## **Growth and Nutrient Concentration of Lychee Grown on an Acid Ultisol**

Ricardo Goenaga<sup>1</sup>, Angel Marrero<sup>1</sup>, and Delvis Pérez<sup>1</sup>

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ABSTRACT. Little is known about the adaptability of lychee (*Litchi chinensis*) to acidic soils high in aluminum (Al). A 2-year greenhouse study was conducted to determine the effects of various levels of soil Al on dry matter production, plant growth, and nutrient concentration in shoots of lychee cultivar rootstock seedlings (maternal half-sibs) of cultivars Brewster, Bostworth-3 (Kwai May Pink), and Kaimana. Soil Al treatments were statistically different for all variables measured in the study but not rootstock seedlings. Total leaf, stem, and root dry weights significantly decreased at soil Al concentrations ranging from 0.42 to 12.69 cmol·kg<sup>-1</sup>. Increments in soil Al resulted in a significant reduction in the concentration of leaf calcium and phosphorus and a significant increase in leaf Al in cultivar rootstock seedlings. The concentration of leaf potassium, magnesium, iron, zinc, and boron were in the optimum range for lychee, whereas leaf nitrogen and manganese concentrations were above optimum. The results of this study demonstrated no cultivar rootstock seedlings differences for dry matter production in lychee trees grown under Al stress and demonstrate that lychee is highly susceptible to acid soils.

ychee (L. chinensis) belongs to the soapberry (Sapindaceae) family and along with other important tropical fruit crops in this family such as longan (Dimocarpus longan) and rambutan (Nephelium lappaceum), it is native to southern China (Zee et al. 1998). The crop is grown commercially from latitude 17° to 32° and is usually found at low elevation in the subtropics and from 300 to 600 m in tropical locations (Menzel and Simpson 1994). China is the leading producer of lychee worldwide but the local demand is so strong that the country imports fresh fruit during the off-season (Huang et al. 2005); other countries such as India, Vietnam, Thailand, Pakistan, and

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R.G. is the corresponding author. E-mail: Ricardo. Goenaga@usda.gov.

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countries in Central America and Africa also produce this fruit commercially for domestic and export markets (Altendorf 2018). The edible portion of the lychee fruit is a fleshy, translucent white sarcotesta, which contains 17% to 20% total soluble solids surrounding a single ovoid to oblong glossy dark-brown seed (Goenaga et al. 2016; Subhadrabandhu and Stern 2005). Depending on production technology and environment, yield can range between 1542 and 9072 kg·ha<sup>-1</sup> (Huang et al. 2005). Goenaga et al. (2016) evaluated six lychee cultivars at two locations in Puerto Rico during 8 years of production. Highest average yield (6567 kg·ha<sup>-1</sup>) was obtained by cultivar Kaimana. As consumers seek healthy and more diverse food products, production and trade of minor tropical fruits such as lychee, are gaining importance globally (Altendorf 2018).

The most common and recommended method of lychee propagation is by air layering. Trees propagated from air layering come into commercial production  $\approx 3$  to 5 years after field planting. Seedlings are slow in growth, not true-to-type, and take many years to bear a crop (Crane et al. 2016). Although air layering is the preferred and fastest method of lychee propagation, it has serious limitations for growing areas that are prone to be affected by tropical storms and hurricanes. For example, in 2004, Tropical Storm Jeanne hit Puerto Rico with sustained winds of 65 mph. One experimental lychee orchard and a longan orchard were completely lost because of severe lodging of air-layered trees lacking a taproot for anchorage. Nearby experimental plantings of rambutan and mamey sapote (Pouteria sapota) propagated by grafting onto rootstocks recovered promptly after suffering only some defoliation. Grafting is difficult in lychee, but techniques using patch grafting have been developed at the U.S. Department of Agriculture, Agricultural Research Service, Tropical Agriculture Research Station (TARS) in Mayaguez, PR, that have resulted in  $\approx$ 80% success rate.

