development and abscission could be related to time of production or to patterns of translocation of the promoters and inhibitors.

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Effects of Flooding and Soil Phosphorus Levels on Pecan Seedlings

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Abstract. **Thirty-six-day-old 'Dodd' pecan seedlings [*Carya illinoensis* (Wangenh) C. Koch] were flooded for 31 days or not flooded in factorial combinations with 0, 215, or 430 g P per m³ of media. Flooding decreased leaf number, leaf area, leaf and root dry weight, and induced stomatal closure. Flooding also reduced leaf, trunk, and root K, Ca, Mg, Zn, Fe, and Mn concentrations. Nitrogen was lower in the leaves and trunk of flooded trees, but higher in the roots of flooded trees than unflooded trees. Flooding decreased the leaf P concentration, but did not affect the P concentration in the roots. Phosphorus application increased leaf P concentration in unflooded trees, but not flooded trees.**

Previous studies have indicated that tolerance to flooding can vary widely among fruit trees (5, 10). Remy and Bidade (10) found apple and pear to be tolerant, plum intermediate, and peach and cherry least tolerant of soil inundation. Jawanda (5) listed pear, mango, and guava trees as tolerant and citrus, loquat, and papaw as being intolerant to waterlogging. Although pecans are native to floodplains, Alben (1) reported that summer and fall flooding caused leaf scorch and partial defoliation in Louisiana pecan orchards. Also , Loustalot (9) reported that 5 days of flooding reduced photosynthetic rates of seedling pecan trees and 35 days of flooding killed as much as 30% of their roots systems.

Our primary objective was to observe how seedling pecan trees respond to flooding. Specifically, comparisons were made between nonflooded and flooded plants with respect to tissue dry weight, elemental absorption and translocation, stomatal responses, and adaptive growth responses to flooding, i.e., the development of adventitious roots or of hypertrophied lenticels.

Work of peas (2), corn (13), and loblolly pine (4, 7) suggested that high P levels could reduce the injurious effects of flooding. One consequence of flooding pecan trees is a reduction in the photosynthetic rate (9). Herold (3) suggested that P_i (inorganic orthophosphate) may be involved in the regulation of photosynthesis, probably via a decreased ATP/ADP ratio, or a direct effect of P_i on ribulose-1, 5-bisphosphate carboxylase activity. Therefore, a flood-induced inhibition of P absorption or translocation could cause a reduction in apparent photosynthesis. Based on this information, our secondary objective was to determine if phosphorus availability would have an effect on the response of pecan seedlings to flooding.

Stratified 'Dodd' pecan seeds were bathed in an aerated aqueous solution of 100 ppm GA_3 for 1 week, then transferred to aerated water until radicle emergence. Seeds with uniform radicles were planted 9 Sept. 1985 in 26-cm-tall by 20-cm-diameter containers filled with a fire-hardened calcite clay (Turface, Wyandotte Chemicals Corp., Wyandotte, Mich.). The medium was amended with 705 g KCl (41.4% K), 4693 g dolomite, 600 g $FeSO_4$ (20% Fe), 92 g $MnSO_4$ (27% Mn), 21 g $CuSO_4$ (25.4% Cu), 3.5 g $NaBO_2$ (20.5% B), 0.5 g $NaMoO_4$ (39% Mo), and 88 g $ZnSO_4$ (36% Zn) per m³ of medium. Five hundred ppm N from ammonium nitrate was applied weekly from 16 Sept. 1985 through 14 Oct. 1985. Three levels of P were incorporated in the media prior to planting (0, 215, and 420 $g \cdot m^{-3}$P) Greenhouse temperatures were maintained near 21° night and 27°C day.

Trees were flooded on 14 Oct. 1985, 36 days after planting, by submerging the pots in individual containers of tap water to about 2.5 cm above the soil surface. Flooded containers were covered with aluminum foil to prevent algae growth. Water was added to flooded containers as required to compensate for evapotranspiration. Normal watering (usually on alternate days, water applied to runoff) was maintained on the unflooded trees. Treatments were replicated five times with one plant in each container in a completely randomized design. Data were analyzed using analysis of variance and the protected LSD.

Table 1. The influence of root flooding on 'Dodd' seedling pecan trees. Means are pooled over P levels.

