Quantifying the Effects of Chelated Calcium and Salicylic Acid on the Postharvest Quality of Poinsettia Cuttings

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ADDITIONAL INDEX WORDS. *Euphorbia pulcherrima*, leaf mechanical strength, nutrient analysis, texture analyzer

SUMMARY. Vegetatively propagated unrooted cuttings typically are grown in equatorial locations and shipped via airfreight to propagators located in temperate climates. Cutting quality, defined as the resistance to external forces, such as physical damage and pathogen infection, impacts postharvest durability during shipping and propagation. During our previous studies, foliar application of calcium (Ca) in the form of Ca chloride was effective at increasing leaf mechanical strength of poinsettia (*Euphorbia pulcherrima*) and zonal geranium (*Pelargonium × hortorum*). Calcium chloride applied at ≥ 800 mg·L⁻¹ Ca caused phytotoxicity symptoms in poinsettia; therefore, in the current work, we investigated the use of chelated Ca by providing Ca at 40, 80, or 160 mg·L⁻¹ and salicylic acid (SA) at 150 or 300 mg·L⁻¹ to increase the mechanical strength of poinsettia leaves. Mechanical strength of leaves was assessed using a force-displacement graph generated from a texture analyzer using a ball probe to penetrate a unit area of a clamped leaf. The peak force to fracture the leaf and work-of-penetration, defined as the area under the force-displacement curve, were used as indicators of mechanical strength. Calcium concentration in the leaves increased by 27% with increased application of Ca from 0 to 160 mg·L⁻¹. Peak force was 26% greater in treatments with Ca at 80 or 160 mg·L⁻¹ compared with the untreated control. Work-of-penetration was 24% and 29% greater for treatments with Ca at 80 and 160 mg·L⁻¹, respectively, compared with the control. Foliar application of SA did not affect leaf mechanical strength. Chelated Ca applied at 160 mg·L⁻¹ Ca caused visual phytotoxicity symptoms; thus, applications of 80 mg·L⁻¹ Ca are recommended to improve resistance to physical damage for poinsettia leaves.

Vegetatively propagated herbaceous ornamental species typically are started from shoot tip cuttings harvested from stock plants, densely packed into plastic bags and cardboard boxes, and transported via airfreight to propagators. The time from harvesting the cuttings to

delivery at the propagation greenhouse is typically 48 to 72 h. Delays during shipping or poor cold-chain management can result in leaf chlorosis, leaf abscission, delayed root initiation, and fungal infections. A goal of the stock plant grower is to produce cuttings that resist these physiological and pathogenic problems. Growers empirically evaluate the cutting quality that impacts postharvest durability during shipping and propagation and refer to the desired characteristics as “toning,” which is defined as the change in

leaf texture required to provide resistance to external forces such as physical damage or pathogen infection. During our previous studies (Samarakoon et al., 2017), techniques were developed to quantify the mechanical strength of leaves with the use of instruments that assess textural properties. Peak force to puncture a leaf (strength) and work-of-penetration (toughness), given by the positive area of the force displacement curve, are two parameters derived from mechanical strength tests that provide an indication of the mechanical resistance of leaves to external forces.

The effect of preharvest foliar Ca chloride (CaCl₂) applications on mechanical strength were quantified with the use of zonal geranium and poinsettia as model species (Samarakoon et al., 2017). Use of Ca at 400 mg·L⁻¹ in the form of CaCl₂ as a preharvest treatment was effective at increasing the mechanical strength of poinsettia; however, concentrations ≥ 800 mg·L⁻¹ caused phytotoxicity symptoms. Chelated Ca that contains ethylenediamine-tetraacetate (EDTA) can improve Ca absorption at lower Ca concentrations with root uptake (Nelson and Niedziela, 1998), as well as with foliar applications (Tang et al., 2007). The commercial crop recommendation of Ca-EDTA foliar application is 20 to 40 mg·L⁻¹ Ca (W.R. Argo, personal communication); however, Ca-EDTA was unable to overcome Ca deficiencies when applied as a foliar application (Alarcón et al., 1998). The first objective of the current research was to quantify the effect of preharvest foliar application of Ca-EDTA on the postharvest leaf mechanical strength of poinsettia cuttings.