As with many other tropical fruit crops, there is a scarcity of information on best management practices and optimum growing conditions for lychee. For example, little is known about the adaptability of lychee to highly acidic soils, common to tropical areas where lychee is grown (Chen et al. 2020). The most productive soils of the world are already under cultivation, and those available for agricultural expansion, particularly in the tropics, are often strongly acidic, possessing toxic levels of soil aluminum (Al) (Kamprath 1984; Laurance et al. 2014; Samac and Tesfaye 2003). The mechanism by which

Units To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1.1209	lb/acre	kg∙ha <sup>−1</sup>	0.8922
1	meq/100 g	cmol⋅kg <sup>-1</sup>	1
1.6093	mph	km⋅h <sup>-1</sup>	0.6214
28.3495	oz	g	0.0353
l	ppm	$mg \cdot kg^{-1}$	1
$(^{\circ}F - 32) \div 1.8$	۰̂F	°C	$(^{\circ}C \times 1.8) + 32$

<sup>&</sup>lt;sup>1</sup>U.S. Department of Agriculture, Agricultural Research Service, Tropical Agriculture Research Station, 2200 P.A. Campos Ave., Suite 201, Mayaguez, PR 00680-5470

soil acidity reduces the yield of many crops has been studied extensively (Foy 1984; Kochian et al. 2002; Marschner 1991; Pérez-Almodovar and Goenaga 2015). A high concentration of Al restricts root growth and hence exploitation of the soil/subsoil by roots for moisture and nutrients. Soil Al concentrations as high as 15 cmol·kg<sup>-1</sup> can be found in tropical acid soils; in the tropical Americas, ≈50% of the soils with potential for agricultural use have been diagnosed with Al toxicity problems (Hoekenga et al. 2006; National Research Council 1993; Villagarcia et al. 2001).

Few studies, if any, have been conducted to screen lychee germ-plasm for acid soil tolerance. The objective of this investigation was to determine the critical soil Al concentrations that affect growth of lychee and to identify potential sources of tolerance that can serve as superior rootstocks to this stress.

## Materials and methods

Greenhouse experiments were established 23 Aug 2016 and 10 Sep 2018 at TARS. The soil used for the study consisted of an extremely acid, Maricao series, Ultisol (very-fine, mixed, subactive, isothermic Typic Haplohumults), collected from Indiera Fria in the northern part of the municipality of Yauco in southwestern Puerto Rico. After air-drying, sieving, and thorough mixing, four Al treatments were established using either sulfuric acid or powdered calcium hydroxide [Ca(OH)<sub>2</sub> (slaked lime)] to acidify or alkalinize the soil, respectively, to obtain soil Al concentrations of 0.42, 2.37, 6.77, and 12.69 cmol·kg<sup>-1</sup> of soil. These Al concentrations fall within the range found in many soils in Puerto Rico and throughout the humid tropics. Two-liter pots were filled with soil and arranged in a split-split plot design with five replications. Years were the main plot treatment, cultivar rootstock seedlings as subplots, and soil Al concentration as sub subplots. Each pot contained two plants.

Soil characteristics are described in Table 1. Samples were air-dried and passed through a 20-mesh screen. Soil pH in water and 0.01 M calcium chloride [CaCl<sub>2</sub> (1 soil: 2 water)] was measured with a glass electrode. Phosphorus (P) and exchangeable cations potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese

