Mineral Nutrient Uptake of Encore Azalea ‘Chiffon’ Affected by Nitrogen, Container, and Irrigation Frequency

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Abstract. Mineral nutrient uptake of Encore® azalea ‘Chiffon’ (*Rhododendron* sp.) affected by nitrogen (N) rate, container type, and irrigation frequency was investigated. One-year-old azalea plants were planted in two types of 1-gallon containers: a black plastic container or a biodegradable container (also referred to as a biocontainer) made from recycled paper. Azalea plants were fertilized with 250 mL of N-free fertilizer twice weekly plus N rates of 0, 5, 10, 15, or 20 mM from ammonium nitrate (NH₄NO₃). All plants were irrigated daily with the same amount of water through one or two irrigations. Plants fertilized without N had the lowest concentrations of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) averaged in the entire plant, which were at deficient levels for azalea species. High N rates of 15 or 20 mM resulted in the highest plant average concentrations of P, K, Ca, and Mg. Concentrations of micronutrients including iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B) showed varied trends affected by different treatments. With high N rates of 15 and 20 mM, paper biocontainers increased uptake of both macro- and micronutrients in terms of total nutrient content (mg or µg per plant) compared with plastic containers. One irrigation per day increased root concentrations of Cu and Zn and root contents of Fe, Zn, Cu, and B, but decreased leaf K concentration compared with two irrigations per day. The beneficial effects of high N rates and biocontainers on mineral nutrient uptake of Encore® azalea ‘Chiffon’ likely indirectly occurred through increasing plant growth.

Nutrient management has been one of the most important practices in commercial nursery production of ornamental plants, and N is often considered a limiting factor for plant growth. Plant N status is mainly improved by application of N fertilizer. Increased N during fertilization increased plant biomass and N uptake and improved flower quality of some species, but it also increased nutrient leaching and decreased uptake efficiency (Bi and Scagel, 2008; Bi et al., 2007; Chang et al., 2012). Higher N fertilization rate promoted shoot growth of container-grown *Rhododen-*

dron L. ‘Karen’ and increased nutrient leaching, whereas decreased N and P promoted root growth and improved nutrient uptake efficiency (Ristvey et al., 2007). Intermediate N rates of 105 and 158 mg·L⁻¹ resulted in greater dry weight, leaf area, and flower number of *Anthurium andraeanum* Lind. than low N (79 mg·L⁻¹) or high N (210 mg·L⁻¹) rates (Chang et al., 2012). An optimum N rate should be determined based on plant requirements (including species, cultivars, and growth stage) as well as growing conditions such as medium composition, temperature, and moisture (Cardarelli et al., 2010; Gastal and Lemaire, 2002; Gómez-López et al., 2006).

Plant demands for mineral nutrients change in response to different N supply and other growing conditions, which should be considered in fertilization management (Pradubsuk and Davenport, 2010; Strik, 2015). Foliar urea spray in the fall altered plant’s demand of nutrients during winter, when uptake of P, Cu, and Mn increased, whereas K and Mg uptake decreased in *Rhododendron* ‘Cannon’s Double’ and ‘P.J.M.’ (Scagel et al., 2008a). Leaf concentrations of N, K, Ca, Mg, Mn, sulfur (S), aluminum (Al), and B in bell pepper (*Capsicum annum* L.) increased with increasing shade level

(Díaz-Pérez, 2013). Uptake of different nutrient elements interact with each other. Excessive N (210 mg·L⁻¹) decreased K and Mg uptake and resulted in poor growth of *Anthurium andraeanum* (Chang et al., 2012). When plants received no N from fertigation, there were deficiencies of P, K, S, and Mn for *Rhododendron* ‘P.J.M.’ and deficiencies of P, K, S, Ca, and Mg for *Rhododendron* ‘Cannon’s Double’ (Scagel et al., 2008b). With a sufficient N supply, plant growth may be limited by other mineral nutrients; therefore, balanced fertilization programs are necessary to optimize plant growth (Marschner, 2012; Scagel et al., 2012).

