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Responses of loblolly pine (mesophyte) and swamp tupelo (hydrophyte) seedlings to soil flooding and phosphorus*

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Summary In a pot study, flooding reduced growth of loblolly pine (LP) seedlings and caused an imbalance of nutrients, particularly Fe, in the tops. Swamp tupelo (ST) suffered none of these problems. Also under anaerobiosis, LP roots leaked 85 percent of total ethanol production into solution whereas ST leaked only 55 percent. Application of phosphorus increased LP growth under flooded and drained conditions but ST only under drained conditions. The differential response of the two species to flooding suggests that poor energetics and the leaky nature of LP roots under anaerobiosis restricts its growth in such habitats. Application of phosphorus (P) appears to amend these conditions in LP by reducing moisture stress, reducing nutrient imbalances, and improving energetics in older seedlings. Our results confirm that ST, the hydrophyte, avoids the problems of soil flooding via adaptations in anaerobic metabolism and oxidation of the rhizosphere.

Introduction

Application of phosphorus fertilizer to poorly drained soils has been reported to increase growth of pine trees[13] and 2 year-old loblolly pine (*Pinus taeda* L.) seedlings[11] and increase the capacity of excised roots of loblolly pine to respire anaerobically[2]. Also, there are phosphorus-zinc interactions in a number of plant species but the nature of the interactions varies among species and site conditions[6]. In lowland rice, applied P reduced Zn uptake but it and applied Zn, individually and in combination, increased dry weight production in waterlogged soils[6].

For further insight into why loblolly pine responds to phosphorus under poor soil aeration, seedlings of loblolly pine and swamp tupelo (*Nyssa sylvatica* var.

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biflora (Walt.) Sarg.), were grown in flooded and drained pots with and without applied P in a growth chamber. Growth and physiological responses were compared.

Methods

Loblolly pine* and swamp tupelo seedlings from coastal South Carolina sources were grown separately in 4 l stone crocks filled with a 1 : 1 volume of sand and finely ground peat. The pots, 12 for LP and 8 for ST, were arranged in a growth chamber in a complete randomized design as a 2 × 2 × 2 factorial experiment. They were grown under well drained conditions for 48 days and one-half were flooded (0–4 cm above the soil surface) until day 118.

Hoagland's nutrient solution was modified by substituting NH_4NO_3 in place of $NH_4H_2PO_4$ for the non-phosphorus treatment by doubling the standard amount of NH_4NO_3 in the final solution. In the phosphorus treatment the amount of $NH_4H_2PO_4$ was doubled so the amount of nitrogen in each treatment was 14 g. A total of 740 ml of one strength with and without applied P was added to each pot on four separate occasions at 3-to-4 week intervals; this amounted to 200 mg P per treated pot. The photoperiod was 16 h ($20 \pm 1 \times 10^3$ lux at 18″ from lights) and temperatures were 30°C ± 2.5 (light) and 20°C ± 1.5 (dark).

Height growth was measured periodically and at harvest. Soil redox potential was sampled 4 times (6 cm depth) only in the flooded pots during the final 30 days of the experiment. The stem of one of the four seedlings from each pot was clipped at harvest, at the beginning of the light cycle, and water potential was measured with a pressure bomb.

Soil solutions were sampled from the bottom of drained and top of flooded pots and stabilized in dilute HNO_3. Soil samples were taken from the pots as seedlings were washed out and seedling parameters were measured. CO_2 production was measured on excised root tips for 4 h in N_2. After incubation, root tips and reaction solutions were analyzed separately for ethanol[7] and malate[5].

Plant nitrogen was determined with a modified micro Kjeldahl procedure[12]. Phosphorus was determined by the molybdovanadate reduction[9] and K, Ca, Cu, Mn, Fe, Mg, and Zn by atomic absorption.

Soil exchangeable bases were determined by atomic absorption on extracts made with 1 *N* NH_4OAC[9] and available phosphorus by extracting 2.5 g of soil with 20 ml of Bray P2 solution[1]. Soil pH was measured with a glass electrode. Total soil nitrogen was determined by micro Kjeldahl digestion[12]. Soil solution samples were analyzed for Ca, Mg, K, Na, Mn, Cu, Fe, and Zn by atomic absorption. Phosphorus was determined by molybdate reduction[9] and nitrogen was determined by Kjeldahl digestion[12].

