Impact of Nitrogen Concentration on Stock Plant Yield and Cutting Performance of 'Purple Small Leaf' and 'Raspberry Ice' Bougainvilleas[®]

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Effects of nitrogen (N) concentration on yield, cutting quality, and rooting performance were investigated to establish a nitrogen-based fertility program for bougainvillea stock plants. Stock plants of 'Raspberry Ice' and 'Purple Small Leaf' were fertigated with N at concentrations of 100, 200, or 300 mg·L⁻¹. Overall 'Purple Small Leaf' stock plants produced more and longer cuttings than 'Raspberry Ice', however rooting performance was better with 'Raspberry Ice'. The greatest cutting yields were achieved with N at 200 mg·L⁻¹, and shorter cuttings with smaller leaf areas occurred when stock plants were fertilized with N at 100 mg·L⁻¹. Cutting diameter was not influenced by N concentration. Undesirable leachate nitrate nitrogen values were observed from stock plants fertilized with N at 300 mg·L⁻¹ after cuttings were harvested 14 and 22 weeks after potting. Based on these results, stock plants of 'Raspberry Ice' and 'Purple Small Leaf' should be fertilized with N at 100 to 200 mg·L⁻¹.

INTRODUCTION

Because the popularity of gardening with tropical annuals, perennials, and vines has grown in the U.S., there has been a considerable increase in production and sales of tropical plants over the past several years (Bowden, 2004). Shortages and poor quality cuttings supplied by domestic propagation firms has become a concern for growers wishing to produce tropical plants for the spring season. Propagators have expressed a need for standardizing production practices to maximize cutting yield and subsequent adventitious root formation in propagules; however, little is published on how to effectively manage tropical stock plants (Dole and Wilkins, 1999; Hartmann et. al, 2002).

A tropical species that has potential to be more widely used by gardeners is bougainvillea (*Bougainvillea glabra*). This multi-use plant grows best in U.S. landscapes during summer months with attractive bracts displayed during short-days. Unfortunately because of its warm temperature production requirements and difficulty in propagation, commercial availability is limited (Czekalski, 1989). Bougainvillea is generally propagated from stem cuttings or air-layering, but often fail to produce roots, and rooting percentage is low, even during the cultivated seasons (Chakraverty, 1970). The root systems are also known to be extremely fine and fragile. Bougainvillea root best from semihardwood to hardwood cuttings depending on temperature (Schoellhorn and Alvarez, 2002), location (Auld, 1987), and time of year (Chakraverty, 1970). These plants have been documented to benefit from bottom heat with rooting substrate temperatures of 30 °C (86 °F) (Singh et al., 1976).

Nitrogen (N) concentration is crucial in cuttings as it is important for nucleic acid and protein synthesis in plant tissue (Hartmann et al., 2002). When considering the role N has in these metabolic processes such as protein synthesis, one could strongly argue its importance in root initiation (Blazich, 1988). Nitrogen concentration also affects cutting yield of stock plants and rooting performance of cuttings. While excessive N may negatively affect cutting propagation (McAvoy, 1995), heavy fertilization in some tropical plants such as bougainvillea, has been observed to inhibit flowering and promote growth (Schoellhorn, 2002).

Although optimum nutrient levels of tropical stock plants remains unclear, it is crucial that propagules come from a nutritionally healthy source (Hartmann et al., 2002). Limited research has been conducted in the management of bougainvillea stock plants for purposes of maximizing quantity and quality of cuttings suitable for propagation. Our objective was to evaluate the impact of three nitrogen concentrations on stock plant yield and cutting performance of *Bougainvillea* 'Purple Small Leaf' and *B. × buttiana* 'Raspberry Ice'.

MATERIALS AND METHODS:

Rooted stem cuttings of B. × buttiana 'Raspberry Ice' and B. 'Purple Small Leaf' were transplanted, two per pot, into 2.8-L (0.74-gal) (15.2-cm [6-inch diameter]) round plastic containers on 15 Oct. 2004. The root substrate was Fafard 2 (Fafard, Anderson, South Carolina), which contained 6.5 sphagnum peat : 2 perlite : 1.5 vermiculite (by volume). A continual liquid fertilization program was initiated on 8 Nov., with N concentration levels at 100, 200, or 300 mg·L⁻¹. Phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) were held constant at 20, 200, 100, and 50 mg·L¹, respectively. Micronutrients were provided using soluble trace elements in solution (S.T.E.M., J.R. Peters, Inc., Allentown, Pennsylvania) at a rate of 6.78 mg·L⁻¹. Supplemental iron was applied using FeEDTA (Sequestrine) at a rate of 1.76 mg·L⁻¹. Ammoniacal-nitrogen was 6% to 35% of the total nitrogen in all treatments. Plants were fertigated by a submersible pump drip irrigation system. The experiment was a randomized complete block design with six single-plant replications of each of three treatments. Stock plants were grown in a greenhouse under natural photoperiod and irradiance with day/night temperatures of 21/18 °C (70/65 °F). Stock plants were pinched on 8 Nov. and 6 Dec. to develop a canopy for harvesting cuttings.

