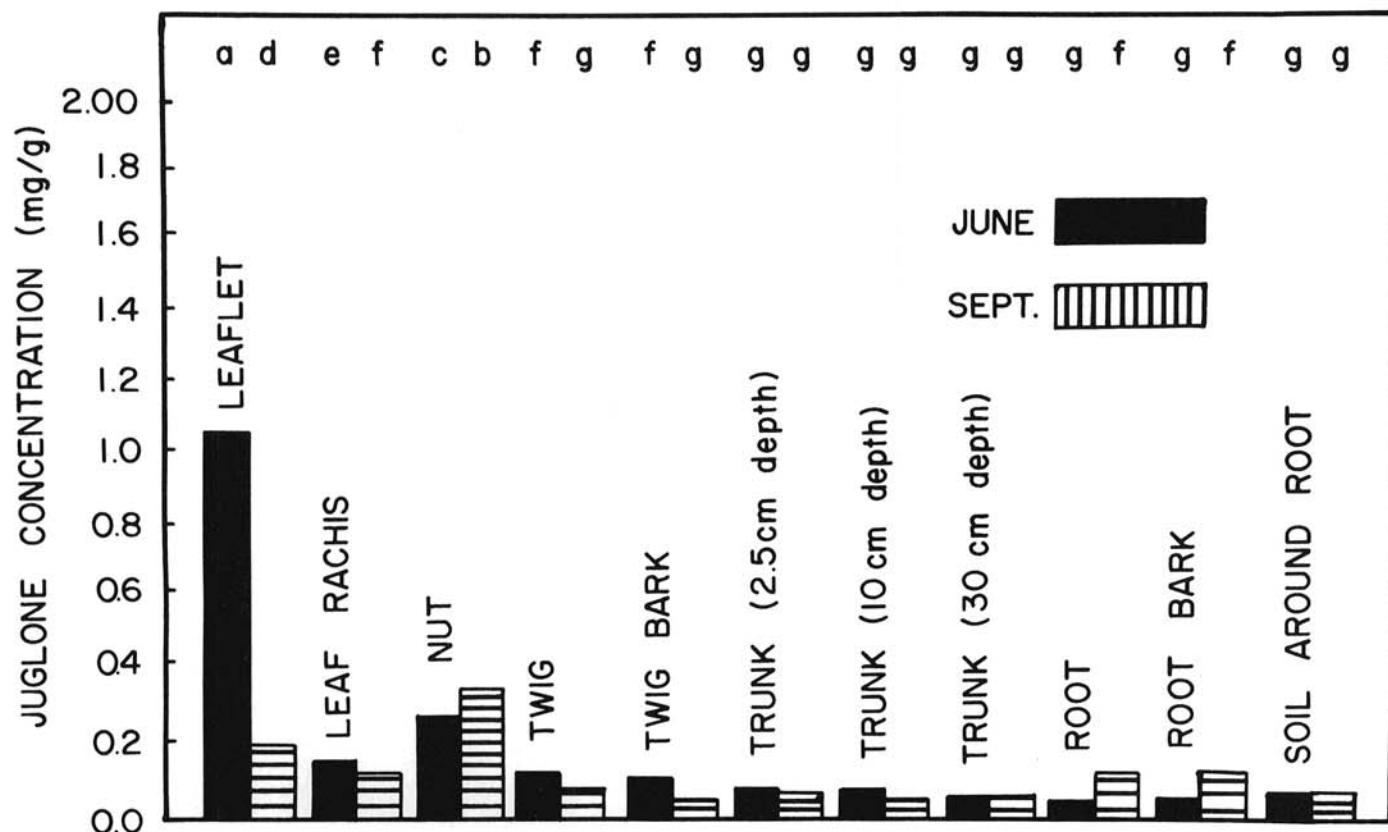


Fig. 1. Juglone concentration (milligrams per gram of fresh tissue or soil) in roots, root bark, leaflets, leaf rachises, twigs, twig bark, trunks, nuts, and soil around roots of pecan cultivar Van Deman in June and September. Each figure represents an average of three replications. Means not followed by the same letter differ significantly according to Duncan's multiple range test ( $P=0.05$ ).