Nutrient availability declines with low soil water content, which becomes a limiting factor for nutrients to become soluble to the root surface (Marschner, 2012). Increased irrigation frequency with the same total amount of water has been used to reduce nutrient leaching and compensate for possible nutrient deficiency, and it has varying effects on plant growth and nutrient uptake (Fare et al., 1994; Scagel et al., 2011). Neilsen et al. (1995) reported that higher frequency irrigation improved tree growth of ‘Gala’ apple (*Malus domestica* Borkh.) compared with lower irrigation frequency. Scagel et al. (2012) reported that net uptake of P, B, and Mn decreased in response to more frequent irrigation in container-grown *Rhododendron* ‘P.J.M. Compact’, ‘English Roseum’, and ‘Gibraltar’, but increased Ca uptake of ‘P.J.M. Compact’ and ‘English Roseum’. Plant species vary in their growth and nutrient uptake responses to irrigation frequency (Li et al., 2018, 2019).

Biocontainers made from various biodegradable materials have been investigated as sustainable alternatives to conventional plastic containers for various crops and production systems (Beeks and Evans, 2013a, 2013b; Evans and Hensley, 2004; Evans and Karcher, 2004; Kuehny et al., 2011; Li et al., 2015, 2018, 2019; Nambuthiri et al., 2015; White, 2009). They were reported to have varying physical properties, water consumption characteristics, and influences on plant growth. A variety of biocontainers made from peat, manure, coir, straw, and wood fiber have been found to produce plants with quality similar to that of plants grown in traditional plastic containers (Koeser et al., 2013; Kuehny et al., 2011). Decomposition of feather containers provides an additional N source and resulted in greater dry shoot weights of ‘Janie Bright Yellow’ marigold (*Tagetes patula* L.), ‘Cool Blush’ vinca (*Catharanthus roseus* L.), and ‘Orbit Cardinal’ geranium (*Pelargonium xhortorum* L.H. Bailey) compared to those grown in peat containers (Evans and Hensley, 2004). The effects of specific biocontainers on plant nutrient uptake require investigation. Therefore, the objective of this study was to investigate the influence of N rate, container type, and irrigation frequency on mineral nutrient uptake of Encore® azalea ‘Chiffon’.

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Materials and Methods

Plant culture and treatments. One hundred 1-year-old liners of Encore® azalea ‘Chiffon’ were planted in two types of 1-gallon containers: a black plastic container (GL 400; top diameter, 17.8 cm; bottom diameter, 18.1 cm; volume, 3.8 L; Nursery Supplies, Chambersburg, PA) and a biodegradable container (also referred to as a biocontainer) made from a mix of recycled paper (7 × 7 RD; interior top, diameter 18.7 cm; bottom diameter, 14.9 cm; height, 17.1 cm; volume, 3.9 L; Western Pulp Products, Corvallis, OR). All azalea plants were maintained outdoors with full sun conditions on the campus of Mississippi State University (USDA hardiness zone 8a; lat. 33.4552°N, long. 88.7944°W). Pine bark (100%) amended with 1 lb/yard of lime was used as the growing substrate. Each azalea plant was fertilized with 250 mL of N-free fertilizer (Cornell No. N Eq. 0–6–27; GreenCare Fertilizers, Kankakee, IL) at a rate of 1.06 mg·mL⁻¹ twice weekly from 23 April to 15 Sept. 2013 plus 0, 5, 10, 15, or 20 mm N from NH₄NO₃ (Li et al., 2019). Plants were drip-irrigated either once per day at 8:00 AM or twice per day at 8:00 AM and 2:30 PM with the same total daily water amount. Plants were irrigated to replace daily water loss plus a 15% leaching fraction. Irrigation volume was determined by calculating daily water use on three dates during the growing season.

Nutrient analyses. All plants were destructively harvested on 12 Nov. 2013, ~32 weeks after transplanting, cleaned free of debris and substrate using deionized water, and separated into leaves, stems, and roots. All plant samples were then oven-dried at 60 °C. Dry weight of each sample was measured and used to calculate N content (mg or µg per plant) in leaves, stems, roots, or the entire plant. Each plant sample was then ground to pass a 1-mm sieve (20 mesh) with a Wiley mini mill (Thomas Scientific, Waltham, MA) for nutrient analyses. Oven-dried tissue samples of 0.5 g were digested by 1 mL of 6 M hydrochloric acid (HCl) and 50 mL of 0.05 M HCl for the concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, and B using inductively coupled plasma optical emission spectrometry (SPECTROBLUE; SPECTRO Analytical Instruments, Kleve, Germany). Plant samples were tested at the Mississippi State University Extension Service Soil Testing Laboratory. Concentrations of macronutrients (%) and micronutrients (ppm) were both presented on a dry weight basis.

