pH Dominates *Leucadendron* 'Safari Sunset' Growth

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Abstract. The objectives of the present research were to study the effects of pH, $\text{NH}_4\text{:NO}_3$ ratio, and P concentration in the nutrient solution on development of *Leucadendron* R. Br. 'Safari Sunset' [*L. salignum* Bergius x *L. laureolum* (Lam.) Fourc.]. The experiment was conducted in aero-hydroponic systems and involved six treatments in a nonfactorial design: two pH levels (5.5 and 7.5), two P levels (7 and 20 $\text{mg}\cdot\text{L}^{-1}$), and two $\text{NH}_4\text{:NO}_3$ ratios (60:40 and 25:75). The pH of the root environment was the most important factor controlling growth. Root cells were longer in plants grown at pH 5.5 than at pH 7.5, but width was not affected. Altering the $\text{NH}_4\text{:NO}_3$ ratio did not affect development regardless of pH. Increasing the P concentration from 7 to 20 $\text{mg}\cdot\text{L}^{-1}$ significantly decreased root fresh weight at the low pH and slightly reduced shoot growth. Nitrogen, P, K, Zn, and Mn concentrations were higher, while that of Fe was lower in plants grown at low pH. Reducing the $\text{NH}_4\text{:NO}_3$ ratio did not affect N concentration but increased P and K concentrations in the shoots. Increasing the P concentration significantly raised the P content of shoot and root tissues but reduced the content of Fe, Zn, and Mn.

The Proteaceae family originated in Australia and South Africa, where most species grow on leached, acidic soils, which are poor in available minerals. Growth reduction and leaf necrosis or chlorosis are generally attributed to phosphorus toxicity (Buining and Cresswell, 1993; Nichols et al., 1979). However, little information is available on the nutritional requirement of plants of this family (Parks et al., 1996).

In the last decade, an effort has been made in Israel to cultivate Proteaceae species (proteas) for cut flowers (Ben-Jaacov, 1986; Ben-Jaacov et al., 1989). Despite the suitable climate, Israeli protea growers have encountered problems because of unfavorable soil characteristics, such as high pH and high free lime content. To avoid soil limitations *L. 'Safari Sunset'*, the main commercially cultivated protea produced in Israel, is grown in tuff, a volcanic pyroclastic material characterized by high porosity (0.6 $\text{L}\cdot\text{L}^{-1}$) and high saturated hydraulic conductivity (Wallach et al., 1992), or is grafted on lime-resistant rootstocks (Ben-Jaacov et al., 1992).

Growing plants on an artificial substrate and using modern irrigation and fertilization equipment can provide appropriate conditions for plant growth, including control of the pH in the rhizosphere. The pH in the root environment can affect plant growth through two major mechanisms: 1) a direct effect on cell

elongation and root hair growth (Taiz, 1984; Tang et al., 1992; White, 1990); and 2) indirect effects through nutrient availability and ion uptake by plants (Marschner, 1995). Rhizosphere pH is affected by the cation and anion uptake ratio (Marschner, 1995) in the nutrient solution, and by microbial activity (mainly nitrification and denitrification). Altering the nitrogen source, e.g., the $\text{NH}_4\text{:NO}_3$ ratio, can also influence the pH in the rhizosphere.

In a previous study, Silber et al. (1998) found that nutritional treatments affected the growth of *L. 'Safari Sunset'* planted in pots filled with tuff. However, since N levels (at fixed $\text{NH}_4\text{:NO}_3$ ratio) or the $\text{NH}_4\text{:NO}_3$ ratios (at fixed N level) affected the rhizosphere pH as well, distinguishing between the main treatment effects (NPK levels and $\text{NH}_4\text{:NO}_3$ ratio) and that of pH changes was not feasible. Differentiating between these two effects is only possible by using a system such as the aero-hydroponic system.

The objectives of this study were to determine the effects of nutrient solution pH, $\text{NH}_4\text{:NO}_3$ ratio and P concentration on nutrient uptake and growth of *L. 'Safari Sunset'*, grown in an aero-hydroponic system.

Materials and Methods

The experiment was conducted in a screen house (10% shade) in Bet Dagan, Israel (35°E, 31°N, 50 m altitude), irradiated by natural sunlight at a temperature range between 12 and 35 °C. Two-month-old *L. 'Safari Sunset'* plants were transplanted into an aero-hydroponic system (Feigin et al., 1984). Each plot consisted of 12 plants placed in two separate polystyrene boxes mounted on a 140-L, covered container. Roots were continuously

exposed to the nutrient solution, which was spread on the roots by means of a plastic tube system. The solution was collected in the bottom of the container and continuously recirculated. The experiment included six treatments (Table 1) with two pH levels (5.5 and 7.5), two P levels (0.23 and 0.65 $\text{mmol}\cdot\text{L}^{-1}$) and two $\text{NH}_4\text{:NO}_3$ ratios (60:40 and 25:75).