TABLE 3. Juglone concentrations in certain tissues of black walnut, McCallister hican, and selected hickory species including several pecan cultivars

Trees	Juglone concentration (mg/g of fresh tissue)			
	Husk	Kernel	Leaflets	Pollen
Black walnut	2.94 a <sup>y</sup>	0.007 a	0.550 a	0.008 b
Shagbark hickory	1.96 b	0.004 b	0.058 c	0.0075 b
Nutmeg hickory	...	...	...	0.015 a
Red hickory	...	...	...	0.0025 bc
Hican <sup>z</sup>	...	...	...	0.001 c
Pecan cultivars:				
Stevens	1.04 c	0.001 c	0.337 b	0.001 c
Lewis	1.31 bc	0.001 c	0.114 c	0.006 b
Moore	1.86 b	0.000 d	0.101 c	0.005 b
Desirable	0.86 c	0.000 d	0.090 c	0.006 b
Stuart	...	...	...	0.009 ab
Frotscher	...	...	...	0.001 c

<sup>y</sup> Means within columns followed by different letters differ significantly according to Duncan's multiple range test ( $P = 0.05$ ). Dots (...) indicate value was not measured.

<sup>z</sup> Hican (interspecific hybrids of unknown *Carya illinoensis* × *Carya* sp.) cultivar McAllister.

Juglone levels were low within pollen of the several hickory genotypes (Table 3). Among the hickory species tested, highest levels of juglone in pollen were found in nutmeg hickory, followed by shagbark, and red hickory. Among pecan cultivars, highest levels were found in Stuart. McCallister hican contained almost no juglone.

Juglone content was higher in husks than in leaflets of black walnut, hickory, and pecan as reported by other workers (5,7), and juglone levels in kernels were very low (Table 3). This high juglone level in the husks of all samples supports the finding of Graves et al (5), but the low level found in kernels emphasizes the inadequacies of grinding whole tissues (such as the whole nuts) for juglone determinations as was done in prior studies (5,7). These results also confirm that juglone content in nuts is not proportional to that found in leaves among hickory species or cultivars of pecan.

Studies of greenhouse-grown girdled seedlings revealed an increase in juglone concentration in leaflets, stems, and stem bark above the girdled area (Table 4), accompanied by a decrease in the stem bark, roots, root bark, and fibrous roots below the girdled area. These data suggest that juglone synthesis occurs in leaves and that juglone is translocated to other parts of the plant. This would seem consistent with the suggestion of Lee et al (8) that juglone is translocated from leaves to the fruit. Small seedlings apparently follow the pattern of mature trees in that there is a considerably higher level of juglone in leaflets than in stems and roots.

Much speculation exists about the potential role of juglone in causing resistance to fungi in the Juglandaceae. Previous works appear to support this role (3,5-7). For instance, in vitro, juglone was fungitoxic toward *C. caryigenum* at levels as low as 0.05 mg/g of liquid culture (7). Our data indicate that juglone concentrations in whole ground tissues such as leaflets and husks are much higher. Thus, the precise role of this host plant constituent in resistance to diseases would seemingly depend on its presence and availability at or near infection sites, and critical histochemical localization studies are needed for better understanding. Further, prior observations (1,2,5,7) have indicated some positive correlations with low disease incidence in the field. Strong negative correlations would not be expected assuming factors other than juglone are involved. Our studies have indicated a low and consistent level of juglone on twigs throughout the growing season, suggesting an unlikely role in overwintering of the pathogen. Thus, it would seem that the occurrence and availability in leaf development, as

TABLE 4. Juglone content of greenhouse-grown pecan seedlings at 17 April, 18 July, and 25 September 1981 and of girdled seedlings at 18 July 1981<sup>x</sup>

