https://doi.org/10.26525/jtfs2017.29.4.530540

INFLUENCE OF PHOSPHORUS LIMITATIONS ON THE GROWTH, NUTRIENT PARTITIONING AND PHYSIOLOGY OF BALSA (*OCHROMA PYRAMIDALE*) SEEDLINGS

CEBC Seabra[1], A Osiecka[2], PC Andersen[2], CAF Tucci[1], BFF Pereira[1] & PJ Minogue[2,] *

[1]Federal University of Amazonas, 69077-000, Minicampus, Coroado, Setor Sul, Manaus, AM, Brazil
[2]University of Florida, North Florida Research and Education Center, 155 Research Rd. Quincy, FL 32351, USA

**pminogue@ufl.edu*
Submitted December 2016; accepted June 2017

Effects of P fertilisation rates (0, 0.56, 5.6 and 56 mg L^{-1}) on the growth and physiology of balsa seedlings in soilless medium were examined. Phosphorus application rate significantly affected most biometric and physiologic variables, and the greatest response occurred with the increase from 5.6 to 56 mg L^{-1}. All growth response parameters were greatest at 56 mg L^{-1}. Foliar P concentration was within sufficiency range only at 56 mg L^{-1}; therefore a minimum sufficiency level was between 5.6 and 56 mg L^{-1} applied P. All foliar macro- and micronutrient concentrations were influenced by P supply, except Mn. Foliar P, Ca and Mg concentration increased with increasing P, and the concentration of most other nutrient decreased. Phosphorus, N and C content in each tissue increased with increasing P application rate due to dry mass increase. The highest concentrations of malic, succinic and lactic acids in xylem fluid occurred at the highest P rate. Physiological adaptations of balsa to P limitations included the preferential allocation of dry mass, P and N to plant roots, reduced specific leaf area, and increase in P-utilisation efficiency, root phosphatase activity and foliar boron concentration.

Keywords: Phosphorus utilisation, phosphorus fertilisation, growth parameters, nutrient-use efficiency, macronutrients, micronutrients, root phosphatase activity, organic acids, xylem fluid

INTRODUCTION

Balsa (*Ochroma pyamidale*) is a fast growing, early successional, tropical tree species characterised by very low wood density, large leaves, rapid leaf turnover, narrow crown and short life cycle (15 to 20 years) (Vleut et al. 2013). Balsa is a light-demanding species, and crown competition induces height growth; an increase in trunk diameter is promoted after attaining optimal height. Plants grown in infertile soils often have lower maximum growth rates and greater biomass allocation to roots than shoots compared with those grown in fertile soils (Grime 2002).

Vincent and Tanner (2013) showed positive response of balsa seedlings with increase in P fertilisation from 5.8 to 50 kg ha^{-1} under shade house conditions. Balsa seedlings actually acquired more P than was quantified in the soil by inductively coupled plasm (ICP) after nitric acid digestion, suggesting that balsa has very high P-acquisition efficiency (Vincent & Tanner 2013). Rapid growth rates, strong root system and sufficiently rigid stem are major goals of nursery production (Shono et al. 2007). There are several justifications for maximising P-utilisation efficiency in balsa. Superphosphate and other P sources are expensive in Brazil (Cella & Rossi 2010), mined fertiliser P sources will soon be depleted based on current rates of usage, and inefficient use of P can result in adverse ecological consequences (Edixhoven et al. 2014).

Irradiance level is often cited as the most limiting resource in tropical rain forests (Pearcy 2007), although there are often trade-offs between radiation-use efficiency and P-use efficiency (Gleason et al. 2011). In tropical forest ecosystems P availability is often more limiting than N (Vitousek 1984, Tanner et al. 1998). Holste et al. (2011) provided data on 522 tree species in Costa Rica and showed that irradiance level and soil NO_3^-, NH_4^+ and PO_4^- were significant predictors of growth for 52, 32, 34 and 29% of the species, respectively. Nutrient-use efficiency, defined as the ratio of biomass production to nutrient uptake, is an important determinant of plant species distribution in soils with varying fertility levels (Vitousek 1984, Hiremath et al. 2002).

© *Forest Research Institute Malaysia*

Plants regulate nutrient uptake by root epidermal cells, parenchyma cells and the casparian strip. Long-distance ion transport occurs predominantly via the non-living xylem vessels (Marschner 2012). Compounds (mainly organic acids) are exuded by root epidermal cells and facilitate P uptake from the soil (Marschner 2012). The flux of ions and organic acids in xylem fluid are interdependent (Ferguson & Turner 1981, Clark et al. 1986). However, there are little data on the influence of soil nutrient status on organic acid concentrations in the rhizosphere. Organic acids are especially important to the uptake, transport and accumulation of PO_4^{3-}.

Many tropical soils have low concentrations of bioavailable inorganic phosphate, but often contain considerable amounts of organic P compounds (Clinebell et al. 1995). Plants can use organic P efficiently after the enzymatic hydrolysis to inorganic phosphate (Tarafdar & Claassen 1988) by phosphatase enzymes that are released by roots and microorganisms in the rhizosphere (Das et al. 2014). There is little information concerning the effect of root phosphatase activity on the growth and physiology of tropical forest tree species (Kroehler & Linkins 1988, MacFall et al. 1991), and data on balsa could not be found.

The objective of this study was to examine the effect of soil P supply on the regulation of P utilisation by assessing growth, nutrient partitioning and physiology of balsa seedlings in the greenhouse. The variables that we examined included plant height, stem diameter, leaf area, leaf dry mass, stem dry mass, root dry mass, height/diameter ratio, specific leaf area, Dickson quality index, the partitioning of P, N and C into plant tissues, leaf macro- and micronutrient concentrations and contents, the concentrations of organic acids in xylem fluid, root phosphatase activity, P-use efficiency, N-use efficiency and P-acquisition efficiency.