The treatments were chosen to reflect different conditions that take place in the rhizosphere of *L. 'Safari Sunset'* because of changing NPK levels or $\text{NH}_4\text{:NO}_3$ ratio (Silber et al., 1998). Potassium concentration in the nutrient solution of all the treatments was 1.3 $\text{mmol}\cdot\text{L}^{-1}$. The solutions were prepared with commercial fertilizers [KNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , KCl, KH_2PO_4], and typical tap water, as used in production greenhouses in Israel. The tap water contained ($\text{mmol}\cdot\text{L}^{-1}$): $\text{NO}_3\text{-N}$ –0.7, P–0.01, K–0.15, Ca–1.25, Mg–0.8, Na–4.3, and Cl–3.9. Microelement concentrations were ($\mu\text{mol}\cdot\text{L}^{-1}$): Fe–12.3, Mn–6.2, Zn–2.6, Cu–0.4, Mo–0.2 and B–23, all EDTA-based, plus 36 $\mu\text{mol}\cdot\text{L}^{-1}$ Fe as EDDHA-Fe. The pH was monitored daily and adjusted to the desired pH level by adding 0.1 M H_2SO_4 or NaOH. The electrical conductivity was $\approx 2 \text{ dS}\cdot\text{m}^{-1}$ and was not changed significantly by addition of H_2SO_4 or NaOH. The solutions were renewed weekly, and water lost was replaced daily. The experiment included five replicates arrayed in a completely randomized nonfactorial design.

At the end of the experiment the plants were harvested and root and shoot fresh and dry weights (after drying at 60 °C) were determined. Dry plant material was ground to pass a 20-mesh sieve. Total N, P, and K in tissue were determined after digesting with $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$, using an autoanalyzer (Technicon Corp., Tarrytown, N.Y.) (for N and P) or a flame photometer (for K). Calcium, Mg, Fe, Zn, and Mn were determined by atomic absorption after digesting the dry tissue with $\text{HNO}_3\text{-HClO}_4$.

Scanning electron microscopy (SEM) was used to examine roots grown in nutrient solution (N1P1) at the two pH treatments. The samples were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, and dehydrated in a graded acetone series up to 100%. Directly after this step, the samples were critical-point-dried. They were mounted on SEM stubs and sputter-coated with gold to a thickness of 0.1 μm . The samples were viewed and photographed with a JSM-T330A instrument (JEOL, Tokyo).

Data were subjected to analysis of variance using the GLM procedure of SAS (SAS Inst., Cary, N.C.).

Table 1. Nitrogen and P concentrations ($\text{mmol}\cdot\text{L}^{-1}$) added to the irrigation water.

Treatment		Nutrient solution		
pH		$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	P
5.5	N1P1	2.5	1.1	0.23
	N1P2	2.5	1.1	0.65
	N2P1	1.1	2.5	0.23
7.5	N1P1	2.5	1.1	0.23
	N1P2	2.5	1.1	0.65
	N2P1	1.1	2.5	0.23

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Results and Discussion

Yield and plant growth. The development of plants grown at pH 5.5 was normal, while at pH 7.5 it was poor (Fig. 1). Both shoot and root weights (fresh and dry) were higher at the lower pH (Table 2). Altering the $\text{NH}_4\text{:NO}_3$ ratio did not affect growth at the two pH levels examined, in agreement with the findings of a previous study (Silber et al., 1998). Phosphorus concentration in the shoots was higher in plants grown in the higher P nutrient solution (Table 3). However, no symptoms of P toxicity were observed. The highest P content in shoots of plants fertilized with the high P concentration was 0.32 mol·kg⁻¹ (Table 3), about half the level considered toxic for many plants by Marschner (1995). However, increasing the P concentration in the nutrient solution to 0.65 mmol·L⁻¹ significantly reduced root fresh weights at the low pH and slightly affected shoot fresh and dry weight

(Table 2). In a previous study, increasing the P concentration in the irrigation solution to 0.65 mmol·L⁻¹ consistently improved growth of *L. 'Safari Sunset'* in a tuff substrate (Silber et al., 1998). Those results do not contradict the present findings because P retention by the solid phase in an aero-hydroponic system is negligible whereas in tuff it is considerable (Silber, 1991). Hence, P concentration in the rhizosphere was markedly lower in a tuff substrate (0–0.2 mmol·L⁻¹) than in the irrigation solution.

Root morphology. Proteoid roots are dense clusters of rootlets, typical of the Proteaceae family (Lamont, 1972). Usually, the abundance of proteoid roots is a sign of health in Proteaceae plants (Lamont, 1986); however, in the present study at sufficient concentrations of nutritional elements, no proteoid roots developed, in agreement with the findings of Silber et al. (1998) for *L. 'Safari Sunset'*, Lamont (1972) for *Hakea* and Racette

et al. (1990) for species of Casuarinaceae. Root development of plants grown at pH 7.5 was restricted, resulting in inhibition of development of root hairs, poor branching, and root death (Fig. 1).