Calculations. Nutrient content in each sample was calculated by multiplying dry weight of a structure (leaves, stems, or roots) by the concentration of a certain nutrient element. Total content of a given nutrient in one plant was estimated by summing the nutrient content from leaves, stems, and roots. Plant average concentration of a given nutrient was calculated by dividing the total nutrient content by total plant dry weight. Both nutrient concentration (% for macronutrients and ppm for micronutrients) and

content (mg per plant for macronutrients and µg per plant for micronutrients) were presented in results to analyze effects of N rate, container type, and irrigation frequency on mineral nutrient uptake of the tested azalea cultivar.

Experimental design and data analyses. The experimental design was a completely randomized design with five single-plant replications. The treatment design was a complete factorial with N rate (five rates), container type (two types), and irrigation frequency (two frequencies) as the three experimental factors, providing 20 treatment combinations. Significance of any main effect or interaction was determined by an analysis of variance (ANOVA) using the Proc GLM procedure of SAS (9.4; SAS Institute, Cary, NC). Where indicated by ANOVA, a multiple comparison was conducted using the LSMEANS statement with the stimulate method at $P \leq 0.05$. When analyzing allocations of nutrient content among leaves, stems, and roots, structure type was considered as a factor to analyze its main effect.

Results

Macronutrient concentrations (%). Plant average concentrations of P and K, concentrations of Mg and Ca in leaves, P concentration in stems, and concentrations of P, K, Mg, and Ca in roots were affected by the interaction between N rate and container type. Plant average Ca and Mg, leaf P and K, and stem Mg concentrations were affected by the main effect of N rate, but not by container type or irrigation frequency, except that plastic containers and two irrigations per day increased leaf K concentration compared with biocontainers and one irrigation per day, respectively (Tables 1 and 2).

Phosphorus. Plant average P concentration ranged from 0.08% in plants fertilized without N grown in biocontainers to 0.14% in plants fertilized with 20 mm N grown in plastic containers (Fig. 1). Plastic containers resulted in higher plant P concentration than biocontainers at N rates of 10, 15, and 20 mm. Stem P concentration had a trend similar to that of the plant average P concentration. An N rate of 5 mm resulted in the highest leaf P concentration (0.28%) compared to other N rates. Two container types resulted in similar P concentrations at a given N rate in roots except that plastic containers resulted in higher P concentration than biocontainers with an N rate of 10 mm.

Potassium. Plant average K concentration ranged from 0.47% in plants fertilized without N grown in biocontainers to 0.69% in plants fertilized with 20 mm N grown in plastic containers (Fig. 2). Plastic containers resulted in higher plant K concentration than biocontainers with N rates of 0 and 5 mm. No N resulted in the highest leaf K concentration of 1.5%, which was higher than the concentrations that occurred with N rates of 5 to 20 mm. Stem K concentration was similar among five N rates. Root K concentration was similar between container types at N rates of 5 to 20 mm. Additionally, plastic containers resulted in a 7% increase in leaf K concentration compared with biocontainers (Table 1). Two irrigations per day resulted in a 6% increase in leaf K concentration compared with one irrigation per day (Table 2).

Calcium. Affected by the main effect of N rate, plant average Ca concentration ranged from 0.41% in plants fertilized without N to 0.46% in plants fertilized with 5 or 15 mm N (Fig. 3). Stem Ca concentration was unaffected by N rate. When affected by the N rate and container type interaction, two container types generally resulted in similar Ca

Table 1. Container type effects on nutrient concentrations and content in leaves or stems of Encore® azalea ‘Chiffon’.

	Concn ²				Content
	K	Zn	B	B	P
	(%)	(ppm)	(ppm)	(ppm)	(mg per plant)
	Leaf	Leaf	Leaf	Stem	Stem
Biocontainer ³	1.14 b	12 a	61 a	10 b	3.69 b
Plastic container	1.22 a	9 b	50 b	11 a	4.60 a
P	0.011	0.018	<0.0001	0.01	0.004

²Different lowercase letters within a column suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

³The azalea plants were planted in either a paper biocontainer or a conventional black plastic container.