Flooding treatment	Tree height (cm)	Leaf area (cm²)	Leaves/tree (no.)	Dry weight (g)		
				Leaf	Trunk	Root
Unflooded	22.5	581	7.7	2.3	0.68	4.8
Flooded	20.9	331	6.5	1.8	0.66	0.8
Significance	NS	***	**	*	NS	***

NS, *, **, *** Nonsignificant or significant at 5%, 1%, or 0.1% levels, respectively, by Fisher's F-test.

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Table 2. The influence of root flooding and P treatment on stomatal resistance of 'Dodd' seedling pecan trees.

Flooding treatment	P ($g \cdot m^{-3}$)	Stomatal resistance ($s \cdot cm^{-1}$) Days flooded 10	17	22
Unflooded	0	1.96	3.06	1.63
	215	2.80	4.32	2.58
	430	2.56	3.17	1.90
Flooded	0	4.10	7.59	5.81
	215	3.70	4.44	4.42
	430	4.79	7.08	3.42
$LSD_{.05}$		1.01	1.58	1.08

Table 3. The influence of root flooding and P treatment on P concentration of 'Dodd' pecan seedlings.

Flooding treatment	P ($g \cdot m^{-3}$)	P dry weight (%) Leaf	Trunk	Root
Unflooded	0	0.25	0.08	0.19
	215	0.35	0.13	0.26
	430	0.40	0.14	0.28
Flooded	0	0.15	0.08	0.18
	215	0.16	0.08	0.26
	430	0.16	0.09	0.21
$LSD_{.05}$		0.04	0.02	0.03

Stomatal resistance to water vapor of the abaxial leaf surface was measured using the most recently expanded leaf of each plant on full sun days between 1300 and 1400 HR. (the time of greatest stomatal resistance) on 24 Oct., 31 Oct., and 5 Nov. 1985 (flooded 10, 17, and 22 days) using a LI-COR model LI-700 transient porometer. The experiment was terminated 14 Nov. 1985 after 31 days of flooding. Plant height was measured, and total leaf area determined with a LI-COR model LI-3100 area meter. Leaves, stems, and roots were separated and washed in tap water, followed by a 0.1% Liquinox solution (P-free soap), then two deionized water rinses, then dried at 75°C. After dry weights were recorded, the tissues were ground in a Wiley mill to pass a 20-mesh screen and stored in airtight jars until analyzed for N using macro-Kjeldahl, P colorimetrically, and K, Ca, Mg, Zn, Fe, and Mn using atomic absorption spectroscopy.

There were no significant interactions between flooding and P levels affecting tree height, leaf number, leaf area, or dry weight of the plant parts. Flooding reduced total leaf area 43% (Table 1). The reduction in leaf area was due to a combination of fewer leaves per tree and inhibited expansion of new leaves (no leaves abscised during the study). Tree height was not significantly different between flooded and unflooded trees.

Leaf dry weight was reduced 22%, and root dry weight 83% as a result of flooding (Table 1). Roots of the flooded trees were thin and dark brown, whereas roots of unflooded trees were bright yellow and profuse. Leaves of flooded trees developed visible anthocyanin pigments after 2 weeks of flooding, which became more intense as flooding continued. Unflooded trees did not develop any visible anthocyanins. Thimann and Edmondson (14) found anthocyanins were formed in the presence of light when sugars, particularly sucrose, were in high concentrations. Anthocyanin development in pecan leaves during flooding suggests that carbohydrate translocation was decreased, thus increasing carbohydrate concentrations in leaves and anthocyanin formation. Flooded trees did not develop other symptoms that are sometimes typical of flooding damage, such as epinasty, chlorosis, or necrosis. Trunk dry weights were not affected by flooding, and P treatments had no affect on tissue dry weight.

Leaf stomatal resistance to water vapor was consistently higher in flooded trees than in unflooded trees (Table 2). Stomatal closure is one of the earliest plant responses to flooding in many plant species (6). Stomata of flooded *Theobroma cacao* seedlings began to close within 2 hr (12), and those of *Fraxinus pennsylvanica* began to close within 2 days after flooding (11). Stomata of *Fraxinus pennsylvanica* began to reopen when adventitious roots developed (11). Stomatal resistance of the pecan seedlings in this study remained high during the experiment, and no adventitous roots formed, although extensive hypertrophic lenticels developed on the trunk below the water surface.