The effects of pH on the development of root epidermal cells and root hairs were examined by SEM. Root cells (at 1 mm from the root tip) were longer in plants grown at pH 5.5 than in those grown at pH 7.5 (30–40 and 20–30 μm , respectively), but cell width (6–8 μm) was not affected by pH. The reduction of *L. 'Safari Sunset'* root growth at pH 7.5 could be attributed to the inhibition of cell elongation without any effect on cell division, similar to the results reported for root growth of lupin (*Lupinus angustifolius* L.) (Tang et al., 1992; White, 1990). The mechanism by which high pH impairs cell elongation and restricts root hair formation is unknown. Cell wall acidification, which would cause loosening of cellulose in the walls, could be a promoter of optimal cell growth (Taiz, 1984).

Elemental concentrations in plant organs.

The damaged root system and the inhibition of root hair growth in plants grown at pH 7.5 resulted in significant changes in element concentrations in the shoots and roots (Table 3). Nitrogen P, K, Zn, and Mn concentrations were higher in shoots of plants grown at low pH (Table 3). The same trend was observed in the roots for P and K but not for N. The pH effect on Fe concentration was inconsistent, and, since Fe uptake was little affected by pH (810 $\pm$ 50 mg Fe/plant), we assumed that Fe did not limit *L. 'Safari Sunset'* growth.

Potassium concentration in shoots and roots (Table 3) was very low compared with norms reported for many other plants (Jones et al., 1991), and was in agreement with the findings of Parks et al. (1996) and Silber et al. (1998) for several Proteaceous plants. The low-K requirement of such plants may be attributed to an adaptation to the low-K soils on which they originated, as suggested previously by Parks et al. (1996).

Table 2. Effects of pH and nutrient content of solutions on fresh and dry weights (g/plant) of shoot and root of 'Safari Sunset' plants.

pH	Treatment	Fresh wt		Dry wt	
		Shoot	Root	Shoot	Root
5.5	N1P1	67.2a ²	21.7 a	11.0	1.7 a
	N1P2	51.5 ab	13.0 bc	10.4	1.5 a
	N2P1	<u>58.6 a</u>	<u>20.3 ab</u>	<u>9.1</u>	<u>1.4 a</u>
Mean		59.1	18.3	10.2	1.5
7.5	N1P1	34.3 bc	7.9 c	8.0	0.8 b
	N1P2	23.8 c	5.7 c	6.4	0.6 b
	N2P1	<u>34.5 bc</u>	<u>8.4 c</u>	<u>8.0</u>	<u>0.8 b</u>
Mean		30.9	7.3	7.5	0.7
Mean	N1P1	50.7	14.8	9.0	1.2
	N1P2	37.7	9.3	8.4	1.0
	N2P1	46.6	14.3	8.6	1.1
	LSD-pH ³	11.6	4.2	1.9	0.3
	F-pH	16***	16***	5.3*	15***
	LSD-Tr ⁴	20.1	7.3	3.4	0.6
	F-Tr	6.6***	8.1***	NS	5.7**

²Mean separation within columns by LSD test, $P \leq 0.05$.

³LSD-pH and F-pH-F and LSD tests between the two pHs.

⁴LSD-Tr and F-Tr-F and LSD tests between treatments.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Effects of pH and nutrient content of solutions on element concentrations in shoot and root of *Leucadendron* 'Safari Sunset' plants.