MATERIALS AND METHODS

Plant material and growing conditions

Balsa fruits were collected in June 2012, 41 km north of Manaus, Amazon State, Brazil (2° 45' S, 60° 1' W) and air-dried. Following extraction, the seeds were treated with 1% sodium hypochlorite for 5 min and refrigerated till sowing in March 2013 at the North Florida Research and Education Center in Quincy, Florida, USA (30° 33' N, 84° 36' W). There were approximately 100,000 seeds per 1 kg, measuring 2 to 5 mm in length and 1 to 1.5 mm in width. Breaking dormancy was accomplished by soaking seeds in boiling water for 4 min, followed by room temperature water for 3 hours (Barbosa et al. 2004). The seeds were germinated in a growth chamber at 25 ± 2 °C, 60 ± 4% relative humidity, and 12-hour photoperiod in soilless germination medium (Table 1).

Table 1 Characteristics and phosphorus concentration of the soilless media used for germinating balsa seeds and growing seedlings, analysed by different methods

Method			Phosphorus concentration (mg L^{-1} medium)[1]			
			Germination medium		Growth medium	
Extraction/digestion	Analysis		Mean	SD	Mean	SD
Water	Colorimetry[2]	EPA 365.1	10.91	0.34	0.65	0.09
Water	ICP	EPA 200.7	12.77	0.19	1.32	0.17
Mehlich 1	ICP	EPA 200.7	29.10	1.63	2.73	0.14
Mehlich 3	ICP	EPA 200.7	43.11	3.08	3.15	0.06
			Composition and characteristics of medium			
Canadian peat moss			30%		40%	
Vermiculite			55%		45%	
Perlite			15%		15%	
pH			6.0		5.9	
EC (mS cm^{-1})					0.18	
Starter fertiliser			Yes		No	
Bulk density (g cm^{-3})					0.248	0.001

[1]Prior to P treatment application, [2]quantifies orthophosphates; ICP = inductively coupled argon plasma spectrophotometry, SD = standard deviation

Eight weeks following sowing, seedlings were transferred to the greenhouse and after two weeks of acclimation, transplanted into pots containing 3.8 L of soilless growth medium supplemented with phosphorus at four rates (see below). The medium was supplemented with N, K, Ca, Mg and micronutrient fertilisers at the same rates for all P treatments (Seabra et al. 2017). Soil moisture was maintain at approximately 70% field capacity. Ambient temperature varied between 21.0 and 29.9 °C at night, and 22.5 and 38.3 °C during the day. Relative humidity varied between 63 and 100% at night, and 35 and 100% during the day. Plants were grown under natural photoperiod.

Experimental design and treatment application

Four P fertilisation treatments (0, 0.56, 5.6 and 56 mg P L^{-1} growth medium) were compared in a randomised complete block design with six replications. Each P treatment was applied to growth medium in 10 pots per replication, before transplanting a single 10-week old seedling (4 to 7 cm high). The four P concentrations approximate 0, 1, 10 and 100 kg P ha^{-1} field fertilisation rates (assuming depth to 18 cm), intended to represent a range of P from deficiency to adequate supply. The P was applied in the form of finely-ground triple superphosphate (Tucci et al. 2011) and thoroughly mixed with the growth medium.

Seedling growth

At 11 weeks after treatment application the seedlings were measured for growth and destructively sampled for the concentration of organic acids in xylem fluid, root phosphatase activity, dry mass and nutritional status. Seedling growth was quantified by seedling height, stem diameter at 1 cm height, leaf area, leaf dry mass, stem dry mass and root dry mass. Leaf area was measured with a portable area meter on three randomly selected seedlings per block in each treatment. Leaves, stems, and roots were separated and washed by hand. Tissues were dried at 52 °C for 1 week and ground in a mill to pass a 2-mm screen. Seedling quality indices included height/diameter ratio, specific leaf area (leaf area/leaf dry matter) (Huante et al. 1995) and the Dickson quality index (Dickson et al. 1960).

Nutritional status

Concentrations of total P, total Kjeldahl N and organic C were determined in leaves, stems and roots. For P quantification, 0.5 g of oven-dried tissue was digested on a block digester, using 5 mL HNO_3 + $HClO_4$ (2:1 v/v). The P concentration in the extract was determined with a UV-Vis spectrophotometer at 660 nm wavelength. Nitrogen concentration was quantified by the Kjeldahl method (Bremner 1996). Carbon concentration was determined by the Walkley-Black method (Walkley & Black 1934). All analyses were performed according to procedures adapted by EMBRAPA (1999). Leaf N concentration was additionally determined by combustion method (Colombo & Giazzi 1982). Other macro- (P, K, Ca, Mg and S) and micronutrients (Fe, Mn, Zn, B and Cu) in leaves were analysed using an ICP spectrophotometer (Anonymous 1998).

Organic acids in the xylem fluid

Xylem fluid was collected with a pressure chamber apparatus (Scholander et al. 1965) between 10 a.m. and 12 p.m. for 30 s at 0.25 MPa greater than xylem tension (Andersen et al. 1993). Several seedlings per block were used to obtain 1.5 mL of sample. Samples were stored at -20 °C. The organic acids (oxalic, citric, tartaric, malic, succinic and lactic acids) were separated and detected by cation exchange chromatography (Andersen et al. 1993) using HPLC. Samples in 0.015 mM H_2SO_4 buffer were run isocratically through an Ion-300 polymeric column at 37 °C. Quantification was with a variable wavelength UV detector at a wavelength of 214 nm (Andersen et al. 1993).