Results and discussion

Soil

Soil redox potential in the flooded pots averaged +24.7 mv and ranged from +84.7 mv to −13.7 mv which indicated that reducing conditions in the flooded soil were sufficient to reduce O_2, NO_3^-, Mn^{+4} and Fe^{+3} (ref.[4]). Such conditions probably reduced the extra nitrate added in the nutrient solution. Soil pH for all treatments at harvest averaged 4.65 and ranged from 4.10 to 5.52.

* LP seeds were donated by the Westvaco Nursery at Ravenel, South Carolina.

Table 1. Growth, and water potential of loblolly pine and swamp tupelo seedlings and nutrient concentration of the foliage at harvest

Variable	Species	Treatment*			
		Drained		Flooded	
		P_0	P_{200}	P_0	P_{200}
(a) Height (cm)	LP	16.0a	18.2a	7.9b	9.4b
	ST	45.9c	53.0d	55.3d	46.7c
Root length (cm)	LP	37.5a	37.1a	11.4b	15.4b
	ST	30.0a	28.9a	15.0b	12.3b
Dry weight (g)					
Foliage	LP	3.02a	4.94b	1.22c	1.76c
	ST	1.90d	4.29b	1.91d	1.97dc
Stem	LP	0.59a	1.15b	0.31a	0.30a
	ST	3.13c	4.44d	4.45d	4.37d
Root	LP	1.30a	1.59b	0.22a	0.28a
	ST	3.80b	4.28c	2.14d	2.11d
(b) Water potential (MPa)	LP	−1.05a	−1.35a	−1.40a	−1.15a
	ST	−0.93b	−0.85b	−0.73b	−0.77b
(c) Major element (mg/g)**					
N	LP	15.6	14.5	14.2	8.7
	ST	15.4	17.5	9.9	11.4
P	LP	1.7	2.3	0.9	1.2
	ST	1.0	1.6	1.0	1.7
K	LP	12.8	12.4	5.9	5.9
	ST	10.3	9.7	12.4	12.9
Ca	LP	2.3	2.0	0.9	0.9
	ST	5.4	5.1	6.5	5.3
Mg	LP	1.3	1.5	0.6	0.7
	ST	3.6	3.6	3.3	2.8
Minor elements (mg/g $\times 10^{-2}$)					
Zn	LP	5.5	6.0	4.1	3.8
	ST	2.1	3.5	3.1	2.2
Mn	LP	25.6	23.6	9.9	8.9
	ST	121.5	50.7	37.4	46.3
Fe	LP	4.9	5.3	17.9	16.9
	ST	4.2	4.9	6.2	4.8

Table 1 (continued)

(d) *Ratio* – Top/root of concentrations (F/D)

Na	LP	.34	.23	.87	1.01	3.2
	ST	.25	.11	.24	.22	1.3
Mn	LP	2.52	1.12	3.05	4.00	1.9
	ST	13.94	10.07	13.03	13.95	1.1
Zn	LP	.57	.59	1.30	1.00	2.0
	ST	.49	.46	.40	.37	0.8
Fe	LP	.14	.18	.34	.28	1.9
	ST	.31	.30	.07	.07	0.2

* The data was analyzed as a completely randomized design with a 2 × 2 × 2 factorial experiment. Mean values are statistically significant at 0.05 level only if they do not have a letter in common.

** Because of the small amount of pine material in the flooded treatments all plant material for each treatment was composited by component (foliage, stem and root) for nutrient analyses, so it was not possible to statistically analyze the data.

The concentration of most nutrients in the soil solution was much lower than in the soil. There was essentially no nitrogen in solution in any treatment and iron concentration in solution from the drained soil was only about one-half that in the soil. However, in the flooded pots, the iron concentration in solution averaged 2882 ppm as compared to 290 ppm in solution in the drained pots.

Nitrogen, sodium, and iron concentrations in the soil were not influenced by treatments but as expected phosphorus concentration was highest in the soil to which P was applied. There was less P, K, and Mg in the soil in which ST grew but this was attributed to greater uptake by the larger ST seedlings. Calcium and potassium concentrations were lower in soils from flooded than drained pots. Applied P resulted in lower concentrations of Mn in flooded soils whereas it tended to be higher in flooded soils without P.