Samples	Juglone concentration (mg/g)			
	17 April 1981	18 July 1981		25 Sept. 1981
	Nongirdled	Girdled	Nongirdled	Nongirdled
Leaflet	... <sup>y</sup>	1.200 Aa	0.420 Ba	0.365 Ba
Stem above ring	0.023 Ba <sup>z</sup>	0.050 Ab	0.025 Bb	0.020 Bd
Stem bark				
above ring	...	0.080 Ab	0.030 Bb	...
Stem bark				
below ring	...	0.020 b	...	...
Root	0.005 Bb	0.005 Bb	0.020 Cb	0.080 Ac
Fibrous root	0.000 Cb	0.000 Cb	0.005 Bc	0.010 Ad
Root bark	0.005 Cb	0.000 Cb	0.010 Bb	0.180 Ab
Soil around bark	0.010 Ab	0.010 Ab	0.016 Ab	0.100 Ac

<sup>x</sup> Girdling was done by removal of a ring of bark down to the cambium region, severing phloem tissues 25 cm above the soil surface.

<sup>y</sup> Dots (...) indicate value was not measured.

<sup>z</sup> Means not followed by the same letter differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test. The capital letters show the mean differences within rows and the lower-case letters those within columns.

affecting early build-up of inoculum potential as well as its presence in developing nutlets, would be worthy of further study.

Even though the results of this and other studies (1,2,5) comparing levels of juglone in pecan with that of other species have generally indicated a slightly higher level of juglone in developing nuts, and in some cases, a lower level in the leaves, determination of the role of juglone as a factor relating to the rare occurrence of *C. caryigenum* on species other than pecan must await more definitive study.

#### LITERATURE CITED

- Borazjani, A. 1981. Occurrence of juglone among walnuts and hickories: seasonal variations, presence among tissues, translocation, and the influence of growth regulators on concentration. Ph.D. dissertation. Mississippi State University, Mississippi State. 68 pp.
- Borazjani, A., Graves, C. H., Jr., and Hedin, P. A. 1983. A survey of juglone levels among walnuts and hickories. *Pecan Quart.* 17(1):9-13.
- Cline, S., and Neely, D. 1984. Relationship between juvenile-leaf resistance to anthracnose and presence of juglone and hydrojuglone glucoside in black walnut. *Phytopathology* 74:185-188.
- Durand, C. R., Cresswell, R., and Nitsch, C. 1978. Factors influencing the regeneration of *Eucalyptus grandis* by organ culture. *Acta Hort.* 78:149.
- Graves, C. H., Hedin, P. A., and Langhans, V. E. 1979. A survey of juglone levels among pecan, hickory, and walnut. *Proc. S.E. Pecan Growers Assoc.* 50:79-81.
- Hedin, P. A., Langhans, V. E., and Graves, C. H., Jr. 1979. Identification of juglone in pecan as a possible factor for resistance to *Fusicladium effusum*. *J. Agric. Food Chem.* 27:92-94.
- Langhans, V. E. 1977. A study of the biochemical nature of resistance of pecan to *Fusicladium effusum*. Ph.D. dissertation. Mississippi State University, Mississippi State. 58 pp.
- Lee, K. C., and Campbell, R. W. 1969. Nature and occurrence of juglone in *Juglans nigra* L. *HortScience* 4:297-298.
- Loomis, W. D. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes. *Phytochemistry* 5:423-438.
- Metha, U., and Mohanram, H. Y. 1980. Regeneration of plantlets from the cotyledons of *Cajanus cajan*. *Ind. J. Exp. Biol.* 18:800-802.
- Taylor, G. G., and Odom, R. E. 1970. Some biochemical compounds associated with rootings of *Carya illinoensis* stem cuttings. *J. Am. Soc. Hort.* 95:146-151.
- Walker, J. R. L. 1964. Studies on the enzymic browning of apples. II. Properties of apple polyphenoloxidase. *Aust. J. Biol. Sci.* 17:360-371.
- Walker, J. R. L., and Wilson, E. L. 1975. Studies on the enzymic browning of apples. Inhibition of apple *o*-diphenol oxidase by phenolic acids. *J. Sci. Food Agric.* 26:1825-1831.