pH	Treatment	Shoot						Root		
		N	P	K	Fe	Zn	Mn	N	P	K
		mol·kg ⁻¹ dry mass			mmol·kg ⁻¹ dry mass			mol·kg ⁻¹ dry mass		
5.5	N1P1	2.14 a	0.19 c	0.23 ab	1.9 abc	1.8 a	4.7 b	2.44	0.21 b	0.22 ab
	N1P2	1.78 b	0.32 a	0.20 b	1.1 d	1.2 b	3.6 c	2.43	0.27 a	0.18 bc
	N2P1	<u>2.05 a</u>	<u>0.24 b</u>	<u>0.25 a</u>	<u>1.15 cd</u>	<u>1.8 a</u>	<u>6.6 a</u>	<u>2.09</u>	<u>0.29 a</u>	<u>0.26 a</u>
Mean		1.99	0.25	0.23	1.5	1.6	5.0	2.31	0.26	0.22
7.5	N1P1	1.47 c	0.09 d	0.14 cd	1.6 bc	0.9 b	1.9d	2.59	0.11 c	0.12 cd
	N1P2	1.20 d	0.11 d	0.12 d	1.9 ab	1.1 b	1.5 d	2.10	0.09 c	0.09 d
	N2P1	<u>1.29 d</u>	<u>0.08 d</u>	<u>0.17 c</u>	<u>2.0 a</u>	<u>1.8 a</u>	<u>1.2 e</u>	<u>3.08</u>	<u>0.77 c</u>	<u>0.16 bc</u>
Mean		1.32	0.09	0.14	1.8	1.3	1.5	2.57	0.10	0.12
Mean	N1P1	1.80	0.14	0.19	1.7	1.4	3.3	2.52	0.16	0.17
	N1P2	1.49	0.22	0.16	1.5	1.2	2.6	2.26	0.18	0.13
	N2P1	1.67	0.16	0.21	1.8	1.8	3.9	2.27	0.2	0.21
	LSD-pH ³	0.02	0.11	0.02	0.24	0.24	0.47	0.54	0.03	0.04
	F-pH	126***	211***	65***	8.3***	8.5***	211**	NS	98***	21***
	LSD-Tr ⁴	0.04	0.19	0.03	0.38	0.38	0.54	0.93	0.06	0.06
	F-Tr	43***	55***	34***	6.1***	9.4***	122***	NS	22***	9.3***

²Mean separation within columns by LSD test, $P \leq 0.05$.

³LSD-pH and F-pH-F and LSD tests between the two pHs.

⁴LSD-Tr and F-Tr-F and LSD tests between treatments.

NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

At acid pH, lowering the $\text{NH}_4\text{:NO}_3$ ratio affected only the P and Mn concentrations (Table 3). Reducing the $\text{NH}_4\text{:NO}_3$ ratio usually increases cation and reduces anion concentrations because of anion-cation balance (Marschner, 1995). Thus, the higher Mn concentration in the shoots is consistent with the above mechanism, but the higher P concentration (shoot and root) is not (Marschner, 1995; Mengel and Kirkby, 1987). The effects of $\text{NH}_4\text{:NO}_3$ ratio on elemental concentrations at the basic pH were inconsistent (increasing Fe and Zn while decreasing that of Mn), and were probably secondary effects of injury of the root system at basic pH.

The effects of varying the P concentration in the nutrient solution on elemental concentrations in the plants were more significant for plants grown at pH 5.5, probably because their roots developed normally. Increasing the P concentration in the nutrient solution significantly raised the P content in shoot and root, but reduced the concentrations of the Fe, Zn, and Mn (Table 3). Micronutrient contents in shoots were not in the deficient or toxic range, according to Marschner (1995) or Jones et al. (1991), and, therefore, we assumed that the micronutrient supply did not limit plant growth. Phosphorus concentration in *L. 'Safari Sunset'* plants increased because of increased P concentration in the nutrient solution, and not because of the "Zn deficiency-enhanced P uptake" mechanism, as proposed by Cakmak and Marschner (1986) for cotton (*Gossypium hirsutum* L.). The extension of the term "P-induced zinc deficiency" (Cakmak and Marschner, 1986, 1987; Loneragan and Webb, 1993; Marschner and Cakmak, 1986) to Fe and Mn is probably incorrect because of the relatively high metal content in shoots, even in plants with high P content (Table 3). Growing the plants for a longer time might have increased the differences between high- and low-P plants and have permitted signs of metal deficiency or "P toxicity" to become visible.

The exact mechanism causing lower metal contents in the shoot of the high-P-fed plants is not clear. No conditions for adsorption or precipitation of metal-P prevail in aerohydroponic systems at low pH. No "dilution effect" (Loneragan and Webb, 1993; Marschner, 1995) can be invoked, since shoot and root dry weights were not significantly affected by elevation of the P concentration (Table 2). The lower content of metals may have resulted from an inhibition of metal absorption by the root system or a translocation of metals to the shoots, or it might have resulted from internal immobilization because of the formation of metal-phosphate compounds, such as the formation of Zn-phytate in several crops reported by van Steveninck et al. (1993).

Conclusions

The pH of the root environment was the most important factor affecting *L. 'Safari Sunset'* growth in the present study. Whether pH affects plant development directly through physiological mechanisms that influence root

hair formation, or indirectly through mechanisms of nutrient availability, is not clear. Apparently, element solubility in an aerohydroponic system is excellent and does not restrict the uptake of elements. In fact, precipitation of insoluble compounds of metal-P or metal oxides may occur on the external surface of the roots grown in high pH, which reduces metal solubility. However, the fact that Fe, Zn, and Mn concentrations, even in shoots of plants grown at pH 7.5, were within the sufficiency range supports the hypothesis that pH mainly affected physiological factors.

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Fig. 1. pH effect on *Leucadendron* 'Safari Sunset' growth at N1P1 treatment. (left) plant growth at pH 5.5; (right) plant grown at pH 7.5.

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