P-use efficiency, N-use efficiency and P-acquisition efficiency

Phosphorus-use efficiency was calculated by dividing total seedling dry mass by total seedling P content. Nitrogen-use efficiency was calculated by dividing total seedling dry mass by total seedling Kjeldhal N content. Phosphorus-acquisition efficiency was calculated as total P content in the seedling divided by the sum of P amount supplied in the germination medium, growth medium and the triple super phosphate. Four P-acquisition efficiency values were calculated

© *Forest Research Institute Malaysia*

for each P fertilisation treatment using P concentrations in the media as determined by four different extraction/analysis protocols: water/colorimetric, water/ICP, Mehlich 1/ICP, Mehlich 3/ICP.

Root phosphatase activity

The method of Das et al. (2014) was used with modification to determine phosphatase (E.C. 3.1.3.2) activity of balsa roots. The roots were randomly selected from all 10 seedlings of each treatment within each block and divided into five replications for phosphatase determination. The method was modified as follows: excised 3 to 6 cm-long root fragments were incubated for 4 hours at 30 °C in para-nitrophenylphosphate, as a single organic P source, and sodium acetate-acetic acid buffer adjusted to a pH 4.5. Mineralised organic P was quantified by the analysis of inorganic P with a double beam UV-Vis spectrophotometer at 880 nm wavelength (Murphy & Riley 1962). Phosphatase activity was calculated as mg organic P per g of root dry mass.

Statistical analysis

Statistical analyses were performed with SAS 9.3 software (2007). Analysis of variance (ANOVA) for all dependent variables was conducted using a mixed model (proc mixed), with blocks treated as random effect, to determine the significance of the P application rate effect. Critical level of significance for ANOVA and least squares-means comparison with Tukey adjustment was $\alpha = 0.05$.

RESULTS AND DISCUSSION

Seedling growth and morphology

The range of P rates was chosen to investigate P regulation under conditions of P deficiency. There was strong positive response to increasing P supply for every measured variable of seedling growth and morphology (Table 2). The most dramatic increase in growth was observed between the 5.6 and 56 mg L^{-1} levels of applied P. There were differences between the 0 or 0.56 mg L^{-1} and the 5.6 mg L^{-1} rates for most growth parameters, but no differences occurred between 0 and 0.56 mg L^{-1} treatments. Increasing P rate from 5.6 to 56 mg L^{-1} resulted in a 6- to 12-fold increase in leaf area, leaf dry mass, stem dry mass, root dry mass, shoot dry mass and total seedling dry mass, as well as 1.8- to 4-fold increase in seedling height, stem diameter, and specific leaf area. The Dickson quality index was 20 times greater at 56 mg L^{-1} compared with the two lower P fertilisation rates.

Phosphorous limitations were associated with greater reduction in shoot than root dry mass. Lower shoot:root ratio may be a plant adaptation to utilise greater soil volume to obtain additional P (Crick & Grime 1987, Medina

Table 2 Balsa seedlings growth parameters 11 weeks after applying four rates of phosphorus (P)

Growth parameter	Unit	P application rate (mg L^{-1} soilless medium)				Rate effect
		0	0.56	5.6	56	p value
Seedling height (H)	cm	10.2 c	10.6 c	22.5 b	92.6 a	< 0.0001
Stem diameter (D)	mm	4.54 c	4.66 c	8.53 b	19.66 a	< 0.0001
Leaf area (LA)	cm^2	102 b	129 b	630 b	7297 a	< 0.0001
Leaf dry mass (LDM)	g	1.43 b	1.55 b	5.67 b	36.36 a	< 0.0001
Stem dry mass (SDM)	g	0.42 b	0.46 b	2.2 b	27.23 a	< 0.0001
Root dry mass (RDM)	g	0.66 c	0.75 c	2.78 b	16.64 a	< 0.0001
Shoot dry mass (ShDM)	g	1.84 c	2.02 c	7.87 b	63.59 a	< 0.0001
Total dry mass (TDM)	g	2.5 c	2.76 bc	10.65 b	80.22 a	< 0.0001
H/D ratio	cm mm^{-1}	22.5 b	22.87 b	26.44 b	47.09 a	< 0.0001
Specific leaf area (SLA)	$cm^2 g^{-1}$	67 c	83 c	110 b	202 a	< 0.0001
Dickson quality index		0.45 c	0.55 c	1.95 b	9.39 a	< 0.0001

Least squares-means within a row followed by the same letter are not significantly different at $\alpha = 0.05$ using Tukey's adjustment for multiple mean comparisons, $p \leq 0.05$ denotes significant effect; ShDM = LDM + SDM, TDM = ShDM + RDM, SLA = LA/LDM, Dickson quality index = TDM/(H/D + (ShDM/RDM))

& Cuevas 1989). There was a 2- and 3-fold reduction in plant height:stem diameter ratio and specific leaf area respectively in response to P deficiency. Leaf morphology can adapt to conditions of water or nutrient stress to optimise leaf functionality (Cramer et al. 2000). The proportion of dry matter in the stem doubled, and the concentration of carbon in the stem increased from 41 to 46% with increasing P application rate from 0 to 56 mg L^{-1}. The structural integrity of seedlings improved with increasing P supply, as evidenced by the 20-fold increase in Dickson quality index in the 56 mg L^{-1} treatment. In a companion study involving P limitations in mahogany (*Swietenia macrophylla*) seedlings, the Dickson quality index was less affected by fertilisation rate, but young mahogany seedlings had much slower initial growth rates (Seabra et al. 2017).