Table 2. Irrigation frequency effects on nutrient concentrations and contents in leaves and roots of Encore® azalea ‘Chiffon’.

	Concn ²				Content			
	K	B	Cu	Zn	Fe	Zn	Cu	B
	(%)	(ppm)	(ppm)	(ppm)	µg per plan			
	Leaf	Leaf	Root	Root	Root	Root	Root	Root
Once ³	1.14 b	58 a	6 a	17 a	288 a	87 a	30 a	34 a
Twice	1.21 a	54 b	5 b	16 b	241 b	71 b	23 b	29 b
P	0.023	0.035	0.029	0.020	0.025	0.004	0.004	0.007

²Different lowercase letters within a column suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

³The azalea plants were irrigated once or twice per day with the same total amount of water.

Fig. 1. Concentrations (%) and contents (mg per plant) of phosphorus (P) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (A, C, D, E, F, and H), or by the main effect of N rate (B and G). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 2. Concentrations (%) and contents (mg per plant) of potassium (K) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by interaction between nitrogen (N) rate and container type (A, D, E, F, G, and H), the main effect of N rate (B), or unaffected by N rate (C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 3. Concentrations (%) and contents (mg per plant) of calcium (Ca) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (B, D, E, F, G, and H), by the main effect of N rate (A), or unaffected by N rate (C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

concentrations in leaves and roots, except that biocontainers resulted in higher leaf Ca concentration than plastic containers without N.

Magnesium. Affected by the main effect of N rate, plant average Mg concentration ranged from 0.19% in plants fertilized without N to 0.25% in plants fertilized with 20 mM N (Fig. 4). Plants fertilized with any nitrogen rate had higher average Mg concentrations than those fertilized without N. The N rate of 10 mM resulted in the highest stem Mg concentration of 0.25%. Concentrations of Mg in leaves and roots were not separated distinctly, and they were generally similar among N rates within one container type. Container type also resulted in similar Mg concentrations with each N rate in leaves and roots, except that biocontainers resulted in higher Mg concentration than plastic containers in leaves without N and in roots with 20 mM N.

Macronutrient contents (mg per plant). Content of macronutrients, including P, K, Ca, and Mg, were generally affected by the interaction between N rate and container type, except that stem P content was influenced by the main effects of N rate and container type without interaction (Tables 1; Figs. 1–4). Content of all macronutrients in the entire plant, leaves, stems, and roots shared similar trends. In biocontainers, macronutrient contents generally increased with increasing N rate from 0 to 15 mM N, with similar contents found for N rates of 15 and 20 mM. In plastic

containers, macronutrient contents increased from 0 to 10 mM N; then, they remained similar or decreased with 15 or 20 mM N. The different trends in two container types resulted in higher contents of P, K, Ca, and Mg in biocontainers than those grown in plastic containers in the entire plant or in any structure type with N rates of 15 and 20 mM. Macronutrient contents were similar between container types with N rates of 0, 5, or 10 mM. Stem P contents were higher with N rates of 10 to 20 mM compared to 0 or 5 mM N. Plastic containers also resulted in a 24.7% increase in stem P contents compared to biocontainers.

Micronutrient concentrations (ppm). Iron concentration averaged in the plant ($P = 0.047$) and in roots ($P = 0.015$) were affected by the interaction between N rate and container type, ranging from 43 ppm to 53 ppm averaged in the plant, without difference among all treatment combinations, and from 47 ppm to 74.1 ppm in roots, respectively (Fig. 5). Fe concentrations in leaves and stems were affected by the main effect of N rate, ranging from 37.9 to 48.5 ppm in leaves and from 31.6 to 37.6 ppm in stems. Irrigation frequency did not affect Fe concentration in the entire plant or in any structure type.

Manganese. Concentrations of Mn averaged in the plant, leaves, stems, and roots were affected similarly by the interaction between N rate and container type (Fig. 6). Plant average Mn concentration ranged from

59 ppm in plants fertilized with 20 mM N grown in plastic containers to 112 ppm in plants fertilized without N grown in biocontainers. Biocontainers resulted in higher Mn concentrations compared with plastic containers in the entire plant and in stems with N rates of 10, 15, and 20 mM, in leaves with N rates of 0 and 15 mM, and in roots with N rates of 15 and 20 mM. Due to the low mobility of Mn in phloem, concentrations of Mn in stems (range, 66.3–155.4 ppm) and roots (range, 71.3–126.8 ppm) were relatively high compared with those in leaves (range, 29.7–59.3 ppm). The lowest leaf Mn concentration of 29.7 ppm was found in plants fertilized with 5 mM N grown in plastic containers.