SA mediates plant defense responses against biotic and abiotic stresses (Hayat et al., 2010; Rivas-San

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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
318.3268	fl oz/ft ²	mL·m ⁻²	0.0031
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
645.1600	inch ²	mm ²	0.0016
28.3495	oz	g	0.0353
720.0779	oz-inch	g·mm	0.0014
1	ppm	mg·kg ⁻¹	1
1	ppm	mg·L ⁻¹	1
0.001	ppm	mL·L ⁻¹	1000
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Vicente and Plasencia, 2011) and increases the postharvest life of cut roses (*Rosa ×hybrida*) when applied as a pre- and postharvest application (Alaey et al., 2011). The effect of SA in increasing leaf mechanical strength has not been reported; however, fruit firmness increased when SA was added to the nutrient solution supplied to strawberries [*Fragaria ×ananassa* (Shafiee et al., 2010)] and when SA was applied as a foliar spray to grapes [*Vitis vinifera* (Champa et al., 2014)]. Therefore, the second objective of the current research was to quantify the effect of preharvest SA application on postharvest leaf mechanical strength of poinsettia cuttings.

Materials and methods

STOCK PLANT MANAGEMENT.

Poinsettia ‘Prestige Red’ rooted cuttings were transplanted into 2.8-L containers, one plant per container. Six weeks following planting, the shoot tips were pinched (about 1 cm below the shoot apex) to leave six nodes on the remaining stem, and treatment applications started 2 weeks later. The plants were grown in a glass greenhouse in which the heating and ventilation set points during day and night were 27 and 22 °C, respectively. Plants were shaded with retractable curtains [55% photosynthetic photon flux (PPF) reduction] when PPF measured outside the greenhouse exceeded 968 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were grown under long days provided through day-length extension lighting with metal halide lamps when PPF measured outside was below 387 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 1000 to 2400 HR to maintain the poinsettia stock plants in a vegetative state for cutting production. A peat-based growing medium (Fafard 3B; Conrad Fafard, Agawam, MA) was used for all experiments. A constant liquid fertigation schedule was used with 15N–2.2P–12.5K–5Ca–2Mg water-soluble fertilizer (Peter’s Excel; ICL Specialty Fertilizers, Dublin, OH) to provide 200 $\text{mg}\cdot\text{L}^{-1}$ N and 66 $\text{mg}\cdot\text{L}^{-1}$ Ca at each irrigation.

TREATMENT APPLICATION. Six treatments comprising different concentrations of either Ca or SA were arranged as a completely randomized design with six individual stock plants (replicates) per treatment. A Ca-EDTA [Ca disodium EDTA (CaNa_2EDTA), 9% Ca chelate (Sequestar; Blackmore Co., Belleville, MI)] solution was

prepared with deionized water to provide Ca at 0, 40, 80, or 160 $\text{mg}\cdot\text{L}^{-1}$. An SA [2-hydroxybenzoic acid, >99.0% (MP Biomedicals, Solon, OH)] solution was prepared with 150 or 300 $\text{mg}\cdot\text{L}^{-1}$ SA in deionized water after being dissolved in 10 mL of heated water (60 °C). A non-ionic surfactant (CapSil; Aquatrols, Paulsboro, NJ) was added to each solution at a concentration of 0.4 $\text{mL}\cdot\text{L}^{-1}$ to improve leaf contact. All treatments were applied weekly to poinsettia stock plants as a foliar spray to glisten the foliage, between 0800 and 1100 HR at an application rate of 200 $\text{mL}\cdot\text{m}^{-2}$ bench space. Shoot tip cuttings (5-cm stem length with four to five leaves) were harvested weekly from the stock plants to provide cuttings for analysis and to stimulate further shoot formation.

