Surveying Changes in Sectional Tissue Nutrient Concentrations of *Hibiscus acetosella* ‘Panama Red’ during Adventitious Root Formation

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Abstract. A study was conducted to observe changes in mineral element concentrations within different sections of leafy stem cuttings of *Hibiscus acetosella* ‘Panama Red’ (PP20121) during a 21-day propagation period under standard industry propagation conditions. Concentrations of 13 mineral elements were analyzed in leaves, lower stems (below substrate), upper stems (above substrate), and roots at 3-day intervals. Before root emergence (day 0–6), P, K, Zn, Ca, and Mg concentrations decreased in the shoots (including upper stems and leaves), whereas Zn, Ca, and B concentrations decreased in the lower stems. Sulfur increase occurred in lower stems before root emergence. After rooting (day 9–21), N, P, Zn, Fe, Cu, and Ni concentrations decreased in the roots; K, S, B, and Mg concentrations increased. In the lower stems, N, P, K, S, and Zn concentrations decreased, whereas B increased. Potassium concentration decreased in the leaves; P, K, S, and Zn decreased in the upper stems. Calcium and Mg increased in leaves. This study indicates specific nutrients are important in adventitious rooting, and that it is important to analyze rooting as a function of fine-scale temporal measurements and fine-scale sectional measurements.

Adventitious root formation (ARF) is the process by which roots arise from nonroot plant tissue (Bellini et al., 2014; Davis and Haissig, 2013). Effectively using ARF for plant propagation is a cornerstone of the ornamental horticulture industry as it allows for plants with preferred traits to be propagated asexually and thereafter perpetuated indefinitely. The process by which excised stem sections develop adventitious roots can be divided into two distinct stages; cells of the existing tissue must first dedifferentiate, and then these cells must redifferentiate into root cells (Hartmann et al., 1990).

Several elements, including N, P, B, Ca, Mg, Mn, and Zn, are necessary for ARF (Anger, 1986; Blazich, 1988). Calcium is crucial to ARF because it is required for root primordia formation, cell expansion, and

cell division (White and Broadley, 2003). Indole-3-acetic acid (IAA) is the primary hormone involved in ARF (Bellini et al., 2014; Verstraeten et al., 2014). Micronutrients, including B and Zn, are necessary for IAA metabolism (Jarvis et al., 1984; Kazan, 2013; Martín-Rejano et al., 2011). Root formation is stimulated by Fe availability (Giehl et al., 2012) and inhibited by S deficiency (Dan et al., 2007), and these effects are probably related to auxin activity (Kazan, 2013). There is some evidence that Cu can promote root formation and growth when applied at subtoxic concentrations, but the precise role of endogenous Cu in root formation is not known (Arnold et al., 1994; Chang and Lin, 2006; Dunn et al., 1997; Schwambach et al., 2005). Other macro- and micronutrients are required for subsequent growth and development after roots have formed (Bellini et al., 2014; Zhang and Forde, 2000).

Several studies have examined tissue nutrient concentrations of leafy stem cuttings (henceforth referred to as “cuttings”) during vegetative propagation in nutrient-free media. Good and Tukey (1967) showed that P was mobilized from older leaves to lower stems during the initial stages of ARF in *Chrysanthemum morifolium* (Ramat.). Nitrogen and K also became mobile after roots developed; N and P translocated from older leaves to expanding leaves and roots,

whereas K moved from the older leaves to expanding leaves only. Calcium concentrations remained constant throughout the study. Blazich and Wright (1979) analyzed N, P, K, Ca, and Mg concentrations in the leaves, upper, and lower stems of box-leaved holly [*Ilex crenata* (Thunb.)]. Concentrations of all nutrients in the leaves, upper, and lower stems remained constant over a 27-d propagation period; no nutrient mobility was observed. In a later study, nutrient movement was characterized in box-leaved holly cuttings with and without auxin treatment under intermittent mist (Blazich et al., 1983). No nutrient mobilization occurred while the roots were forming. However, following root initiation and subsequent budbreak, N, P, K, Ca, and Mg were translocated from leaves to upper stems in untreated cuttings to support new shoot growth. In auxin-treated cuttings, N, P, and K were mobilized to the roots, probably to support enhanced root proliferation. Svenson and Davies (1995) analyzed several macro- and micronutrient concentrations in the leaves and basal stems of poinsettia stem cuttings during ARF. Before root development; Cu, Fe, and Mo accumulated in the basal stems, whereas concentrations of P, K, Ca, and Mg declined. After root initiation, B, Cu, Fe, Mg, Mn, Mo, and Zn concentrations increased in the basal stems. Foliar N, Fe, and Mo concentrations declined over the course of the study, suggesting that Fe and Mo may have been translocated from the leaves to lower stems.