Copper. Concentrations of Cu averaged in the plant, leaves, and roots were affected similarly by the interaction between N rate and container type (Fig. 7). Stem Cu concentration was affected by the main effect of N rate, but not by container type or irrigation frequency. In general, there was a decreasing trend of Cu concentration with increasing N rate averaged in plants and in leaves in both container types, in roots in biocontainers, or in stems averaged over two container types. Root Cu concentration in plastic containers did not vary dramatically among five N rates. One irrigation per day resulted in root Cu concentration of 6 ppm compared with 5 ppm with two irrigations per day (Table 2). Plant average Cu concentration ranged from 3.4

Fig. 4. Concentrations (%) and contents (mg per plant) of magnesium (Mg) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (B, D, E, F, G, and H), or by the main effect of N rate (A and C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 5. Concentrations (ppm) and contents (µg per plant) of iron (Fe) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (A, D, E, F, G, and H), or by the main effect of N rate (B and C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 6. Concentrations (ppm) and contents (µg per plant) of manganese (Mn) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type. One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 7. Concentrations (ppm) and contents (µg per plant) of copper (Cu) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (A, B, D, E, F, G, and H) or by the main effect of N rate (C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

ppm in plants fertilized with 20 mM N grown in plastic containers to 6.3 ppm in plants fertilized without N grown in biocontainers. With the N rate and container type interaction, biocontainers resulted in higher Cu concentrations than plastic containers averaged in the plant and in leaves without N, and in roots with N rates of 0 and 5 mM. Plastic containers resulted in higher Cu concentration than biocontainers in leaves with 10 mM N.

Zinc. Concentrations of Zn averaged in the plant, stems, and roots were affected similarly by the interaction between N rate and container type (Fig. 8). Leaf Zn concentration was unaffected by N rate or irrigation frequency. Biocontainers resulted in leaf Zn concentration of 12 ppm, which was 33% higher compared with 9 ppm in plastic containers. Both the highest and lowest plant average Zn concentrations were found in plants fertilized with 5 mM N, with the highest, 15.3 ppm, in plants grown in biocontainers and the lowest, 10.2 ppm, in those grown in plastic containers. In addition, one irrigation per day resulted in higher root Zn concentration (17 ppm) than two irrigations per day (16 ppm) (Table 2).

Boron. Plant average B concentration (range, 15–27.8 ppm) was affected by the interaction between N rate and container type (Fig. 9). With such an interaction, biocon-

tainers resulted in higher plant B concentrations than plastic containers with N rates of 15 and 20 mM. Leaf B concentration was affected by the main effects of N rate, container type, and irrigation frequency without interaction. Nitrogen rates of 0, 5, and 10 mM resulted in comparable leaf B concentrations but higher leaf B concentration than 15 or 20 mM N. Biocontainers resulted in a 22% increase in leaf B concentration (61 ppm) compared with plastic containers (50 ppm) (Table 1). One irrigation per day resulted in a 7.4% increase in leaf B concentration (58 ppm) compared with two irrigations per day (54 ppm) (Table 2). Stem B concentration was affected by the main effects of N rate and container type without interaction; plastic containers resulted in a 10% increase in B concentration compared with biocontainers. Root B concentration was affected by the interaction among N rate, container type, and irrigation frequency. Plants fertilized with 5 mM N grown in biocontainers irrigated with both frequencies and those fertilized with 20 mM N grown in biocontainers irrigated once per day resulted in higher root B concentrations than any other treatment combination.

Micronutrient contents (μg per plant). Micronutrient contents in the entire plant, leaves, stems, or roots generally shared similar trends, which were all affected by the

interaction between N rate and container type (Figs. 5–9). Total micronutrient content in plants grown in biocontainers had an increasing trend with increasing N rate, without differences between 15 and 20 mM N. In plastic containers, there was no difference in total plant micronutrient contents among N rates of 10, 15, or 20 mM N. Therefore, biocontainers resulted in higher total plant micronutrient content than plastic containers with N rates of 15 or 20 mM. Plants fertilized with 15 and 20 mM N grown in biocontainers had the highest micronutrient contents in the entire plant or in any structure type among all treatment combinations; however, the Zn content in roots did not vary as much among treatment combinations.