(Mn), zinc (Zn), boron (B), and Al were extracted with Mehlich III solution (Amacher 2007; Mehlich 1984) and determined by inductively coupled plasma spectrometry (Soltanpour et al. 2007). Potassium chloride (KCl) extractable Al was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES), organic carbon was determined by the Walkley-Black chromic acid wet oxidation method (Nelson and Sommers 2007). Soil ammonium-nitrogen (NH<sub>4</sub>-N) and nitrate N (NO<sub>3</sub>-N) were determined by steam distillation (Mulvaney 2007). Percentage Al saturation of the soil was calculated on the assumption that exchangeable Ca + Mg + K + Al + hydrogen (H) was the effective cation exchange capacity of the soil (Kamprath 1984). All plots were planted to open-pollinated seedlings (maternal half-sibs) of lychee clones 'Brewster', 'Bostworth-3' ('Kwai May Pink'), and 'Kaimana'. Cultivars from which these rootstock seedlings originated from have unique characteristics. 'Brewster' is a vigorous midseason commercial cultivar in Florida; 'Bosworth-3' is an Australian selection that bears fruit regularly; 'Kaimana' is a seedling selection of the Chinese cultivar Hak Ip. In contrast to others (e.g., 'Salathiel'), these cultivars exhibited vigorous growth when grown in heavy soils typical of some mountain regions of Puerto Rico and having a slightly acidic pH of ≈5.6 (Goenaga et al. 2016). Cultivar rootstock seedlings were  $\sim$ 2.5 months old and had an average (over cultivar

rootstock seedlings) height, stem diameter, and leaf number of 13.1 cm, 2.8 mm, and 16.8 leaves, respectively, when the experiment started. Plants were fertilized with soluble fertilizer (Plant Foods, Inc., Vero Beach, FL, USA) 3 months after transplanting with 20N-8.7K-16.6K plus micronutrients by dissolving 7 g of fertilizer per gallon and applying 300 mL of solution to each pot. Cultivar rootstock seedlings were harvested for biomass accumulation on 24 Apr 2017, in Expt. 1, and 23 Sep 2019 in Expt. 2. At each harvest, plant height was measured with a ruler and stem diameter with a digital caliper at 2.0 cm from the soil. Soil was then loosened and plants from each treatment were pulled from the soil, washed, and separated into leaves, stems, and roots. Plant parts from each cultivar rootstock seedlings were dried at 70 °C to constant weight for dry matter determination. The dry samples were ground to pass a 1.0-mesh screen and analyzed for N, P, K, Ca, Mg, Fe, Mn, Zn, Al, and B concentration using recommended digestion procedures (Perkin-Elmer 2013). For this purpose, leaf samples were digested using a microwave assisted acid digestion method. The samples were digested with 10 mL of concentrated nitric acid (HNO<sub>3</sub>) and 10 mL of distilled water. After digestion was completed, each sample was filtered through filter paper (Whatman No. 541; GE Healthcare Life Sciences, Piscataway, NJ, USA) into a 50-mL volumetric flask. The

Table 1. Preplant soil characteristics of experimental Ultisol soil amended to obtain four aluminum (Al) concentrations to evaluate growth of lychee rootstock seedlings under greenhouse conditions.

	Al treatment (cmol·kg <sup>-1</sup> ) <sup>i</sup>				
Soil characteristic <sup>i</sup>	0.42	2.37	6.77	12.69	
pH in water	4.96	4.42	4.00	3.82	
pH in calcium chloride	4.80	4.31	3.91	3.76	
Ammonium nitrogen (mg·kg <sup>-1</sup> )	42	40	127	206	
Nitrate nitrogen (mg·kg <sup>-1</sup> )	111	103	169	24	
Phosphorus (mg·kg <sup>-1</sup> )	76	75	66	46	
Potassium (mg·kg <sup>-1</sup> )	526	644	584	485	
Calcium (mg·kg <sup>-1</sup> )	4,877	3,870	4,574	1,868	
Magnesium (mg·kg <sup>-1</sup> )	403	535	618	435	
Iron $(mg \cdot kg^{-1})$	5 <i>7</i>	61	108	123	
Manganese (mg·kg <sup>-1</sup> )	116	323	494	620	
Zinc $(mg \cdot kg^{-1})$	1.17	1.12	3.88	8.24	
Al (mg·kg <sup>-1</sup> )	38	213	609	1142	
Organic carbon (%)	0.63	0.63	0.60	0.58	
Al saturation (%)	0.65	4.00	9.50	29.00	