This study was conducted to survey changes in nutrient concentrations in *Hibiscus acetosella* (Hiern.) Welw. ‘Panama Red’ (PP20121), a fast-rooting species, under standard commercial production conditions. Cuttings were harvested once every 3 d and divided into four sections for analysis: leaves, upper stems, lower stems, and roots (including callus). Tissue concentrations of 13 mineral elements (N, P, K, B, Ca, Cu, Fe, Mg, Mn, Mo, Ni, S, and Zn) were analyzed. Cuttings were stuck in a standard growing medium with starter fertilizer and kept under shade with intermittent mist to replicate industry practices.

Materials and Methods

Plant material. Cuttings of *H. acetosella* ‘Panama Red’ (PP20121) were obtained from breeder stock at the University of Georgia and rooted in a Fafard Nursery Mix (Conrad Fafard, Inc., Anderson, SC). Rooted liners were potted into 1671-cm³ containers (Nursery Supply, Kissimmee, FL) filled with Fafard Nursery Mix, then top-dressed with 15 g of a 19–6–12 (19.0N–2.6P–11.6K plus micronutrients) 5–6 month slow-release fertilizer (Harrell’s LLC, Lakeland, FL). Liners were then grown for 4 weeks in a greenhouse with an average daily light integral of 21.04 ± 1.31 mol·m⁻²·d⁻¹ and average temperature of 26.1 ± 0.17 °C. Terminal cuttings of ≈ 10 cm with five nodes were harvested from stock plants and set to a depth of 3 cm in 72-cell

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flats, one cutting per cell, filled with substrate (Fafard 3B mix, Conrad Fafard, Inc.) containing an industry-standard starter fertilizer containing (in ppm) 7.84 Ca, 5.99 K, 1.75 Mg, 3.73 $\text{NH}_4\text{-N}$, 4.53 $\text{NO}_3\text{-N}$, 3.84 P, and 0.3 Zn. All but the top two fully expanded leaves were removed, and cuttings were placed on a mist bench in a glass-covered greenhouse and shaded with a 60% shade cloth (Griffin Greenhouse & Nursery Supplies, Inc., Ball Ground, GA). Cuttings were misted every 10 min for 6 s from 0700 to 1900 HR with water at a pH of 7.1 and hardness of 5.9; containing (in ppm) <0.03 Al, 0.1 B, 1.36 Ca, 12.4 S, <0.01 Cr, <0.01 Cu, <0.01 Fe, <0.20 K, 0.62 Mg, <0.01 Mn, <0.01 Mo, 187.3 Na, <0.01 Ni, <0.06 P, 1.91 Si, and 0.03 Zn.

Cutting harvest and sample preparation. Cuttings were harvested from stock plants on day 0 and set. Beginning at day 0, cuttings were harvested every 3 d for 21 d. Six cuttings per replication among three replications were randomly collected at each harvest date. The samples were bulked by replication for each treatment and placed in a freeze dryer for 48 h, then ball-mill ground to less than 250- μm particle size. Five additional cuttings were harvested, divided, oven-dried, and weighed for dry weight determination. The study was ended after 21 d, when the cuttings were fully rooted.

Nutrient analysis. Samples were submitted to the University of Georgia Agricultural and Environmental Services Laboratory for analysis. A 250-mg (± 2 mg) subsample of each tissue sample was weighed and placed into a microwave digestion vessel (CEM Corporation, Matthews, NC). Ten mL HNO_3 was added to each vessel and the mixture was allowed to sit for 15 min. Samples were then placed in a microwave digestion system (CEM Mars 5; CEM Corporation) for acid digestion. Once cooled, samples were placed in 100-mL volumetric flasks and diluted with deionized water. This solution was used for the analysis of B, Cu, Fe, Mn, Mo, Ni, and Zn, then diluted further in deionized water (1:10), for P, K, Ca, and Mg analysis. Solutions were analyzed using an inductively coupled argon plasma spectrometer (Thermo Jarrell-Ash Enviro I; Thermo Jarrell-Ash, Franklin, MA). An additional 150–200 mg of each sample was weighed, and S content was determined using a carbon, nitrogen, and sulfur analyzer (CNS 2000; LECO Corporation, St. Joseph, MI). Nitrogen was measured by weighing 1.5–2.5 mg of sample (± 0.001 μg) into 5 $\times$ 9 mm tin capsules (Cole-Parmer, Vernon Hills, IL) and analyzed at the Stable Isotope/Soil Biology Laboratory of the University of Georgia Odum School of Ecology using an elemental analysis isotope ratio mass spectrometer.