Seedling nutrition

The partitioning of total P, Kjeldahl N and C to leaves, stems and roots of balsa seedlings was assessed as a function of P supply (Table 3). Total P concentration increased and Kjeldhal N concentration decreased in all three tissues with increasing P application rate, while C concentration was much less affected. In all tissues significant changes in total P and Kjeldahl N concentrations resulted from increasing P application rate from 5.6 to 56 mg L^{-1}, but there were no differences between the three lower P rates, except for Kjeldahl N concentration. The effect of applied P on seedling contents of P, Kjeldahl N and C was magnified due to the great impact of P supply on plant dry mass. Thus, there was a 17-, 5- and 8-fold increases in the seedling content of P, Kjeldahl N and C respectively for seedlings in the 56 compared to the 5.6 mg L^{-1} treatment.

Leaf nutrient concentrations were examined in greater detail (Table 4). The results for leaf total P and total N (by combustion) concentrations confirmed the same trends as those results reported in Table 3. At the highest P application rate, there was a 3-fold increase in total P concentration, whereas total N concentration was reduced to 1.6% compared with 1.8 to 1.9% for the rest of the treatments. The greatest differences in nutrient concentrations usually occurred between the 5.6 and 56 mg L^{-1} treatments. Foliar concentrations of K, B, Zn and Cu decreased with increasing P application rate, Ca and Mg tended to increase, S and Fe did not exhibit a clear pattern, and Mn was not significantly affected. Total foliar content of all macro- and micronutrients increased with increasing P application rate due to increases in leaf dry mass (Table 2).

Table 3 Total phosphorus (P_t), total Kjeldahl nitrogen (TKN), and total carbon (C) concentration and content in leaf, stem, and root tissue of balsa seedlings 11 weeks after applying four rates of phosphorus (P)

Nutrient	Tissue	P application rate (mg L^{-1} medium)				Rate effect	P application rate (mg L^{-1} medium)				Rate effect
		0	0.56	5.6	56	p value	0	0.56	5.6	56	p value
		Nutrient concentration (%)					Nutrient content (mg $seedling^{-1}$)				
P_t	Leaf	0.07 b	0.07 b	0.07 b	0.19 a	< 0.0001	0.96 b	1.02 b	4.03 b	68.59 a	< 0.0001
P_t	Stem	0.04 b	0.04 b	0.04 b	0.10 a	< 0.0001	0.17 b	0.18 b	0.93 b	26.79 a	< 0.0001
P_t	Root	0.06 b	0.06 b	0.06 b	0.11 a	< 0.0001	0.43 b	0.48 b	1.79 b	18.14 a	< 0.0001
P_t	Seedling total						1.55 b	1.69 b	6.75 b	113.52 a	< 0.0001
TKN	Leaf	2.02 a	1.99 a	2.01 a	1.76 b	0.0016	28.8 b	30.8 b	114.5 b	639.7 a	< 0.0001
TKN	Stem	1.84 a	1.86 a	1.49 b	0.61 c	< 0.0001	7.7 c	8.6 c	32.1 b	164.0 a	< 0.0001
TKN	Root	1.82 a	1.78 a	1.67 a	0.93 b	< 0.0001	12.0 c	13.3 c	46.8 b	154.6 a	< 0.0001
TKN	Seedling total						48.3 c	52.7 c	193.4 b	958.2 a	< 0.0001
C	Leaf	43.5 a	44.7 a	45.1 a	46.1 a	0.4584	618 c	693 c	2,565 b	16,716 a	< 0.0001
C	Stem	40.9 c	40.8 c	43.0 b	46.2 a	< 0.0001	171 b	189 b	945 b	12,602 a	< 0.0001
C	Root	42.9 a	41.7 a	41.6 a	42.3 a	0.2296	282 c	311 c	1157 b	7,025 a	< 0.0001

$p \leq 0.05$ denotes significant effect, least squares-means within a row followed by the same letter (for concentration or content) are not significantly different at $\alpha = 0.05$ using Tukey's adjustment for multiple mean comparisons

Table 4 Nutrient concentration and content in the leaves of balsa seedlings 11 weeks after applying four rates of phosphorus

Nutrient	Phosphorous application rate (mg L^{-1} medium)				Rate effect	Phosphorous application rate (mg L^{-1} medium)				Rate effect
	0	0.56	5.6	56	p value	0	0.56	5.6	56	p value
	Nutrient concentration (%)					Nutrient content (mg $seedling^{-1}$)				
P	0.06 b	0.06 b	0.06 b	0.18 a	< 0.0001	0.80 b	0.90 b	3.51 b	65.47 a	< 0.0001
N	1.93 a	1.78 ab	1.87 a	1.61 b	0.0098	27.4 b	27.5 b	106.6 b	585.0 a	< 0.0001
K	1.48 a	1.52 a	1.31 b	1.15 c	< 0.0001	21.1 c	23.7 c	74.0 b	413.4 a	< 0.0001
Mg	0.92 b	0.96 ab	0.85 c	1.00 a	< 0.0001	13.0 b	14.8 b	48.3 b	363.2 a	< 0.0001
Ca	1.55 b	1.59 ab	1.47 b	1.70 a	0.0026	22.1 b	24.6 b	83.7 b	615.2 a	< 0.0001
S	0.19 a	0.17 b	0.16 ab	0.17 ab	0.0052	2.66 b	2.61 b	8.89 b	62.35 a	< 0.0001
	Nutrient concentration (mg kg^{-1})									
B	59.2 a	61.7 a	45.2 b	22.2 c	< 0.0001	0.08 c	0.10 c	0.26 b	0.80 a	< 0.0001
Zn	59.3 a	59.5 a	61.8 a	31.5 b	< 0.0001	0.08 c	0.09 c	0.35 b	0.05 a	< 0.0001
Mn	88.7 a	88.3 a	90.0 a	85.0 a	0.8801	0.12 b	0.14 b	0.52 b	3.05 a	< 0.0001
Fe	426 a	399 a	231 a	248 a	0.0408	0.62 b	0.63 b	1.31 b	9.18 a	< 0.0001
Cu	8.83 a	8.67 a	6.83 ab	5.17 b	0.0002	0.01 b	0.01 b	0.04 b	0.19 a	< 0.0001