Leachate samples were initially collected 4 weeks after potting (WAP) and biweekly thereafter until 28 WAP via a modified Virginia Tech Extraction Method (VTEM) (Wright, 1986) to determine pH, electrical conductivity (EC), and nitrate nitrogen (NO₃-N). Distilled water (85 ml) was applied to the substrate surface to displace 50 ml of leachate. Leachate solutions were analyzed for pH (pHep pH meter; Hanna Instruments, Woonsocket, Rhode Island), EC (DiST WP4 EC meter; Hanna Instruments), and NO₃-N (Horiba Compact Ion Meter C-141 Spectrum Technologies, Inc., Plainfield, Illinois).

Stock plants were maintained in Milton, Florida, and harvested 14, 19, 22, 26, 31, and 36 WAP on 17 Jan. (H1), 22 Feb. (H2), 15 March (H3), 11 April (H4), 16 May (H5), and 13 June (H6). Harvesting protocols consisted of removing the shoot tip 0.5 cm above the first recently mature leaf node, and excised thereafter 1 cm below the fourth node.

The number of cuttings was recorded at each harvest, and three cuttings per replicate from each treatment were selected randomly 14, 22, and 31 WAP to measure stem base diameter (mm) and shoot length (cm). Cutting leaf area was also measured with a LI-COR LI-3100 portable area meter (LI-COR, Lincoln, Nebraska).

Cuttings harvested 19, 26, and 36 WAP were packaged in moist towels, inserted into perforated plastic bags, and box shipped to Gainesville, Florida, for propagation.

Unless otherwise noted, basal portions of cuttings (1 cm) were inserted into indole-3-butyric acid, potassium salt (K-IBA) at 3,000 mg·L⁻¹ for 3-sec then inserted into $3.3 \times 8.8 \times 6$ cm ($1.3 \times 3.5 \times 2.4$ inch) 6-cell containers in a substrate consisting of 4 perlite : 1 vermiculite (v/v) and placed under intermittent mist. Cuttings were maintained under natural photoperiod and irradiance with day/night temperatures of 20/18 °C (68/64 °F) and misted daily for 10-sec every 30 min (7:00 AM– 7:30 PM). Bottom heat was provided at 24 °C (75 °F). The experimental design was a completely randomized design with three N treatments with three replications per treatment and six sub-samples per replication. Exceptions to the study included using a rooting substrate of 1 sphagnum peat : 1 perlite (v/v) for H2 and 100% perlite for H4.

Adventitious roots \geq 1.0 mm in length were evaluated 4 and 6 weeks after sticking (WAS). The numbers of primary and secondary roots were counted and recorded 4 WAS. Cuttings were evaluated for rooting quality based on a 0 to 5 scale 4 and 6 WAS: 0 = no roots; 1 = minimal rooting; 2 = minimal, uneven rooting; 3 = moderate, uneven rooting; 4 = moderate, uniform rooting; 5 = well-developed rooting. Root quality values were reassigned relative to each cutting at each evaluation date.

Data were subjected to analysis of variance using general linear model procedures (SAS Inst., Cary, North Carolina). Means were separated by least significant differences (LSD) at $P \le 0.05$.

RESULTS

Leachate Analysis. As N concentration increased, leachate pH decreased for both cultivars. Fertilizing stock plants of 'Raspberry Ice' with N at 100, 200, or 300 mg·L⁻¹ produced pH values of 6.6, 6.3, and 5.8, respectively, while fertilizing 'Purple Small Leaf' with N at 100, 200, or 300 mg·L⁻¹ produced pH values of 6.8, 6.3, and 5.9, respectively. Bougainvillea performed best at pH values of 5.5 to 6.0 (Schoellhorn, 2002). In our study pH values \geq 6.0 may be attributed to the low percentage of NH₄-N in the fertilizer treatment or limestone activation in the root substrate.

There was a significant treatment by week interaction for leachate EC for both cultivars. Raspberry Ice' stock plant EC values peaked for concentrations of N at 200 and 300 mg·L⁻¹ following harvests 14 and 22 WAP. A similar trend occurred for N at 300 mg·L⁻¹ with 'Purple Small Leaf'. Greater differences amongst N concentrations occurred with 'Raspberry Ice' than 'Purple Small Leaf' (data not shown). Similarly, leachate NO₃-N levels increased following the second pinch and after a cutting harvest H1 (14 WAP) and H2 (19 WAP). These differences were greater with N at 300 mg·L⁻¹; significant differences in treatment were less common when stock plants were fertilized with 200 mg·L⁻¹ and almost nonexistent when fertilized with 100 mg·L⁻¹. Leachate NO₃-N levels were not significantly different between cultivars.