Three different evaluations were performed to determine the treatment effects on plant tissues. The evaluations included leaf texture analysis, leaf nutrient analysis, and moisture loss. Sampling for each analysis method started 6 weeks following initiation of treatment applications and continued as required for each analysis described in the sections to follow.

TEXTURE ANALYSIS FOR MECHANICAL PROPERTIES. A 3-cm-long leaf that developed on each axillary shoot following pinching was tagged on the same day across all stock plants. Each stock plant had one leaf marked on each of six shoots. When those tagged leaves reached a mature size, they were harvested in the morning (0800–1000 HR), wrapped in moist paper towel, placed in a sealed polyethylene bag (17.7 × 19.5 cm), and placed in a 5 °C room until the start of measurement the following day. Texture analysis was performed within 24 to 32 h from leaf removal, and there were six leaves per replicate.

The texture analysis procedure involved forcing a probe of a known cross-sectional area through a leaf causing the leaf to fracture (Gutiérrez-Rodríguez et al., 2013). A texture analyzer (TA-XT Plus; Stable Microsystems, Godalming, UK) was used with a TA-1085 small film extension fixture (Stable Microsystems) with a 10-mm-diameter opening (78.5 mm^2) to hold the leaf and 6.35-mm-diameter ball probe (rounded end) to penetrate the leaf. The test specifications were

a pre-test speed of 2 $\text{mm}\cdot\text{s}^{-1}$, test speed of 1 $\text{mm}\cdot\text{s}^{-1}$, and post-test speed of 10 $\text{mm}\cdot\text{s}^{-1}$. The probe moved a standard distance of 8 mm. Each leaf was placed between two metal plates and clamped to keep the leaf flat. Leaves were cut at the midrib immediately before the measurement and the test was performed on both halves of each leaf with the probe penetrating from the adaxial side, avoiding conspicuous veins. A force-displacement graph was generated for each test. From this graph, the peak (maximum) force to puncture the leaf and area under the force displacement curve, which also is referred to as the work-of-penetration, were identified for each leaf analyzed.

LEAF NUTRIENT ANALYSIS. The newest, fully developed leaf on each of six shoots per stock plant was collected between 0800 and 1000 HR, washed with deionized water, air dried at 20 °C for 48 h, and placed in an oven at 60 °C until the weight was constant. The dried samples were ground to a fine powder for analysis of macro and micronutrients of leaves (Nelson, 1988) using inductively coupled plasma optical emission spectrometry (iCAP 6300; Thermo Fisher Scientific, Waltham, MA) and a carbon nitrogen analyzer (Vario MI-CRO cube CHNS; Elementar, Mt. Laurel, NJ) at the U.S. Department of Agriculture, Agricultural Research Service laboratory in Toledo, OH.

WATER LOSS. Six shoot tip cuttings per stock plant were harvested for postharvest simulation. Three cuttings were placed in one zip-sealed polyethylene bag (17.7 × 19.5 cm) with 15 round holes (0.65 mm diameter) on each side and two bags per replicate/plant; hence, there were 12 bags per treatment. A simulated postharvest environment was provided by placing the bags of cuttings in dark storage at 10 °C [70% to 80% relative humidity (RH)] for 48 h followed by 20 °C for 24 h (70% to 80% RH) in a growth room with temperature and humidity sensors connected to a data logger.