Stomatal resistance of unflooded trees was not affected by P treatments (Table 2). However, stomatal resistance of flooded trees was lower with 215 g P/m^3 17 days after flooding, and 215 and 430 g P/m^{-3} 22 days after flooding compared to no P application. The reduction in stomatal resistance with added P occurred without an apparent increase in leaf P concentration (Table 3). However, the trees were grown for 36 days in the P treatments and flooded 31 days, then P concentrations were determined when the study was terminated. Therefore, P concentrations reported here are probably lower than when stomatal resistance was measured. Thus, P may have been active in reducing stomatal resistance.

P treatments increased leaf, trunk, and root P concentrations in unflooded trees. Phosphorus addition did not affect P concentration of tissues in flooded trees, except root P concentration was greater in flooded trees using 215 g P/m^{-3} than no P (Table 3). Phosphorus concentrations were lower in tissues of flooded trees than unflooded trees.

Elemental concentrations were reduced in the leaves, trunk, and roots of flooded trees in most instances (Table 4), which is consistent with the effects of flooding stress on other plant species (6). However, N concentrations were 10% higher in the roots and 53% lower in leaves of flooded trees compared to unflooded trees. This difference suggests that N translocation was inhibited more than N absorption. Phosphorus concentrations were similarly reduced in the leaves and trunk of flooded trees, but not in the roots (Table 3). Shapiro et al. (13) reported a similar response in corn and hypothesized that reduced O_2 availability to the roots inhibits translocation of some elements to the shoots more so than the absorption of those elements.

Loucks and Keen (8) reported that survival of pecan seedlings after 4 weeks flooding was less than that of green ash, baldcypress, and silver maple, but greater than black walnut, siberian elm, boxelder, and cottonwood. Observations in this study indicate that pecan seedlings will tolerate extended flooding without death; however, significant decreases in root mass and leaf area could severely restrict growth or decrease survival upon termination of flooding.

Table 4. The influence of root flooding on elemental concentrations of 'Dodd' seedling pecans. Means are pooled over P levels.

Flooding treatment	Dry weight (%) N	K	Ca	Mg	Dry weight ($\mu g \cdot g^{-1}$) Zn	Fe	Mn
			Leaf				
Unflooded	4.02	1.03	1.28	0.41	81	202	1330
Flooded	1.87	0.50	0.52	0.20	27	90	274
Significance	***	***	***	***	***	***	***
			Trunk				
Unflooded	1.29	0.64	1.06	0.29	84	51	421
Flooded	1.01	0.24	0.80	0.19	28	18	22
Significance	*	***	***	***	***	***	***
			Root				
Unflooded	1.98	0.98	0.38	0.21	64	393	351
Flooded	2.20	0.54	0.23	0.08	45	220	47
Significance	*	***	***	***	***	***	***

*,*** Significant difference at 5%, or 0.1% levels, respectively, by Fisher's F-test.

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Development of Anthocyanin Pigments in Muscadine Grapes

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Abstract. **The development of individual anthocyanins (Acy) in black muscadine grapes (*Vitis rotundifolia* Michx) during maturity was followed. Most of the grapes investigated contained the nonacylated 3,5-diglucoside of delphindinin (Dp), cyanidin (Cy), petundin (Pt), Peonidin (Pn), and malvidin (Mv) when pigment extracts were separated on a reverse phase HPLC C_{18} column. Dp was the most abundant anthocyanin (Acy) in most cultivars at maturity. The development of individual Acy, which is indicative of the nature of enzyme activity, occurred at different rates for each of the cultivars. A very high flavonoid 3′ hydroxylase activity was indicated by the fact that, in most instances, Dp content was higher than those of the other Acy at the onset of pigmentation, including Cy, which is the precursor of the other pigments. The total Acy content at maturity ranged from 12 to 338 mg/100 g of berries.**