In addition to the effects created by the N rate and container type interaction, root contents of Fe, Cu, Zn, and B were also affected by the main effect of irrigation frequency. One irrigation per day resulted in 19.5%, 30.4%, 22.5%, and 17.2% increased contents of Fe, Cu, Zn, and B compared with two irrigations per day, respectively (Table 2).

Allocation of nutrients in different structures. In general, content of macronutrients (P, K, Ca, and Mg) were the highest in leaves, higher than those in stems, with roots having the lowest macronutrient contents (Table 3). In comparison, the highest contents of Fe, Mn, Zn, and Cu were found in roots, higher than

Fig. 8. Concentrations (ppm) and contents (μg per plant) of zinc (Zn) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction between nitrogen (N) rate and container type (A, C, D, E, F, G, and H), or unaffected by N rate (B). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When unaffected by the N rate (B), the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Fig. 9. Concentrations (ppm) and contents (µg per plant) of boron (B) in the entire plant, leaves, stems, or roots of Encore® azalea 'Chiffon' affected by the interaction among nitrogen (N) rate, container type, and irrigation frequency (D), by the interaction between N rate and container type (A, E, F, G, and H), or by the main effect of N rate (B and C). One-year-old liners of Encore® azalea 'Chiffon' were fertilized with N rates of 0, 5, 10, 15, or 20 mM from NH_4NO_3 , grown in 1-gallon black plastic containers or paper biocontainers, and irrigated once or twice per day with the same total irrigation volume. When affected by the N main effect, the average at each N rate was obtained by averaging data from both container types and irrigation frequencies. Different lowercase letters within a chart suggest significance differences indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

Table 3. Allocation of macro- and micronutrients among structure types within Encore® azalea 'Chiffon' plants.

	Macronutrient content (mg per plant) ²				Micronutrient content (µg per plant)				
	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
Leaf	8.1 a	50.4 a	36.6 a	18.5 a	202 b	204 c	46 b	9 c	253 a
Stem	4.1 b	24.6 b	15.9 b	9.2 b	139 c	423 b	51 b	15 b	43 b
Root	2.8 c	7.4 c	7.3 c	4.6 c	264 a	481 a	79 a	26 a	32 b

²Different lowercase letters within a column suggest significance differences in nutrient contents among structure types of azalea plants indicated by the LSMEANS procedure in SAS using the stimulate adjustment with $P \leq 0.05$.

those in leaves or stems. For instance, roots had the highest content of Mn of 481 µg per plant, which was higher than those in stems of 423 µg or in leaves of 204 µg per plant. Different from most micronutrients, leaf B content was the highest (average, 253 µg per plant) when compared with those in stems (average, 43 µg per plant) and roots (average, 32 µg per plant).

Discussion

Plant average concentrations of tested macronutrients (including P, K, Ca and Mg) in Encore® azalea 'Chiffon' were the lowest without N and were at deficient levels for container-grown azalea plants (Mills and Jones, 1996; Scagel et al., 2012), suggesting that N deficiency has limited uptake of other nutrients. As described by Marschner (2012), increasing the supply of just one nutrient with

a deficient level will stimulate growth but may induce deficiency of other nutrients by dilution. In our previous study, we found that increasing N rate from 0 increased plant growth index (PGI) and plant dry weights of Encore® azalea 'Chiffon' (Li et al., 2018). The highest concentrations of P, K, Ca, and Mg were found in plants fertilized with 15 mM or 20 mM N, suggesting that the increased N supply did not cause a significant diluting effect on the uptake of other macronutrients in this study. The increasing effects of high N rate on nutrient uptake likely resulted from increased plant growth. The trends of plant micronutrient concentrations (including Fe, Mn, Cu, Zn, and B) were not as clearly associated with N rate. However, plant average concentrations of Fe, Mn, Cu, Zn, and B tested in our study were generally lower than those reported for container-grown azalea cultivars (Mills and Jones,

1996). A possible reason for this might be that Encore® azalea 'Chiffon' was one of the most dwarf cultivars and may have low micronutrient requirements.