Cutting Quantity. For H1 the cultivar × treatment interaction was not significant, therefore only the main effects are presented. Stock plants of 'Purple Small-Leaf' (6.3) produced 150% more cuttings than 'Raspberry Ice' (4.1) (LSD = 1.0, n = 18). Stock plants produced 147% more cuttings when fertilized with N at 200 mg·L⁻¹ N than with N at 100 mg·L⁻¹ (data not shown). For H2, the cultivar × treatment interac-

tion was significant for stock plant yield. 'Purple Small-Leaf' fertilized with N at 200 $mg \cdot L^{-1}$ or 300 $mg \cdot L^{-1}$ produced 162% more cuttings than when fertilized with N at 100 mg·L⁻¹ (Table 1). 'Raspberry Ice' produced six cuttings per plant, regardless of N concentration. For H3 the cultivar × treatment interaction was not significant with 'Purple Small Leaf' stock plants producing 195% more cuttings than 'Raspberry Ice' (9.1 vs. 4.7) (LSD = 1.5, n = 18). When fertilized with N at 200 mg·L⁻¹, stock plants of bougainvillea produced 8.1 cuttings per plant, which was 142% more than when fertilized with N at 100 mg mg·L¹, which yielded 5.7 (LSD = 1.9, n = 18). For H4, the cultivar \times treatment interaction was not significant. 'Purple Small Leaf' stock plants produced 16.3 cuttings, while 'Raspberry Ice' produced 5.6 (LSD = 2.1, n = 18). Stock plants produced 143% more cuttings at 200 or 300 mg·L^{·1} when compared to 100 mg·L^{\cdot 1} (data not shown). For H5 the treatment × cultivar interaction was significant for yield. For 'Raspberry Ice' no significant differences in treatment existed with 7.7 cuttings produced per plant. When 'Purple Small Leaf' was fertilized with $200 \text{ mg}\cdot\text{L}^{-1}$, 131% to 137% more cuttings were generated than with the 100 or 300 mg L^{-1} treatments, respectively (Table 1). For H6 the treatment × cultivar interaction was significant. When 'Raspberry Ice' was fertilized with N at 300 mg·L⁻¹, 139% more cuttings were produced than when fertilized with N at $100 \text{ mg} \cdot \text{L}^{-1}$ (data not shown). Stock plants of 'Purple Small Leaf' when fertilized with N at 200 mg L^1 or $300 \text{ mg} \cdot \text{L}^{-1}$ yielded at least 180% more cuttings than when fertilized with N at 100 mg·L^{·1} (Table 1).

	Harvest						
N conc. (mg·L ^{·1})	H1	H2	H3	H4	H5	H6	
100	5.50	7.83	7.83	12.83	24.33	25.00	
200	7.67	12.67	10.33	18.67	31.83	45.00	
300	5.83	13.67	9.16	15.50	23.17	46.67	
Significance ^z	*	*	NS	NS	*	*	
LSD	2.64	3.62	_	_	6.58	14.22	

Table 1. Effect of N concentration on cutting yield of 'Purple Small Leaf' bougainvillea harvested 14 (H1), 19 (H2), 22 (H3), 26 (H4), 31 (H5), and 37 (H6) weeks after potting.

^{*z*} *, Significance at $P \le 0.05$.

Cutting Quality. Only the first harvest was significantly different in basal stem diameter, with 'Purple Small Leaf' having a diameter of 1.9 mm, while 'Raspberry Ice' had a diameter of 2.2 mm (LSD = 0.02 n = 18). For H1 stock plants fertigated with N at 100 mg·L⁻¹ produced 16% shorter cuttings than cuttings generated from stock plants fertigated with N at 200 or 300 mg·L⁻¹ (Table 2). The treatment × cultivar interaction was significant for leaf area. Cuttings harvested from stock plants of 'Raspberry Ice' had a leaf area of 36.1 cm² and was not statistically different amongst N concentrations. When stock plants of 'Purple Small Leaf' were fertilized with N at 200 or 300 mg·L⁻¹, leaf area was significantly greater, 27.5 or 31.4 cm², respectively, than 100 mg·L⁻¹ (21.4 cm²) (LSD = 5.19 n = 6). For H3, 'Purple Small-Leaf' produced 16% longer stems than 'Raspberry Ice' (8.82 vs. 7.4 cm) (LSD = 0.87,

N conc.		Harvest	
$(mg \cdot L^{\cdot 1})$	H1	H3	H5
100	5.56	7.21	6.79
200	6.58	8.29	7.33
300	6.95	8.90	7.83
Significance ^z	*	*	NS
LSD	0.82	1.09	_

Table 2. Effect of N concentration on cutting length (cm) harvested 14 (H1), 22 (H3), 31 (H5) weeks after potting.