$p \leq 0.05$ denotes significant effect, least squares-means within a row followed by the same letter for concentration or content are not significantly different at $\alpha = 0.05$ using Tukey's adjustment for multiple mean comparisons

Foliar analysis is the preferred method to diagnose nutrient deficiencies; however, we did not observe a dark green or purple colour of older leaves, which is symptomatic of P deficiency (Bryson et al. 2014). Phosphorus at 0, 0.56 and 5.6 mg L^{-1} represented P deficiency levels, and 56 mg L^{-1} was a sufficiency level based on foliar P concentrations, which were 0.06–0.07% for the 0 to 5.6 mg L^{-1} rates, and 0.18–0.19% for the 56 mg L^{-1} treatment. Foliar P concentrations of 0.14 to 0.20% have been reported for balsa grown in perlite, vermiculite and sand (Dalling et al. 2013). Foliar P concentration for the 56 mg L^{-1} P treatment is within the sufficiency range of many broad-leaved forest tree species (Bryson et al. 2014).

The availability of P is often more limiting than that of N in the soils of many tropical ecosystems (Tanner et al. 1998, Lawrence 2003, Hedin et al. 2009). In the current study, increasing P application rate was associated with decreasing N concentration in all plant tissues and a consistent 60% increase in N-use efficiency. Foliar total N concentrations of 1.8 to 1.9% for the 0, 0.56 and 5.6 mg L^{-1} treatments would be in the low end of normal range for many tree species (Bryson et al. 2014). Differences in total quantity of nutrients partitioned to various tissues were magnified by concomitant changes in dry mass. Total P content in the leaves, stems, roots and the whole seedling increased 71-, 158-, 42- and 73-fold respectively with increase in P fertilisation rate from 0 to 56 mg L^{-1}, and 17-, 29-, 10- and 17-fold with an increase from the 5.6 to 56 mg L^{-1} treatment respectively.

Highest P application rate decreased foliar concentrations of N, K, B, Zn and Cu, increased concentrations of Ca and Mg, and had no effect on S, Fe and Mn. The decrease in N, K, B and Zn concentrations at the 56 mg L^{-1} P treatment is a result of nutrient dilution due to great increases in plant growth. High levels of P in potting medium can cause Cu and Zn deficiencies (Bryson et al. 2014). It is also known that B has positive impact on P absorption (Marschner 2012), and we suggest that greater foliar B concentrations at low levels of P supply may reflect the plant adaptation to P deficiency. Besides P, the greatest increase in elemental concentration with increasing P supply was observed for Ca, which may be due, in part, to the application of triple superphosphate which contains 15% Ca.

Organic acids in xylem fluid

Accurate quantification of organic acids in the rhizosphere is not possible, although organic acids in xylem fluid are easily quantified. Xylem fluid is dilute and mainly consists of monomeric compounds (amino acids and organic acids) and inorganic ions (Ferguson & Turner 1981, Clark et al. 1986, Andersen et al. 1993, 1995). The concentrations of organic acids in xylem fluid were significantly influenced by P treatment, although the response was not consistent across the range of P rates (Table 5). For example, the lowest concentrations of organic acids were recorded at 5.6 and the highest at 56 mg L^{-1} P treatment. Citric, malic and succinic acids were predominant in all treatments, which is consistent with the organic acid profiles of many plant species (Clark et al. 1986, Andersen et al. 1993, 1995). Oxalic and tartaric were minor organic acids in all treatments.

The flux of organic acids and inorganic ions to xylem vessels are interdependent (Ferguson & Turner 1981, Clark et al. 1986). The highest concentration of organic acids occurred at 56 mg L^{-1} P rate, except for citric acid, which was numerically higher at the two lower P rates. Phosphorus uptake from the soil can be enhanced by organic acids exuded from plant roots (Marschner 2012), and citric acid is the most efficient organic acid at solubilising P in the rhizosphere (Hinsinger 2001). The observed trend of greater citric acid concentration under deficient P supply may be an adaptation to promote P uptake. We cannot explain why organic acid concentrations were lowest at the 5.6 mg L^{-1} rate; however, this may not be an artefact since both leaf Ca and Mg concentration were also lowest for this treatment. Both organic acids and PO_4^{-3} can form ionic bonds with Ca, Mg, Zn or Mn (Ferguson & Turner 1981, Clark et al. 1986). Clark et al. (1986) calculated that 35% of the Ca and 56% of the Zn in xylem fluid of kiwi (*Actinidia deliciosa*) was translocated in the form of malate complexes. Thus, perhaps organic acids and PO_4^{-3} were competing for the binding sites of divalent cations.

P-use efficiency, N-use efficiency, P-acquisition efficiency and root phosphatase activity

There was great increase in balsa seedling dry mass and total P content as P supply increased (Table 6). Both P-use efficiency and N-use efficiency were similar for the three lower P rates. However, P-use efficiency was reduced and N-use efficiency was increased at the high P fertilisation rate. Although seedling dry mass increased over 7-fold with an increase in P fertilisation rate from 5.6 to 56 mg L^{-1}, the corresponding increase in total P content was 17-fold. Consequently, P-use efficiency for seedlings at the 56 mg L^{-1} rate was reduced by more than 50% compared with the rest of the treatments. There was more than 50% increase in N-use efficiency in the 56 mg L^{-1} treatment, despite the same amount of N supplied to all treatments.