Statistics. Data were analyzed using analysis of variance (ANOVA) and post hoc Tukey's honestly significant difference (HSD) using SAS 9.2 (SAS Institute Inc., 2012) to determine whether there were differences in nutrient concentrations within each tissue between different measurement days. To distinguish between changes in nutrient concentration before (day 0–6) and

after (day 9–21) root initiation, the data were separated into two sets for each tissue and nutrient combination. The separated data were analyzed by ANOVA and post hoc Tukey's HSD. Dry weight data were analyzed using generalized linear model to ascertain whether growth occurred in shoots (leaves and upper stems) or roots (lower stems and roots) between day 0 and day 21. Paired *t* test was used to compare means of N content between day 6 and day 9.

Results and Discussion

Tissue nutrient concentrations before root initiation. Visible callus formation was observed on day 9. Shoot dry mass (leaf, upper and lower stems) did not increase during the 21-d course of the experiment ($0.48 \text{ g} \pm 0.13$). From days 0–6 (precalls and root formation), foliar concentrations of P, K, Zn, Ca, and Mg decreased. Zinc and Mg were also lost from the upper stems (Table 1). Nutrient leaching typically occurs in cuttings under intermittent mist (Tukey, 1978; Wilkerson et al., 2005; Wott and Tukey, 1967). Although decreases in tissue nutrient concentration can also be due to dilution, this would require growth and expansion of the cutting (Blazich, 1988; Blazich et al., 1983; Svenson and Davies, 1995).

In this study, Zn, Ca, and B concentrations decreased in the lower stems before root formation (Table 1). Although nutrient leaching can occur through stems (Good and Tukey, 1966; Tukey, 1970), it is not clear why only these nutrients were lost in this manner. Calcium loss from the basal stems was also observed in poinsettia stem cuttings in previous research (Svenson and Davies, 1995). Since these elements are crucial for root formation and growth, but were lost from the site of root development, perseverance tissue concentrations could influence ARF success and should be optimized in stock plants. Calcium concentration of the cuttings is likely to have an influence on root primordia formation and elongation, since it is an essential component of cell walls and root caps, and roots rapidly proliferate soon after emergence in cuttings (Bellini et al., 2014; Falasca et al., 2004). Although a great deal of research has been conducted on perseverance nutrition and nutrient content of cuttings, it has focused almost exclusively on stock plant and cutting N fertilization rates, and does not offer insight into the potential influence of other nutrients on successful propagation (Santos et al., 2011c). Further research is necessary to understand the effect of stock plant Ca fertility on successful rooting, and to develop perseverance (stock plant) on Zn, Ca and B fertilization guidelines.

Generally, substrate nutrient content has been shown to have little or no effect on rooting percentage or cutting survival (Buwalda and Kim, 1994; Carney and Whitcomb, 1983; Rowe and Cregg, 2002; Santos et al., 2011a, 2011b); only on postrooting nutrient uptake and subsequent plant growth. This suggests that nutrients used for root initiation must be present within the cutting tissue at the time

of excision. Calcium may be an exception. It is thought that some amount of exogenous Ca must be present for rooting to occur, since Ca cannot be redistributed via the phloem (Falasca et al., 2004; White and Broadley, 2003). Eliasson (1978) showed that external Ca supply had a positive effect on root elongation in pea (*Pisum sativum* L.) cuttings, although it did not affect the number of roots formed, as compared with deionized water. Bellamine et al. (1998) found a 42% reduction in rooting percentage of auxin-induced hybrid aspen (*Populus tremula* L. $\times$ *Populus tremuloides* Michx.) cuttings grown in a calcium-free medium. Since Ca was lost from the lower stems in this study, further research may be necessary to develop Ca fertility guidelines for propagation substrates.