 $<sup>\</sup>frac{1}{1} \text{ mg} \cdot \text{kg}^{-1} = 1 \text{ ppm}, 1 \text{ cmol} \cdot \text{kg}^{-1} = 1 \text{ meq}/100 \text{ g}.$ 

solution was used for nutrient determination using an inductively coupled plasmaoptical emission spectrometer (PE 8000; Perkin-Elmer, Shelton, CT, USA). Total N was determined by a modification of the micro-Kjeldahl method (Foss Tecator 2002). For this purpose, 0.2 g of tissue was weighed and transferred to a Kjeldahl tube. The following compounds were added to each tube: 6-mm Hengar granules (Fisher Scientific, Fair Lawn, NJ, USA) for smooth boiling, one catalyzing tablet (1.5 g potassium sulfate + 0.15 g copper sulfate), 5 mL of concentrated sulfuric acid, and 3 mL of 30% hydrogen peroxide. Samples were digested in a digestion block for 2 h at 400 °C.

Analyses of variance and regression analyses were done using the general linear model procedure of the SAS program package (version 9.4; SAS Institute Inc., Cary, NC, USA). Only coefficients at  $P \le 0.05$  were retained in the models.

## Results and discussion

At the end of the experimental period, differences among soil Al treatments were highly significant ( $P \le 0.01$ ) for total, leaf, stem, and root dry weight; however, rootstock seedlings and the year × rootstock seedlings interaction were not significant (analysis of variance not shown). Therefore, results were averaged over years and cultivar rootstock seedlings.

Trees attained maximum total dry weight at the lowest soil Al concentration. Increasing soil Al concentration from 0.42 to 12.69 cmol·kg<sup>-1</sup> resulted in a decrease in total dry weight of 77.5% (Fig. 1A). Even a small increase in soil Al concentration from 0.42 to 2.37 cmol·kg<sup>-1</sup> resulted in almost a 30% decline in total dry weight. These results are similar to those obtained in field-grown longan, also a member of the soapberry family, where increasing the soil Al concentration from 5.1 to 12.2 cmol⋅kg<sup>-1</sup> resulted in a significant reduction in total dry weight of rootstock seedlings ranging between 68% and 87% (Goenaga 2013). Results are also similar to those obtained by Xiao et al. (2002) in nutrient culture, which showed significant reductions in biomass production when longan seedlings were exposed to increasing concentrations of Al in the solution. Factors such as root membrane leakage of solutes (Wan 2007) and increased proteolysis in roots and leaves (Xiao

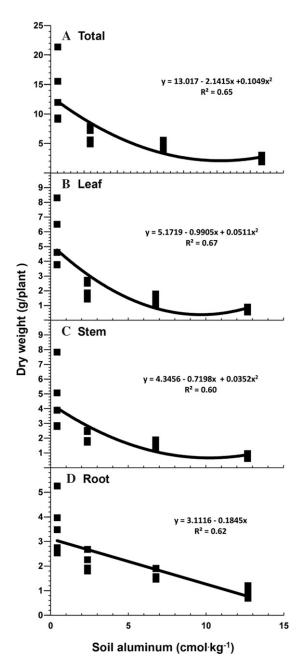


Fig. 1. Average total (A), leaf (B), stem (C), and root (D) dry weight of lychee rootstock seedlings grown under four soil aluminum (Al) concentrations in the greenhouse. The experimental Ultisol soil was amended to obtain four Al concentrations equivalent to 0.42, 2.37, 6.77, and 12.69 cmol·kg $^{-1}$ . Values are means of rootstock seedlings of three cultivars, five replications, and 2 years. Graphs are best fit regression curves significant at  $P \le 0.05$ ; 1 cmol·kg $^{-1} = 1$  meq/100 g, 1 g = 0.0353 oz.

et al. 2006) have been shown to increase in longan seedlings when exposed to high Al concentrations in the nutrient solution.