Growth

Average height growth of ST and LP was 0.43 cm d^{-1} and 0.15 cm d^{-1} respectively (Table 1a). This rapid initial height growth of ST is apparently an inherent adaptative survival characteristic of hydrophytic tree species[3], which enables them to extend their foliage as far above the soil surface of the swamp as possible during dry periods. Seeds of swamp tree species do not germinate under water[3].

Flooding reduced the root length of both species but it had a more pronounced effect on LP roots (Table 1a). Water potential (Table 1b) was lower in ST than LP seedlings and applied P tended to decrease it in LP and increase it in ST in the flooded pots but had the reverse effect in drained pots (3 way interaction was significant). The lower stress for LP correlates with increased growth in the flooded treatment.

Applied P increased dry weight of both species in drained pots but only of LP in the flooded pots (Table 1a). LP weight was primarily in foliage whereas ST had a high percentage of weight in the stems. Even though P increased total dry weight of ST in the drained pots it had no more stem weight than in flooded pots. LP total dry weight responded slightly more to applied P in the drained than the flooded pots.

Anaerobic metabolism of roots

Excised roots of swamp tupelo produced more CO_2 (3.53 µl/mg ODW roots/h) than LP (2.14 l/mg ODW roots/h) and higher concentrations of ethanol and malate than LP from both drained and flooded pots (Fig. 1). ST roots from flooded pots produced slightly more ethanol and much more malate than roots from drained pots, but LP roots from flooded pots produced no detectable

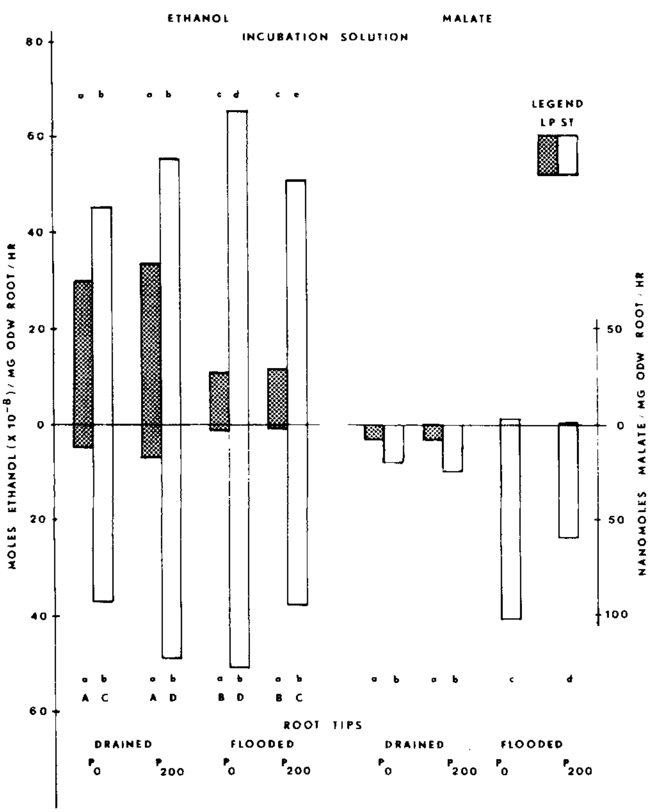

Fig. 1. Ethanol and malate concentrations in excised roots and incubation solution in relation to water and phosphorus treatments. Bars with similar letters were not significantly different at the 0.10 level; small letters refer to incubation solution and root tips; capital letters refer to total production.

malate. LP roots leaked about 85 percent of the total ethanol produced into solution whereas ST leaked only about 55 percent. Essentially no malate leaked into solution from either species. The relatively small increase of ethanol production in ST roots from flooded compared with drained pots and lack of malate production in LP roots from flooded treatment is attributed to age of the seedlings. It has been reported that between one month and one year of age, ethanol production in flooded ST roots decreased significantly and oxygen diffusion rate increased[10]. Based on the data presented here and in a companion paper[2], accumulation of end products of anaerobic metabolism by LP roots also appears to change with age. Roots incubated in the same manner as above from two year-old LP seedlings which were grown in flooded tanks (tanks were located outside hence plants and soil were exposed to natural environmental factors) and harvested in early spring (April 15), contained much higher concentrations of ethanol and malate than from well drained tanks under the same conditions. Also, under these semicontrolled conditions applied phosphorus was associated with increased ethanol and malate content in the incubated roots from flooded tanks. However, no influence of applied P on anaerobic metabolism was found in the experiment reported here with seedlings of approximately 4 months age, even though roots from applied P treatments had three times higher P concentrations than non-phosphorus treatments.