Sulfur concentration increased in lower stems before root formation (Table 1). Since no decrease in S concentration was observed in the leaves or upper stems, translocation is an unlikely cause of this observation (Table 1). Uptake may have occurred in the unrooted stems via substrate-based starter fertilizer or S contained in irrigation water, although water testing (12.4 ppm S) nor soil testing (3 ppm S) indicated appreciable levels of S (data not shown). No other nutrient accumulated in the lower stems before root emergence. Santos et al. (2009) demonstrated that N uptake occurred through the base of petunia cuttings before visible root development. Some nutrient uptake may also occur via diffusion across the epidermis (Fernández and Brown, 2013; Schönherr, 2006). Sulfur may be required in higher quantities to support differentiation and root development, since it is an essential component of cysteine, glutathione, and methionine, as well as a common component of proteins and metabolites (Leustek and Saito, 1999). There is also evidence that H_2S is a signal molecule involved in IAA metabolism and ARF. Exogenous applications of H_2S and the H_2S donor NaHS increased the number and length of adventitious roots formed when applied at low concentrations (0.1 or 0.2 mM) to *Ipomoea batatas* L., *Salix matsudana* Koidz., and *Glycine max* L. stem cuttings (Zhang et al., 2009). Sodium hydrosulfide also induced ARF in IAA-depleted *Cucumis sativus* L. explants (Lin et al., 2012). Hydrogen sulfide likely acts as an endogenous gasotransmitter that promotes root organogenesis by working downstream of IAA and stimulating the expression of cell cycle regulatory genes via plant heme oxygenase and calmodulin (Fang et al., 2014; Li et al., 2014).

Changes in tissue nutrient concentration after root emergence. Root initiation was observed as visible callus formation on day 9. Root dry mass increased from 0.015 ± 0.007 g on day 9 to 0.077 ± 0.029 g on day 21 ($P = 0.05$). Shoot dry mass did not significantly change during this time.

Sulfur concentrations declined in the upper and lower stems after root formation, and increased in the roots (Table 2). These data support the possibility that S was translocated. Potassium concentration similarly declined in

Table 1. Sectional tissue nutrient concentrations before adventitious root formation (day 0–6).

Day	Preadventitious root formation nutrient concentrations by section								
	N (g·kg ⁻¹)	P (g·kg ⁻¹)	K (g·kg ⁻¹)	S (g·kg ⁻¹)	Zn (mg·kg ⁻¹)	Ca (g·kg ⁻¹)	B (mg·kg ⁻¹)	Mg (g·kg ⁻¹)	Fe (g·kg ⁻¹)
	Leaves								
0	27.54 a	2.73 a	11.29 a	1.92 a	79.23 a	9.58 a	27.47 a	4.09 a	1,141 a
3	24.58 a	2.36 ab	10.00 b	1.80 a	37.84 b	8.89 a	21.55 a	3.75 a	1,255 a
6	22.65 a	2.22 b	8.38 c	1.79 a	33.55 b	6.69 b	20.54 a	3.21 b	545 a
<i>P</i>	NS	0.0327	0.0012	NS	0.0101	0.0184	NS	0.0040	NS
	—	L	L	—	L	L	—	L	—
	Upper stems (above soil line)								
0	14.43 a	2.55 a	24.62 a	1.69 a	63.63 a	8.98 a	23.45 a	4.36 a	894 a
3	13.98 a	2.45 a	23.96 a	1.73 a	44.71 b	8.98 a	19.44 a	3.90 b	1,085 a
6	12.38 a	2.65 a	22.79 a	1.64 a	42.30 b	6.96 a	20.64 a	3.19 c	485 a
<i>P</i>	NS	NS	NS	NS	0.0068	NS	NS	0.0003	NS
	—	—	—	—	L	—	—	L	—
	Lower stems (below soil line)								
0	7.82 a	1.52 a	20.00 a	1.14 b	50.05 a	7.62 a	21.69 a	3.51 a	1,331 a
3	9.56 a	1.74 a	20.92 a	1.74 a	39.02 ab	7.49 a	16.78 b	3.63 a	1,100 a
6	10.59 a	1.95 a	20.42 a	1.91 a	36.55 b	6.19 b	13.26 b	3.44 a	1,017 a
<i>P</i>	NS	NS	NS	0.0009	0.0445	0.0165	0.0058	NS	NS
	—	—	—	U	L	L	L	—	—

NS = nonsignificant; L = leaching; U = uptake.

Values are means of three observations. Lowercase letters represent differences from Tukey's honestly significant difference test at $P \leq 0.05$. Results from analysis of variance are listed. Proposed cause for difference between measurement days is shown for each nutrient–tissue combination.

Table 2. Sectional tissue nutrient concentrations after adventitious root formation (day 9–21).