^z *, Significance at $P \le 0.05$.

Table 3. Rooting performance values four weeks after sticking from stock plants of bougainvillea for cuttings harvested 37 weeks after potting (H6).

	Survival (%)	Root quality rating	Primary roots (no.)	Lateral roots (no.)
'Raspberry Ice'	94	2.8	3.5	10.7
'Purple Small Leaf'	72	1.4	0.4	1.7
Significance ^z	*	*	*	*
LSD	17	3.2	1.4	3.2

^{*z*} *, Significance at $P \leq 0.05$.

n = 18). Stock plants fertilized with N at 300 mg·L⁻¹ produced 19% longer cuttings than 100 mg·L⁻¹ (Table 2). No significant differences in leaf area were observed between cultivars or amongst treatments with a mean of 34.4 cm². For H5, stock plants produced shoots 7.3 cm long (Table 2). 'Purple Small Leaf' (28.8 cm²) had a larger leaf area than 'Raspberry Ice' (23.7 cm²) (LSD = 2.98, n = 18).

Rooting Performance. For H2, no significant differences at four or six WAS were detected among N concentrations or between cultivars for percentage survival and root quality rating. At 4 WAS there were 13.8 lateral roots on cuttings harvested from stock plants fertilized at 100 mg·L⁻¹, when compared to 3.7 or 2.7 lateral roots on cuttings from 200 or 300 mg·L¹, respectively (LSD = 6.7 n = 6). For H4, the cultivar \times treatment interaction for number of roots was not significant, however 'Raspberry Ice' produced 250% more primary roots and 460% more lateral roots than 'Purple Small Leaf' (data not shown). Nitrogen concentration had no influence on primary or lateral root number. There was a cultivar × treatment interaction for cutting percent survival. For 'Raspberry Ice', no differences were measured, however 'Purple Small Leaf' produced cuttings that survived better with $100 \text{ mg} \cdot L^{-1}(17\%)$ than with 300 mg·L⁻¹ (0%). The highest percentage occurred with 200 mg·L⁻¹ (39%) (LSD = 0.13, n = 3). Cuttings evaluated 6 WAS from plants fertilized with N at 300 $\text{mg}\cdot\text{L}^{-1}$ had higher survival percentage (61%) than those fertilized with 100 mg $\cdot\text{L}^{-1}$ (33%) (LSD = 0.21, n = 6). For H6, the root quality rating 4 WAS higher with N at $200 \text{ mg} \cdot \text{L}^{-1}$ (2.6) than 100 (2.0) or $300 \text{ mg} \cdot \text{L}^{-1}$ (1.8) (LSD = 0.52, n = 6). A similar trend occurred with number of lateral roots: 9.3, 3.8, 4.7, for 200, 100, and 300 mg L^1 , respectively (LSD = 3.96 n = 6). Percentage survival was higher at 200 mg L¹ than $100 \text{ mg} \cdot \text{L}^{-1}$ (data not shown). Percent survival and root quality rating was higher,

and number of roots was greater for 'Raspberry Ice' when compared to 'Purple Small Leaf' (Table 3). Rooted cutting quality ratings at 6 WAS were better at 100 (3.14) or 200 mg·L⁻¹ (3.43) than 300 mg·L⁻¹ (2.40) (LSD = 0.69, n = 6). Similar to 4 WAS, cuttings from stock plants of 'Raspberry Ice' demonstrated higher root quality ratings than cuttings of 'Purple Small Leaf' 6 WAS (4.20 vs. 1.78) (LSD = 0.65, n = 9).

DISCUSSION

While 'Purple Small Leaf' stock plants produced more cuttings at each harvest than the variegated cultivar Raspberry Ice, rooting performance was lower in this predominantly green-leafed cultivar. In order to improve cutting performance, stock plants of 'Purple Small Leaf' should be fertilized at 100 mg·L⁻¹ during the first few harvest dates, then increased to 200 mg·L⁻¹ to improve root number and quality as stock plants age.

Propagators of tropical plants need to adopt a nutrient program that produces the maximum quantity of high quality cuttings so that growers can profit from the specialized cultivation of stock plants. Relating yield, quality, and rooting performance of cuttings from stock plants fertilized at different N concentrations was a means of achieving this goal. Overall, stock plants of bougainvillea had the greatest yield and optimal cutting performance with N at 100 to 200 mg·L⁻¹. Fertilizing with 300 mg N per L did not enhance yield and higher, undesirable leachate nitrogen levels compared to 200 mg N per L, after cuttings were harvested 14 and 22 WAP.

Future studies will investigate the impact of nitrogen concentration on cutting quantity and quality during warm/high irradiance and cool/low irradiance periods of the year using 'Raspberry Ice' and 'Purple Small Leaf'.

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