Table 5 Organic acid concentration in xylem fluid of balsa seedlings 11 weeks after applying four rates of phosphorus

Organic acid	Phosphorous application rate (mg L^{-1} medium)				Rate effect p value
	0	0.56	5.6	56	
	Acid concentration (μM)				
Oxalic	7 a	7 a	4 a	7 a	0.0365
Citric	312 a	361 a	140 b	233 ab	0.0037
Tartaric	3 a	3 a	1 b	1 b	0.0056
Malic	541 ab	540 ab	371 b	828 a	0.0428
Succinic	197 ab	188 ab	123 b	256 a	0.0161
Lactic	79 b	71 b	26 b	275 a	0.0001
Total organic acids	1139 ab	1169 ab	667 b	1599 a	0.0123

$p \leq 0.05$ denotes significant effect, least squares-means within a row followed by the same letter are not significantly different at $\alpha = 0.05$ using Tukey's adjustment for multiple mean comparisons

Table 6 Total seedling dry mass, total phosphorus (P_t) and total Kjeldahl nitrogen (TKN) content; P-use efficiency (PUE); nitrogen-use efficiency (NUE); P-acquisition efficiency (PAE) and root phosphatase activity (RPA) in balsa seedlings 11 weeks after applying four rates of P to soilless growth medium

Variable			Unit	Phosphorous application rate (mg L^{-1} medium)				Rate effect
				0	0.56	5.6	56	p value
Total seedling dry mass			g $seedling^{-1}$	2.50 c	2.76 bc	10.65 b	80.22 d	< 0.0001
Total P seedling content			mg $seedling^{-1}$	1.55 b	1.69 b	6.75 b	113.52 a	< 0.0001
TKN seedling content			mg $seedling^{-1}$	48.34 c	52.73 c	193.40 b	958.20 a	< 0.0001
PUE			g mg^{-1}	1.61 a	1.64 a	1.58 a	0.71 b	< 0.0001
NUE			g mg^{-1}	0.052 b	0.052 b	0.055 b	0.084 a	< 0.0001
	Method for P in soilless medium							
	Extraction	Analysis						
PAE	Water	Colorimetry[1]	mg mg^{-1}	0.593 a	0.355 c	0.283 d	0.527 b	< 0.0001
PAE	Water	ICP	mg mg^{-1}	0.301 b	0.231 c	0.255 bc	0.521 a	< 0.0001
PAE	Mehlich 1	ICP	mg mg^{-1}	0.144 c	0.131 c	0.211 b	0.508 a	< 0.0001
PAE	Mehlich 3	ICP	mg mg^{-1}	0.124 c	0.115 c	0.200 b	0.504 a	< 0.0001
RPA			mg g^{-1}	4.40 a	4.32 a	3.29 b	2.87 b	< 0.0001

Least squares-means within a row followed by the same letter are not significantly different at $\alpha = 0.05$ (except for RPA $\alpha = 0.1$) using Tukey's adjustment for multiple mean comparisons, $p \leq 0.05$ denotes significant effect; [1]quantifies orthophosphates

Phosphorous-acquisition efficiency depended on the P extraction and analysis protocol followed. Baseline P concentrations in the soilless growth medium before P additions were 0.65, 1.32, 2.73, and 3.15 mg L^{-1} for the water/colorimetry, water/ICP, Mehlich 1/ICP and Mehlich 3/ICP protocols respectively (Table 1). There was a decline in P-acquisition efficiency with the protocols that released more P from the medium. For example, seedlings receiving no P addition acquired 59, 30, 14 and 12% of the extractable P contained in the pot, based on the water/colorimetry, water/ICP, Mehlich 1/ICP and Mehlich 3/ICP protocols respectively. When superphosphate was supplied to the medium, the calculated values varied less between extraction/analysis methods, i.e. from 20 to 28% for the 5.6 mg L^{-1} treatment and 50 to 52% for the 56 mg L^{-1} treatment. There was a stepwise decrease in root phosphatase activity with increasing P rate, although the values for the 0 and 0.56 mg L^{-1} treatments or the 5.6 and 56 mg L^{-1} treatments were not significantly different. As P rate increased from 0 to 56 mg L^{-1} total seedling dry matter increased 32-fold, but total P content increased 73-fold, thus reducing P-use efficiency by more than 50%. In contrast, Kjeldahl N content increased 20-fold, considerably less than the increase in dry matter, and N-use efficiency increased by 60%.

Phosphorous-acquisition efficiency is also an important determinant of P efficiency for many crop species (Wang et al. 2010, Penn et al. 2015). For any given P rate, calculated P-acquisition efficiency values decreased with more complete P extractions from the growth medium. This was especially true in the 0 mg L^{-1} P fertilisation treatment where the Mehlich 3/ICP protocol resulted in 5-fold greater P concentration, and a 5-fold smaller P-use efficiency than with water extraction and colorimetric procedure. In contrast, most of the P in the medium supplemented with 56 mg L^{-1} P was in the form of water-extractable orthophosphates, and therefore, the values for P concentration in the medium and for P acquisition efficiency did not differ substantially between extraction/analysis methods. Balsa seedlings were highly efficient in extracting P at the highest P application rate, taking up over 50% of the P supplied in the pot. These results are in sharp contrast with mahogany which showed a reduction in P-acquisition efficiency with increasing P fertilisation rate (Seabra et al. 2017). For balsa, increased P supply resulted in a much greater biomass increase (and consequently seedling P

content) than in mahogany, while the increase in extracted medium P content was the same for the two species. In addition, P fertilisation greatly stimulated balsa seedling root growth, as evidenced by the 25-fold increase in root dry matter, improving the ability to uptake P from greater soil volume.