Soil Al significantly reduced the dry weight of all plant organs (Fig. 1B–D). At a soil Al concentration of 0.42 cmol·kg<sup>-1</sup>, average leaf, stem, and root dry weights accounted for 40%, 34%, and 26%, respectively, of the total dry weight. At the highest soil Al

concentration (12.69 cmol·kg<sup>-1</sup>), these proportions changed to 33%, 36%, and 31%, respectively. Therefore, although high soil Al significantly reduced the dry weight of all plant parts, root dry weight was the least affected (Fig. 1A–D). Similar responses in shoot–root ratios have been found with other crops subjected to acid soil conditions (Bates et al. 2002; Goenaga 2011, 2013; Himelrick 1991). This response may be

indicative of plants translocating metabolites to maintain root function at the expense of shoot growth. These results demonstrate the greater sensitivity of lychee to soil Al when compared with rambutan, which showed that total plant dry weight increased by more than 145% when soil Al concentration was increased from 0.70 to 11.0 cmol·kg<sup>-1</sup> (Goenaga 2011), suggesting the involvement of an Al-sequestration mechanism (Pérez-Almodovar and Goenaga 2015).

Stem diameter and plant height showed a reduction of 47% and 51%, respectively, when soil Al was increased from 0.42 to 12.69 cmol·kg<sup>-1</sup> further demonstrating the susceptibility of this crop to high soil Al (Fig. 2A and B). These values are remarkably similar to those obtained for longan showing stem diameter and plant height reductions of 50% and 49%, respectively, when soil Al was increased from 5.1 to 12.2 cmol·kg<sup>-1</sup> (Goenaga 2013). In contrast, studies with rambutan (Goenaga 2011) showed an increase in plant height and stem diameter with increasing levels of soil Al up to 11.0 cmol·kg<sup>-1</sup> of soil Al and then declined. Therefore, there seems to be genetic diversity for Al tolerance within the soapberry family.

Figure 3 shows the concentration of various nutrients in leaves collected at the end of the experimental period. Increments in soil Al resulted in a significant reduction in the concentration of leaf P, Ca, and Mg, and a significant increase in the concentration of leaf Mn and Al (Fig. 3B, D, E, G, J). High concentration of tissue Al and Mn can limit plant growth and development (George et al. 2012; Gupta et al. 2013; Kochian et al. 2002). These results are similar to those found by others (Goenaga 2013; Goenaga and Smith 2002), in which the concentration of leaf Al increased significantly with increments in soil Al. However, results greatly contrast those obtained with rambutan, which showed Al concentrations in leaf tissue declining with increments in soil Al up to a soil Al concentration of 11 cmol·kg<sup>-1</sup>. Although the primary effect of high Al is on root growth, high Al concentration in the soil solution also induces nutritional imbalances. Al strongly competes with other cations such as Ca and Mg for binding sites in the apoplasm and may inhibit Ca

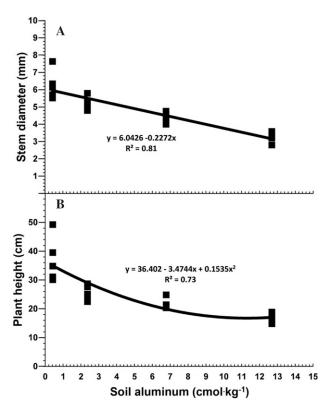


Fig. 2. Average stem diameter (A) and plant height (B) of lychee rootstock seedlings grown under four soil aluminum (Al) concentrations in the greenhouse. The experimental Ultisol soil was amended to obtain four Al concentrations equivalent to 0.42, 2.37, 6.77, and 12.69 cmol·kg<sup>-1</sup>. Values are means of rootstock seedlings of three cultivars, five replications, and 2 years. Graphs are best fit regression curves significant at  $P \le 0.05$ ; 1 cmol·kg<sup>-1</sup> = 1 meq/100 g, 1 mm = 0.0394 inch, 1 cm = 0.3937 inch.