Day	Postadventitious root formation nutrient concentrations by section								
	N (g·kg ⁻¹)	P (g·kg ⁻¹)	K (g·kg ⁻¹)	S (g·kg ⁻¹)	Zn (mg·kg ⁻¹)	Ca (g·kg ⁻¹)	B (mg·kg ⁻¹)	Mg (g·kg ⁻¹)	Fe (g·kg ⁻¹)
	Leaves								
9	18.79 a	1.57 a	7.12 a	1.52 a	31.69 a	7.52 b	15.06 a	3.36 b	763 a
12	18.78 a	1.53 a	6.09 ab	1.46 a	30.50 a	7.96 b	17.00 a	3.46 ab	406 a
15	17.78 a	1.52 a	5.55 b	1.50 a	41.04 a	8.03 b	16.89 a	3.76 ab	450 a
18	17.56 a	1.35 a	5.09 b	1.34 a	20.54 a	8.40 ab	18.35 a	3.83 ab	373 a
21	16.63 a	1.59 a	6.06 ab	1.44 a	26.42 a	10.29 a	22.09 a	4.56 a	450 a
<i>P</i>	NS	NS	0.0166	NS	NS	0.0199	NS	0.0334	NS
	—	—	L/T	—	—	U	—	U	—
	Upper stems (above soil line)								
9	10.92 a	1.98 a	22.79 a	1.33 a	39.47 a	6.84 a	18.09 a	2.66 a	554 a
12	10.09 a	1.87 a	17.77 ab	1.16 ab	34.81 ab	6.76 a	16.98 a	2.44 a	383 a
15	8.13 a	1.66 ab	15.61 bc	1.07 ab	29.78 bc	6.56 a	16.84 a	2.39 a	403 a
18	7.91 a	1.56 ab	16.39 abc	1.09 ab	24.26 c	7.12 a	19.29 a	2.55 a	347 a
21	7.32 a	1.24 b	14.37 c	0.94 b	26.92 bc	6.80 a	18.72 a	2.59 a	399 a
<i>P</i>	0.0395	0.0071	0.0077	0.0164	0.0014	NS	NS	NS	NS
	—	L/T	T	T	L/T	—	—	—	—
	Lower stems (below soil line)								
9	7.36 a	1.39 a	16.49 a	1.19 a	36.54 a	5.37 a	12.77 ab	2.67 a	1,215 a
12	6.58 ab	1.19 ab	14.22 ab	1.04 ab	29.23 b	5.04 a	11.48 b	2.46 a	996 a
15	5.20 ab	1.21 ab	12.85 b	0.92 bc	22.65 bc	4.69 a	12.27 ab	2.29 a	1,224 a
18	5.06 ab	0.99 ab	11.54 b	0.76 c	21.86 c	5.09 a	16.39 ab	2.51 a	794 a
21	4.31 b	0.90 b	11.67 b	0.78 c	24.39 bc	5.11 a	17.07 a	2.67 a	974 a
<i>P</i>	0.0369	0.0328	0.0037	0.0011	0.0003	NS	0.0189	NS	NS
	L/T	L/T	T	T	L/T	—	U	—	—
	Roots								
9	14.73 a	2.37 a	16.29 c	2.87 c	60.44 a	6.65 a	10.15 b	4.01 c	4,375 a
12	14.69 a	2.22 a	21.42 b	2.51 c	42.36 b	6.11 a	34.73 a	4.23 c	2,751 b
15	14.44 ab	2.25 a	30.53 a	3.94 b	28.34 b	4.25 b	50.32 a	5.97 b	2,509 bc
18	13.15 ab	2.02 ab	32.43 a	5.03 a	51.42 b	3.39 c	39.54 a	8.60 a	1,644 cd
21	11.19 b	1.76 b	31.51 a	5.01 a	29.45 b	3.11 c	43.60 a	8.82 a	1,409 d
<i>P</i>	0.0055	0.0091	<0.0001	<0.0001	0.0009	<0.0001	0.0003	<0.0001	<0.0001
	D	D	U	U	D	D	U	U	D

NS = nonsignificant; L = leaching; U = uptake; D = dilution; T = translocation to roots.

Values are means of three observations. Lowercase letters represent differences from Tukey's honestly significant difference test at $P \leq 0.05$. Results from analysis of variance are listed. Proposed cause for change between measurement days is shown for each nutrient–tissue combination.

the leaves, upper stems, and lower stems (Table 2), but increased in the roots. This may have been due in part to translocation. Blazich et al. (1983) found that K was translocated to developing roots of box-leaved holly. However, these changes could also have been due to further leaching as observed before callus/root formation (Table 1), combined with root uptake. Molybdenum concentration

decreased in the leaves as well ($P = 0.0035$), and this was probably due to leaching rather than movement, since no increase was observed in any other tissue.