An increase in root phosphatase activity was an adaptation of balsa seedlings to limited P supply. In the companion study on mahogany, root phosphatase activity was not influenced by P fertilisation rate (Seabra et al. 2017). Tropical soils often contain low concentrations of bioavailable inorganic phosphate but higher concentrations of organic P compounds (Clinebell et al. 1995). Root phosphatase performs the enzymatic hydrolysis of organic phosphate (Tarafdar & Claassen 1988, Das et al. 2014). Balsa is a pioneer tree species with high demand for P and is adapted to rapid growth when conditions of light and soil fertility are favourable (Holste et al. 2011). More work is needed to identify the role of root phosphatase activity in balsa under conditions of P deficiency, particularly in natural habitats.

In conclusion, balsa exhibited several adaptations to P limitations. Root growth was curtailed to a lesser degree than shoot growth, as evidenced by increased percentage of root dry matter, and a greater percentage of total P and N partitioned to root compared with shoot. P-use efficiency increased more than 2-fold when P was not added to the media. Foliar B concentration increased by about 2-fold with extreme P deficiency, and B may promote P absorption by roots (Marschner 2012). The observed increase in root phosphatase activity under P limitations may be an adaptation by roots to utilise organic P which is abundant, but not readily available in many tropical soils. Since native soils have a mixture of inorganic P, organic P, mycorrhizae, bacteria, fungi and other soil organisms (Vitousek 1984, Smith & Smith 2011, Dalling et al. 2013, Vincent & Tanner 2013), additional research is needed to examine the influence of soil P dynamics on the growth and physiology of balsa in plantations and in native habitats.

ACKNOWLEDGEMENTS

The authors thank LGP Seabra, ST Wright, C Bliss, ED Chevasco, NB Comerford, S George and C Mackowiak for assistance in conducting the study; EMBRAPA for help with tissue analyses; and B Brodbeck for help with statistical analysis.

REFERENCES

Andersen PC, Brodbeck BV & Mizell RF III. 1993. Diurnal variations of amino acids and organic acids in xylem fluid from *Lagerstroemia indica*: an endogenous circadian rhythm. *Physiologia Plantarum* 89: 783–790. https://doi.org/10.1111/j.1399-3054.1993.tb05285.x.

Andersen PC, Brodbeck BV & Mizell RF III. 1995. Water stress- and nutrient solution-mediated changes in water relations and amino acids, organics acids, and sugars in xylem fluid of *Prunus salicina* and *Lagertroemia indica*. *Journal of the American Society for Horticultural Science* 120: 36–42.

Anonymous. 1998. AOAC Official Method 985.01. In *Official Methods of Analysis of AOAC International*. 16th edition AOAC International, Gaithersburg.

Barbosa AP, Sampaio P De TB, Campos MAA et al. 2004. Tecnologia alternativa para a quebra de dormência das sementes de pau-de-balsa (*Ochroma lagopus* Sw., *Bombacaceae*). *Acta Amazonica* 34: 107–110. http://dx.doi.org/10.1590/S0044-59672004000100013.

Bremner JM. 1996. Nitrogen—total. Pp 1085–1121 in Sparks DL et al. (eds) *Methods of Soil Analysis. Part 3. Chemical Methods*. Soil Science Society of America, Madison.

Bryson GM, Mills HA, Sasseville DN, Jones JB JR & Barker AV. 2014. *Plant Analysis Handbook III: A Guide to Sampling, Preparation, Analysis and Interpretation for Agronomic and Horticultural Crops*. Micro-Macro Publishing Inc., Athens.

Cella D & Rossi MCL. 2010. Análise de mercado de fertilizantes no Brasil. *Revista Interface Tecnológica* 7: 1–10.

Clark CJ, Holland PT & Smith GS. 1986. Chemical composition of bleeding sap from kiwifruit vines. *Annals of Botany* 58: 353–362. https://doi.org/10.1093/oxfordjournals.aob.a087213.

Clinebell RR, Phillips OL, Gentry AH, Stark N & Zuuring H. 1995. Prediction of neotropical tree and liana species richness from soil and climatic data. *Biodiversity and Conservation* 4: 56–90. https://doi.org/10.1007/BF00115314.

Colombo B & Giazzi G. 1982. Total automatic nitrogen determination. *American Laboratory* 14: 38–45.

Cramer J, Fahey TJ & Battles JJ. 2000. Patterns of leaf mass, area and nitrogen in young northern hardwood forests. *American Midland Naturalist* 144: 253–264. https://doi.org/10.1674/0003-0031(2000)144[0253:POLMAA]2.0.CO;2.

Crick JC & Grime JP. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasting ecology. *New Phytollogist* 107: 403–414. https://doi.org/10.1111/j.1469-8137.1987.tb00192.x.

Dalling JW, Winter K, Andersen KM & Turner BL. 2013. Artefacts of the pot environment on soil nutrient availability: implications for the interpretation of ecological studies. *Plant Ecology* 214: 329–338. https://doi.org/10.1007/s11258-013-0172-3.

Das J, Comerford N, Wright D, Marois J & Mackowiak C. 2014. Development of a phosphatase activity assay using excised plant roots. *Soil Research* 52: 193–202. https://doi.org/10.1071/SR13198.

Dickson A, Leaf AL & Hosner JF. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forestry Chronicle* 36: 10–13.

Edixhoven JD, Gupta J & Savennije HHG. 2014. Recent revisions of phosphate rock reserves and resources: a critique. *Earth System Dynamics* 5: 491-507. https://doi.org/10.5194/esd-5-491-2014.

EMBRAPA (Empresa Brasileira De Pesquisa Agropecuária). 1999. *Manual de análises químicas de solos*. EMBRAPA, Brasília.

Ferguson IE & Turner NA. 1981. Mobilization of nutrients in cuttings of kiwifruit (*Actinidia chinensis* Planch). *Annals of Botany* 47: 229–237. https://doi.org/10.1093/oxfordjournals.aob.a086011.