uptake by blocking Ca channels in the plasma membrane and blocking Mg binding sites in transport proteins (Bose et al. 2011; Huang et al. 1992; Rengel and Robinson 1989). The rapid decline in leaf Ca (Fig. 3D) provides evidence that similar responses may occur in lychee, making it one of the most acid soil-intolerant crops in the soapberry family. Further evidence on the low threshold lychee has to grow on acid soils is the fact that in this experiment, soil Al saturation was only 29% in the highest (12.69 cmol·kg<sup>-1</sup>) treatment (Table 1). Goenaga and Smith (2002) found that increasing soil Al concentration from 0.68 cmol·kg<sup>-1</sup> to just 2.5 cmol·kg<sup>-1</sup> reduced total dry weight of five common bean (Phaseolus vulgaris) genotypes between 25% and 31%. Working with pigeon peas (Cajanus cajan), a crop reputed to be drought tolerant, Abruña et al. (1984) found that increasing the soil Al saturation from 0% to 51% resulted in a yield reduction of 46%. In this study, increasing the Al saturation from 0.65% (0.42 cmol·kg $^{-1}$ ) to just 9.5% (6.77 cmol·kg $^{-1}$ ) reduced total dry matter by 73%. In contrast, total dry matter of rambutan was unaffected until the soil reached an Al concentration of 11.0 cmol·kg<sup>-1</sup>, which represented about 66% soil Al saturation (Goenaga 2011). Except for Ca and P, the concentration of the rest of the elements, including Mg (Fig. 3A–J), were in the optimum range for lychee (Menzel 2005). Leaf N concentration was slightly above optimum. Leaf Mn concentration increased significantly but none of the typical Mn toxicity symptoms (e.g., crinkle leaf, brown speckling) were observed.

The results of this study demonstrated no cultivar rootstock seedling differences for dry matter production in lychee trees grown under Al stress. On average, increasing the soil Al concentration from 0.42 to 12.69 cmol·kg<sup>-1</sup> resulted in a 77% reduction in total dry matter production, which is indicative of how sensitive this crop is to high soil Al. Future studies should be directed to the screening of a wider pool of lychee germplasm as an effort to identify Altolerant genotypes, which could be used as commercial rootstocks in acid soils.

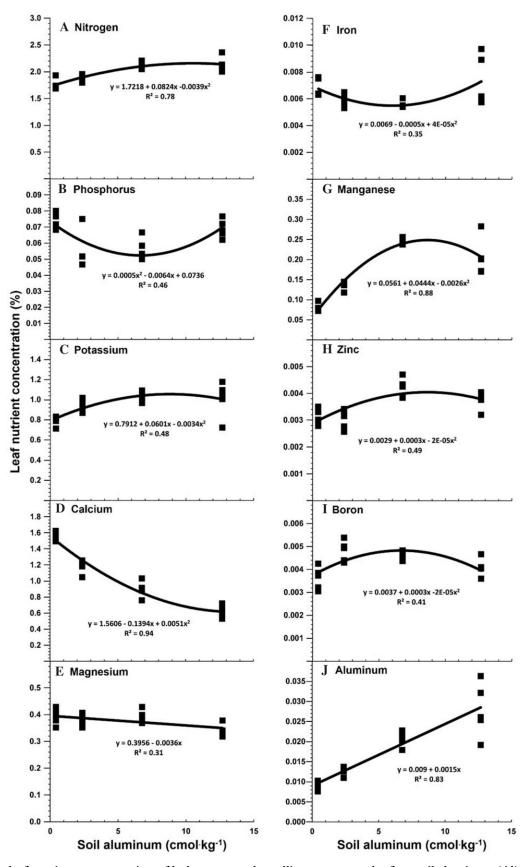


Fig. 3. Average leaf nutrient concentration of lychee rootstock seedlings grown under four soil aluminum (Al) concentrations in the greenhouse. The experimental Ultisol soil was amended to obtain four Al concentrations equivalent to 0.42, 2.37, 6.77, and 12.69 cmol·kg<sup>-1</sup>. Values are means of rootstock seedlings of three cultivars, five replications, and 2 years. Graphs are best fit regression curves significant at  $P \le 0.05$ ; 1 cmol·kg<sup>-1</sup> = 1 meq/100 g.

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