The nature and content of grape anthocyanins (Acy) can serve as important chemical markers and improve understanding of the biochemical reactions leading to ripening of grapes. Ebel and Halbrook (3) and Markakis (7) noted that a chalcone is the first intermediate of the whole classes of flavonoids. Flavonone then is produced from chalcone isomerization by a chalcone isomerase, and this undergoes different enzyme catalyzed reactions leading to flavones, flavonols, isoflavones, or Acy. Although very little is known about the last transformation (3, 5), it appears to be quite evident that chemical modifications, such as hydroxylation, methylation, glycosylation, and esterification of the glucosides, occur. Roggero et al. (10) suggested a reaction pathway of Acy that involves the synthesis of cyanidin (Cy) from a flavonone after the isomerization of chalcone. Cy then can be converted to either delphindin (Dp) or peonidin (Pn) as a result of enzymatic activity. Flavonoid-3-hydroxylase (FH) catalyzes the conversion of Cy to Dp, while the formation of Pn is catalyzed by O-methyltransferase (MT). Dp then can be converted to malvidin (Mv) through Mt activity. Their results suggest that the hydroxylation of Pn to form Pt does not take place, and they noted that the percentage of Dp at the early stages of ripening is an excellent sign of enzyme activity in cultivars.

Liao and Luh (6), and Ribereau-Gayon (9) reported that the color of red muscadine wine bears a direct relationship with the Acy composition. They also reported that these pigments are the nonacylated 3,5-diglucosides of Mv, Pn, Pt, Cy, and Dp. These findings also were supported by the research conducted by Ballinger et al. (1). Nesbitt et al. (8) demonstrated that good red wine color bears a direct relationship to the presence of a large amount of Mv, and, to a lesser degree, of Pt. It was suggested that increased methylation of individual Acy enhanced pigment stability. Although Dp was the most abundant form of all the Acy present, there appeared to be no correlation between the quantity of Dp present in the grapes and good wine quality. Ballinger et al. (2) in a subsequent study also confirmed these results. They reported that Mv is the most stable Acy in wines, followed in decreasing order by Pn, Pt, Cy, and Dp. Similar observations were made in muscadine juices and jellies by Flora (4), who showed that cultivars with relatively high Mv and total Acy contents yielded products with the highest quality and most suitable colors. The objective of this study was to separate and quantify the Acy pigments that are present in some black muscadine grape cultivars at different stages of maturity.

Grapes from the cultivars Chief, Jumbo, Albermarle, Cowart, Noble, Regale, and Pink Hunt were harvested at different stages of maturity from experimental vineyards located on the university campus. Each cultivar then was separated visually into three categories based on the degree of coloration (verasion, half color development, and full color, respectively). Each subgroup again was separated into three batches for analysis. The sugar content of the grapes was determined with an American Optional temperature-compensated hand refractometer.

Color of whole grapes was measured on a Perkin-Elmer Lambda 3B Spectrophotometer equipped with an external integrating sphere and a data station. Grapes were placed in the external sphere, and transmittance values for scans between 780 and 380 nm were determined at 1 nm intervals. Luminance "L' values were determined using the CIE standard source C on Perkin Elmer IFL3 and COLOR software. Total Acy content in the grapes was determined on freeze-dried, dewaxed grapes (30 g). Wax removal was done by soaking whole grapes in ether for 5 hr. Pigments then were extracted exhaustively in 1% HCl in methanol by successive extractions. Absorbance values of these solutions were read at 535 nm and Acy content was estimated using a molar extinction coefficient (E) 2.1×14^4 and a molecular weight of 683 (4).

For HPLC analysis, the dry powders obtained from freeze drying the grapes (30 g) were mixed with acidified methanol (20 ml) and left overnight in the refrigerator. The dissolved pigments were separated from solid materials by centrifugation at 5000 × g for 10 min. Separation of individual Acy in the supernatant was done with a Hitachi 655A-11 system after filteration through a 0.45 Teflon filter, using a 250 mm × 4 mm I.D. Hibar C_{18} column and an L-3000 multichannel UV-VIS photodetector. Acy were monitored at 520 nm. The solvent system used was H_3PO_4 (1%) in acetic acid-water (10:90). Samples (20 µl) were injected into the column and eluted at a flow rate of 1.0 ml/mm (11), and peaks were integrated with a Hitachi D-2000 Chromato-Integrator. Peaks were identified from their retention times using standards obtained from Alpine Chemical Co., England. Statistical analysis of data was carried out using the Student *t*-distribution.

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