Foliar N concentration decreased over the course of the study (days 0–21; $P < 0.0001$). However, there was no decrease from day 0 to day 6, before root formation (Table 1), nor was there a decrease from day 9 to day 21

(Table 2). Yet between day 6 and day 9, there was a significant drop in foliar N concentration ($\text{Pr} > |t| = 0.0067$). It appears that there was rapid N translocation from the existing foliage to the developing roots around the time of callus expansion and root emergence (day 6–9) that explains the overall decrease in leaf N concentration. Good and Tukey (1967) observed similar N translocation to developing

roots after root initiation in chrysanthemum. Conversely, Svenson and Davies (1995) observed that N concentrations declined in poinsettia stem cuttings over the course of the propagation period and attributed this effect to dilution. In the current study, it appears that more frequent 3-d sampling intervals, combined with greater separation of cutting parts in analysis (leaf, upper stem, lower stem, and root vs. leaves and basal stem sections), afforded increased clarity of rapid N translocation within cuttings.

Boron concentration increased in the roots and lower stems after root formation, whereas Mg concentration increased in the roots and leaves (Table 2). This was probably due to nutrient uptake and upward translocation. Concentrations of these nutrients did not decrease in other tissues, and these nutrients were available in the starter fertilizer imbedded in the substrate and the irrigation water applied to the crop. These sources probably facilitated uptake. Svenson and Davies (1995) observed an increase in B and Mg concentrations after root emergence in the basal stems of poinsettia stem cuttings grown in a nutrient-free medium that they attributed to translocation. Regardless of source, both studies corroborate the importance of B and Mg availability for root development.

In this study, foliar Ca concentration increased in the leaves after root initiation but declined in the roots (Table 2). This suggests that root uptake leads to an increase in leaf Ca, that once deposited in the foliage could not be reallocated to developing roots. Thereafter, rapid root growth diluted the Ca present in the substrate and roots. Nitrogen, P, Zn, and Fe concentrations decreased in the roots after day 12, probably due to dilution caused by growth and expansion without a sufficient pool of soil-available nutrients to facilitate uptake (Table 2.). Nickel ($P = 0.0002$) and Cu ($P = 0.0011$) concentrations also declined in the roots, yet not other cutting sections. Additionally, P and Zn concentrations decreased in the upper and lower stems, and N decreased in the lower stems (Table 2). The decrease in root and stem concentrations of N, P, and Zn could have been due to translocation to the growing roots. Leaching may also have caused these changes; however, leaching of N and P from the stem was not apparent in the earlier part of the study (Table 1).

Since tissue nutrient concentrations declined for several elements following root development (Table 2), ensuring that sufficient amounts of these nutrients are provided to stock plants to optimize nutritional health of subsequent cuttings is essential. This could be accomplished using an appropriately formulated soil-based or foliar-applied stock-plant or cutting fertilizer. For example, this study corroborates that Ca concentration decreases in roots due to dilution, and yet Ca is crucial for root development (Bellamine et al., 1998; Eliasson, 1978). Additionally, this study validates Henry et al. (1992) who indicated root dry weight of red cedar stem cuttings was positively influenced by N fertilization rate, but N is readily leached

from cuttings. Future work should focus on developing nutrient-specific fertility guidelines for stock plants that translates into maximizing nutrient availability in subsequent unrooted cuttings.

Conclusions

In a commercial substrate, ARF in *H. acetosella* 'Panama Red' was accompanied by several changes in tissue nutrient concentrations similar to those reported from previous studies conducted with other species in nutrient-free media. In this study, the frequent sampling period allowed for the observation of several changes in nutrient concentration that may have otherwise gone unnoticed in this fast-rooting species. This was particularly true for N that underwent rapid translocation to newly developing callus and roots at the time of root formation. Changes in lower stem nutrient concentrations before root formation were of particular interest; Zn, Ca, and B concentrations declined, whereas S concentration increased. Since these nutrients are directly involved in root development or the IAA pathway, further research is needed to understand how changes in concentrations of these nutrients occur in leafy stem cuttings, as well as to develop stock plant and substrate fertility guidelines for cutting propagation.