In this study, high N rates of 15 and 20 mM increased macro- and micronutrient uptake (in terms of contents in azalea plants) to varying degrees regardless of their concentrations. The increase of macro- and micronutrient uptake were not proportional to N rate (data not shown). Scagel et al. (2008b) reported that when changing N rate in fertilization management, proportional changes of other nutrients may not be necessary. There are various nonspecific and specific interactions between nutrients in plants (Marschner, 2012). Such interactions are more important when certain nutrient elements are at critical deficient levels. Optimal ratios among nutrients vary with plant species, growing stages (vegetative or reproductive), and environmental conditions (Marschner, 2012). Therefore, both nutrient concentrations and ratios among nutrients should be investigated to evaluate plant nutritional stage.

The effects of increasing N rate on nutrient uptake varied between the two container types tested. With N rates of 0 to 20 mM, PGI and total dry weight increased from 0 to 10 mM and peaked in plastic containers, but they increased from 0 to 15 mM N and peaked in biocontainers. As a result, the paper biocontainers resulted in improved vegetative

growth of Encore® azalea ‘Chiffon’, with greater PGI, total plant dry weight, leaf area, root length, and surface area compared with plastic containers with N rates of 10, 15, or 20 mM (Li et al., 2018). In this study, the effects of biocontainers on improving uptake of both micro- and macronutrients, thereby improving plant growth, were possibly similar. As discussed in the previous study, such beneficial effects of paper biocontainers for azalea were likely related to their porous structure, which provides better drainage and possibly evaporative cooling effects through the container side wall (Nambuthiri et al., 2015; Wang et al., 2015).

One irrigation per day improved micro-nutrient uptake by increasing leaf B concentration, root concentrations of Cu and Zn, and root contents of Fe, Cu, Zn, and B. One irrigation per day was also considered beneficial to the growth of azalea compared to two irrigations per day due to the possible improved substrate drainage between irrigations (Li et al., 2018). More frequent irrigation also decreased uptake of P, B, and Mn in three *Rhododendron* species, which was consistent with our results. Such effects of irrigation frequency on nutrient uptake, resulting in effects on plant growth and N uptake, were considered indirect by Scagel et al. (2012). However, one irrigation per day decreased leaf K concentration, which may have resulted from more leaching from the substrate compared with two irrigations per day. The beneficial effect of delivering the same amount of water through one irrigation compared to two irrigations per day was species-dependent, where *Hydrangea macrophylla* ‘Supreme’, a species having high irrigation requirement, showed increased PGI with two irrigations per day compared with one irrigation (Li et al., 2019).

Allocations of macro- and micronutrients were consistent with those reported by Scagel et al. (2012), who studied three *Rhododendron* species. Macronutrients, except for Ca, are generally mobile and accumulated most in aboveground structures compared with roots; however, micronutrients accumulated most in roots. Root accumulation of Fe, Mn, Zn, and Cu may have occurred because of their low mobility (Barker and Pilbeam, 2007; Strik, 2015). The highest content of B in leaves compared with stems or roots likely resulted from the mobility of B in plant phloem (Marschner, 2012). In addition, allocations of different nutrient elements were related to the sink-source relationship within a plant (Marschner, 2012). For example, shoot apices, young leaves, and fruits are sinks of Ca. Although Ca has low phloem mobility, the demand of Ca by these sinks are satisfied by a high rate of xylem volume flow into these organs.

Conclusion

Nitrogen deficiency limits plant’s ability to take up mineral nutrients, with zero N resulting in the lowest concentrations of P, K, Ca, and Mg averaged in Encore® azalea ‘Chiffon’

plants. High N rates of 15 and 20 mM resulted in the highest plant concentrations of P, K, Ca, and Mg. With a sufficient N supply (15 and 20 mM N), biocontainers resulted in higher content of macro- and micronutrients in azalea plants compared to plastic containers. One irrigation per day was beneficial to root micro-nutrient contents (including Fe, Zn, Cu, and B) over two irrigations per day. The beneficial effects of high N rates (15 and 20 mM) combined with the paper biocontainers tested in this study on mineral nutrient uptake were likely to be indirect through stimulating growth of azalea plants.

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