Gleason SM, Read J & Ares A. 2011. Biomass allocation and phosphorus economics of rain-forest seedlings: effects of fertilisation and radiation on soil specialists and soil generalists. *Journal of Tropical Ecology* 27: 147–161. https://doi.org/10.1017/S0266467410000660.

Grime JP. 2002. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*. John Wiley & Sons, Chichester.

Hedin LO, Brookshire NJ, Menge NL & Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 40: 613–635. https://doi.org/10.1146/annurev.ecolsys.37.091305.110246

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. *Plant and Soil* 237: 173–195. https://doi.org/10.1023/A:1013351617532.

Hiremath AJ, Ewel JJ & Cole TG. 2002. Nutrient use efficiency in three fast-growing tropical trees. *Forest Science* 48: 662–672.

Holste EK, Kobe RK & Vriesendorp CF. 2011. Seedling growth responses to soil resources in the understory of a wet tropical forest. *Ecology* 92: 1828–1838. https://doi.org/10.1890/10-1697.1.

Huante P, Rincon E & Acosta I. 1995. Nutrient availability and growth rate of 34 woody species from a tropical deciduous Forest in Mexico. *Functional Ecology* 9: 849–858. https://doi.org/10.2307/2389982.

Kroehler CJ & Linkins AE. 1988. The root suface phosphatases of *Eriophorum vaginatum*: effects of temperature, pH, substrate concentration on inorganic phosphorus. *Plant and Soil* 105: 3–10. https://doi.org/10.1007/BF02371136.

Lawrence D. 2003. The response of tropical tree seedlings to nutrient supply: meta-analysis for understanding a changing tropical landscape. *Journal of Tropical Ecology* 19: 239–250. https://doi.org/10.1017/S0266467403003274.

MacFall J, Slack SA & Iyer J. 1991. Effects of *Hebeloma arenosa* and phosphorus fertility on roots acid phosphatase activity of red pine (*Pinus resinosa*) seedlings. *Canadian Journal of Botany* 69: 380–393. https://doi.org/10.1139/b91-051.

Marschner P. 2012. Long-distance transport in the xylem and phloem. Pp 49–70 in Marschner P (ed) *Marschner's Mineral Nutrition of Higher Plants*. Academic Press, London.

Medina E & Cuevas E. 1989. Patterns of nutrient accumulation and release in Amazonian forests of the upper Rio Negro basin. Pp 217–240 in Proctor J (ed) *Mineral Nutrients in Tropical Forest and Savanna Ecosystems*. Blackwell Scientific, Oxford.

Murphy J & Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Pearcy RW. 2007. Responses of plants to heterogeneous light environments. Pp 213–258 in Pugnaire FJ & Valladares F (eds) *Functional Plant Ecology*. CRC Press, Boca Raton.

Penn CJ, Bell PR, Carver B, Arnall DB & Klatt A. 2015. Comparison of phosphorus use efficiency among various wheat accessions grown in acid and calcarous soils. *Journal of Plant Nutrition* 38: 2279–2293. http://dx.doi.org/10.1080/01904167.2015.1009103.

Scholander PF, Bradstreet ED, Hemmingsen EA & Hammel HT. 1965. Sap pressure in vascular plants. *Science* 148: 339–346. https://doi.org/10.1126/science.149.3687.920.

Seabra CEBC, Osiecka A, Tucci CAF, Minogue PJ, Pereira BFF & Andersen PC. 2017. Influence of phosphorus limitations on the growth, nutrient partitioning, and physiology of mahogany (*Swietenia macrophylla* King) seedlings. *Journal of Plant Nutrition*. https://doi.org.10.1080/01904167.2017.1385803.

Shono S, Davies SJ, & Chua YK. 2007. Performance of 45 native tree species on degraded lands in Singapore. *Journal of Tropical Forest Science* 19: 23–34.

Smith SE & Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth:new paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology* 62: 227–250. https://doi.org/10.1104/pp.111.174581.

Tanner EVJ, Vitousek PM & Cuevas E. 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* 79: 10–22. https://doi.org/10.1890/0012-9658(1998)079[0010:EIONLO]2.0.CO;2.

Tarafdar JC & Claassen N. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils* 5: 308–312. https://doi.org/10.1007/BF00262137.

Tucci CAF, Santos JZL, Júnior CHS, Souza PA, Batista IMP & Venturin N. 2011. Desenvolvimento de mudas de *Swietenia macrophylla* em resposta a nitrogênio, fósforo e potássio. *Floresta* 41: 471–490. http://dx.doi.org/10.5380/rf.v41i3.24039.

Vincent AG & Tanner EVJ. 2013. Major litterfall manipulation affects seedling growth and nutrient status in one of two species in a lowland forest in Panama. *Journal of Tropical Ecology* 29: 449–454. https://doi.org/10.1017/S0266467413000424.

Vitousek PM. 1984. Litterfall, nutrient cycling and nutrient limitation in tropical forests. *Ecology* 65: 285–298. https://doi.org/10.2307/1939481.

Vleut I, Levy-Tacher SI, Boer WF de, Galindo-Gonzalez J & Ramirez-Marcial N. 2013. Can a fast growing early-successional tree (*Ochroma pyramidale*, Malvaceae) accelerate forest succession? *Journal of Tropical Ecology* 29: 173–180. https://doi.org/10.1017/S0266467413000126.

Walkley A & Black IA. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37: 29–38. https://doi.org/10.1097/00010694-193401000-00003.

Wang X, Shen J & Liao H. 2010. Acquisition or utilisation, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Science* 179: 302–306. https://doi.org/10.1016/j.plantsci.2010.06.007.