Literature Cited

- Anderson, A.S. 1986. Environmental influences on adventitious rooting in cuttings of non-woody species, p. 223–253. In: M.B. Jackson (ed.). New root formation in plants and cuttings. Martinus Nijhoff Publishers, Boston, MA.
- Arnold, M.A., R.D. Lineberger, and D.K. Struve. 1994. Copper compounds influence in vitro rooting of birch microcuttings. *J. Amer. Soc. Hort. Sci.* 119:74–79.
- Bellamine, J., C. Penel, H. Greppin, and T. Gaspar. 1998. Confirmation of the role of auxin and calcium in the late phases of adventitious root formation. *Plant Growth Regulat.* 26:191–194.
- Bellini, C., D.I. Pacurar, and I. Perrone. 2014. Adventitious roots and lateral roots: Similarities and differences. *Annu. Rev. Plant Biol.* 65:639–666.
- Blazich, F.A. and R.D. Wright. 1979. Non-mobilization of nutrients during rooting of *Ilex crenata* Thunb. cv. 'Convexa' stem cuttings. *HortScience* 14:242.
- Blazich, F.A., R.D. Wright, and H.E. Schaffer. 1983. Mineral nutrient status of 'Convexa' holly cuttings during intermittent mist propagation as influenced by exogenous auxin application. *J. Amer. Soc. Hort. Sci.* 108:425–429.
- Blazich, F.A. 1988. Mineral nutrition and adventitious rooting, p. 61–69. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). Adventitious root formation in cuttings. Dioscorides Press, Portland, OR.
- Buwalda, F. and K.S. Kim. 1994. Effects of irrigation frequency on root formation and shoot growth of spray chrysanthemum cuttings in small jute plugs. *Scientia Hort.* 60:125–138.
- Carney, M. and C.E. Whitcomb. 1983. Effects of 2 slow-release fertilizers on the propagation and subsequent growth of 3 woody plants. *J. Environ. Hort.* 1:55–58.
- Chang, Y.S. and C.Y. Lin. 2006. Copper hydroxide improved root quality of *Palimara alstonia* in plastic containers. *HortTechnology* 16:357–359.

- Dan, H., G. Yang, and Z.L. Zheng. 2007. A negative regulatory role for auxin in sulphate deficiency response in *Arabidopsis thaliana*. *Plant Mol. Biol.* 63:221–235.
- Davis, T.D. and B.E. Haissig. 2013. Biology of adventitious root formation (Vol. 62). Plenum Press, New York, NY.
- Dunn, G.M., J.R. Huth, and M.J. Lewty. 1997. Coating nursery containers with copper carbonate improves root morphology of native Australian tree species used in agroforestry systems. *Agrofor. Syst.* 37:143–155.
- Eliasson, L. 1978. Effects of nutrients and light on growth and root formation in *Pisum sativum* cuttings. *Physiol. Plant.* 43:13–18.
- Falasca, G., D. Zaghi, M. Possenti, and M.M. Altamura. 2004. Adventitious root formation in *Arabidopsis thaliana* thin cell layers. *Plant Cell Rpt.* 23:17–25.
- Fang, T., Z. Cao, J. Li, W. Shen, and L. Huang. 2014. Auxin-induced hydrogen sulfide generation is involved in lateral root formation in tomato. *Plant Physiol. Biochem.* 76:44–51.
- Fernández, V. and P.H. Brown. 2013. From plant surface to plant metabolism: The uncertain fate of foliar-applied nutrients. *Front. Plant Sci.* 4:289.
- Giehl, R.F.H., J.E. Lima, and N. von Wirén. 2012. Localized iron supply triggers lateral root elongation in *Arabidopsis* by altering the AUX1-mediated auxin distribution. *Plant Cell.* 24:33–49.
- Good, G.L. and H.B. Tukey, Jr. 1966. Leaching of metabolites from cuttings propagated under intermittent mist. *Proc. Amer. Soc. Hort. Sci.* 89:728–733.
- Good, G.L. and H.B. Tukey, Jr. 1967. Redistribution of mineral nutrients in *Chrysanthemum morifolium* during propagation. *Proc. Amer. Soc. Hort. Sci.* 90:384–388.
- Hartmann, H.T., D.E. Kester, and F.T. Davies, Jr. 1990. Plant propagation: Principles and practices. Prentice Hall, Englewood Cliffs, NJ.
- Henry, P.H., F.A. Blazich, L.E. Hinesley, and R.D. Wright. 1992. Nitrogen nutrition of containerized eastern redcedar. I. Growth, mineral nutrient concentrations, and carbohydrate status. *J. Amer. Soc. Hort. Sci.* 117:563–567.
- Jarvis, B.C., A.H.N. Ali, and R. Hunt. 1984. The interaction between auxin and boron in adventitious root development. *New Phytol.* 97:197–204.
- Kazan, K. 2013. Auxin and the integration of environmental signals into plant root development. *Ann. Bot. (Lond.)* 112:1655–1665.
- Leustek, T. and K. Saito. 1999. Sulfate transport and assimilation in plants. *Plant Physiol.* 120:637–644.
- Li, Y., Z. Shi, L. Gan, and J. Chen. 2014. Hydrogen sulfide is a novel gasotransmitter with pivotal role in regulating lateral root formation in plants. *Plant Signal. Behav.* 9(6):e90340.
- Lin, Y., M. Li, W. Cui, W. Lu, and W. Shen. 2012. Haem oxygenase-1 is involved in hydrogen sulfide-induced cucumber adventitious root formation. *J. Plant Growth Regul.* 31:519–528.
- Martin-Rejano, E.M., J.J. Camacho-Cristóbal, M.B. Herrera-Rodríguez, J. Rexach, M.T. Navarro-Gochicoa, and A. González-Fontes. 2011. Auxin and ethylene are involved in the response of root architecture to low boron supply in *Arabidopsis* seedlings. *Physiol. Plant.* 142:170–178.
- Rowe, D.B. and B.M. Cregg. 2002. Effect of incorporating controlled-release fertilizer on adventitious rooting and growth of *Artemisia*, *Gaura*, and *Nepeta*. *J. Environ. Hort.* 20:1–6.
- Santos, K.M., P.R. Fisher, and W.R. Argo. 2009. Stem versus foliar uptake during propagation of *Petunia × hybrida* vegetative cuttings. *HortScience* 44:1974–1977.