Nutrient status

The concentration of P, K, Ca, and Mg in LP foliage in the flooded pots was only about one-half that in the drained pots (Table 1c) whereas ST foliage contained much higher concentrations of Ca, Mg, and Mn than LP in all treatments. However, in comparing the relative concentration within seedlings (concentration top/root), it can be seen that LP appears to distribute relatively more Na, Mn, Zn, and Fe to the tops under soil flooding (cf F/D in Table 1d) than in drained soils. The LP also accumulated higher concentrations of Fe in the shoot under waterlogged than under drained conditions in contrast to ST (Fig. 2). ST oxidizes its rhizosphere in waterlogged soils[8] and presumably it oxidized the iron in the 'free space' of its roots, thereby preventing excessive uptake of this element, even though Fe concentration in the soil solution was 10 times higher in flooded than in drained pots. In the flooded treatments, the nutrients in ST probably entered the symplast and were transported to the top via active processes. However, in LP in the flooded pots several elements appear to be distributed more to the tops, *i.e.*, Na, Mn, Zn, and Fe (Table 1c), hence it appears that under anaerobiosis LP roots not only leak ethanol, a non ionized compound, to the external environment, but may also lose their discriminatory capacity to uptake and transfer to the transpirational stream. Roots of beans and peas (mesophytes) appear to lose their discriminatory capacity under anaerobiosis and lose both inorganic and organic ions[14,15].

The increased concentrations of several elements in the shoot of LP relative to

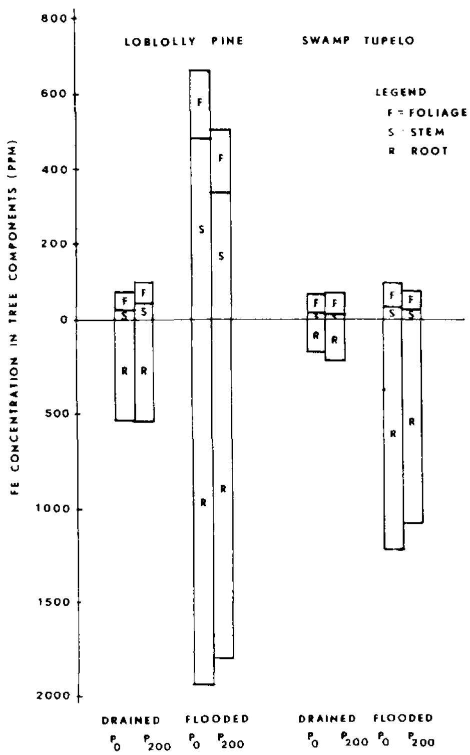

Fig. 2. Iron concentration in foliage (F), stems (S), and roots (R) of loblolly pine and swamp tupelo seedlings in relation to water and phosphorus treatments.

root in the waterlogged soils and not in ST indicates that the mesophyte under anaerobiosis stress may have incurred an ion imbalance between shoot and root and/or even toxic levels of some elements in the shoot.

Since application of P appeared to reduce the concentration of N, Zn, Mn, and Fe in the foliage of LP under waterlogging (Table 1b), its beneficial aspects may be related to lessening the apparent ion imbalance. Considering the improved nutrient relationships in the younger seedlings with applied P and the enhanced anaerobic metabolism of older seedlings [3], the response of LP to applied P may be related to improved energetics which may help maintain the normal structure of the root plasma membrane and thereby stabilize nutrient uptake.

In summary, application of P under drained conditions influenced both species positively but under flooding only LP benefited. It appears from this and companion research that P facilitates the adaption of loblolly to wet sites *via* improving root penetration, lowering water stress, stabilizing nutrient uptake, and improving energetics in older seedlings. Applied P had no noticeable beneficial effect on ST under flooding and may even have been detrimental. This is in contrast to the response reported for lowland rice mentioned previously [6].

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