- Santos, K.M., P.R. Fisher, and W.R. Argo. 2011a. Survey of tissue nutrient levels in vegetative cuttings. *Commun. Soil Sci. Plant Anal.* 42:669–693.
- Santos, K.M., P.R. Fisher, T. Yeager, E.H. Simonne, H.S. Carter, and W.R. Argo. 2011b. Timing of macronutrient supply during cutting propagation of *Petunia*. *HortScience* 46:475–480.
- Santos, K.M., P.R. Fisher, T.H. Yeager, E.H. Simonne, H.S. Carter, and W.R. Argo. 2011c. Effect of petunia stock plant nutritional status on fertilizer response during propagation. *J. Plant Nutr.* 34:1424–1436.
- SAS Institute Inc. 2012. Base SAS 9.2 software. SAS Institute Inc., Cary, NC.
- Schönherr, J. 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. *J. Expt. Bot.* 57:2471–2491.
- Schwambach, J., C. Fadaneli, and A.G. Fett-Neto. 2005. Mineral nutrition and adventitious rooting in microcuttings of *Eucalyptus globulus*. *Tree Physiol.* 25:487–494.
- Svenson, S.E. and F.T. Davies. 1995. Change in tissue mineral elemental concentration during root initiation and development of poinsettia cuttings. *HortScience* 30:617–619.
- Tukey, H.B., Jr. 1970. The leaching of substances from plants. *Annu. Rev. Plant Physiol.* 21:305–324.
- Tukey, H.B., Jr. 1978. The effects of intermittent mist on cuttings. *Acta Hort.* 79:49–56.
- Verstraeten, I., S. Schotte, and D. Geelen. 2014. Hypocotyl adventitious root organogenesis differs from lateral root development. *Front. Plant Sci.* 5:495.
- White, P.J. and M.R. Broadley. 2003. Calcium in plants. *Ann. Bot.* 92:487–511.
- Wilkerson, E.G., R.S. Gates, S. Zolnier, S.T. Kester, and R.L. Geneve. 2005. Transpiration capacity in poinsettia cuttings at different rooting stages and the development of a cutting coefficient for scheduling mist. *J. Amer. Soc. Hort. Sci.* 130:295–301.
- Wott, J.A. and H.B. Tukey, Jr. 1967. Influence of nutrient mist on the propagation of cuttings. *Proc. Amer. Soc. Hort. Sci.* 90:454–461.
- Zhang, H. and B.G. Forde. 2000. Regulation of *Arabidopsis* root development by nitrate availability. *J. Expt. Bot.* 51:51–59.
- Zhang, H., J. Tang, X. Liu, Y. Wang, W. Yu, W. Peng, F. Fang, D. Ma, Z. Wei, and L. Hu. 2009. Hydrogen sulfide promotes root organogenesis in *Ipomoea batatas*, *Salix matsudana* and *Glycine max*. *J. Integr. Plant Biol.* 51:1086–1094.