The fresh weight (FW) of six cuttings was recorded at harvest, or Day 0 (FW_{D0}), and again after 3 d simulated postharvest storage (FW_{D3}). Then cuttings were oven-dried at 60 °C until the weight was constant and dry weight (DW) was recorded. Water content of a cutting at harvest was determined by subtracting DW from FW_{D0} . Moisture

loss was calculated by subtracting FW_{D3} from FW_{D0} . Relative moisture loss from cuttings was calculated as a percentage of moisture loss from the water content at harvest, as it gives an indication of transpiration rate during the postharvest storage as expressed in Eq. [1]. No additional moisture was provided within the bag during postharvest storage.

$$\text{Relative moisture loss} = \frac{FW_{D3} - FW_{D0}}{FW_{D0} - DW} \times 100 \quad [1]$$

DATA ANALYSIS. Data were analyzed using analysis of variance in JMP Pro 10 (SAS Institute, Cary, NC). Trend lines were plotted using a three-parameter, exponential growth nonlinear equation for the leaf mechanical properties and nutrient analysis data, and a two-parameter, exponential decay nonlinear equation for the moisture loss data.

Results

LEAF MECHANICAL PROPERTIES. Peak force increased by 26% greater ($P = 0.01$) as Ca application rate increased from 0 to 80 $\text{mg}\cdot\text{L}^{-1}$, whereas no additional increase was observed at 160 $\text{mg}\cdot\text{L}^{-1}$ Ca. (Fig. 1A). Work-of-penetration increased by 29% ($P = 0.05$) as Ca application rate increased from 0 to 160 $\text{mg}\cdot\text{L}^{-1}$ (Fig. 1B). No change in leaf mechanical properties were observed from the SA applications (data not shown).

LEAF NUTRIENT CONCENTRATION. Calcium concentration in the poinsettia leaves increased by 27% with increased application of Ca from 0 to 160 $\text{mg}\cdot\text{L}^{-1}$ [$P < 0.0001$ (Fig. 2A)]. The other macronutrients measured in the leaf tissue were not different among the Ca treatments ($P > 0.05$). Elemental analysis of the Ca-EDTA solution with surfactant indicated the presence of aluminum, boron (B), Ca, copper (Cu), iron (Fe), potassium, manganese, molybdenum, phosphorus, and sulfur; therefore, the Ca-EDTA applications resulted in increases in several of the micronutrients (data not shown). For example, B increased by 59% ($P < 0.007$), Cu by 68% ($P < 0.0001$), Fe by 38% ($P < 0.05$), and zinc (Zn) by 41% ($P < 0.0001$) with application of Ca-EDTA at 160 $\text{mg}\cdot\text{L}^{-1}$ Ca (Fig. 2B–F). All nutrients, except for Cu,

were within the acceptable range for poinsettia leaf tissue (Ecke et al., 2004). Variation of macro- or micronutrient concentration was not evident following application of SA (data not shown). Phytotoxicity symptoms in the form of yellowing and necrotic patches near the leaf margins were observed following applications of 160 $\text{mg}\cdot\text{L}^{-1}$ Ca and 300 $\text{mg}\cdot\text{L}^{-1}$ SA.

WATER LOSS. Cutting FW and DW at harvest was not different among treatments, with an average of 28.8 ± 3.4 g and 3.9 ± 0.6 g, respectively. Initial relative water content was $86.6\% \pm 1.1\%$. The Ca and SA spray treatments reduced moisture loss during storage compared with the control; however, no differences were observed amongst the various rates of Ca or SA (Fig. 3).

Discussion

Calcium applied to poinsettias at or above 80 $\text{mg}\cdot\text{L}^{-1}$ in the form of Ca-EDTA can improve leaf mechanical strength. As reported in our previous studies, when CaCl_2 was used as the Ca source, the effect on mechanical strength was evident at 400 $\text{mg}\cdot\text{L}^{-1}$ Ca with a 10% increase in work-of-penetration (Samarakoon et al., 2017). Since the work-of-penetration increased by 24% when Ca-EDTA was applied, it could be considered more effective in strengthening the leaves than CaCl_2 . The mode of action of Ca was hypothesized to be via increased lignin and cellulose content in cell walls (Conn et al., 2011) and/or strengthening the middle lamella (Hongo et al., 2012). However, the relative increase of Ca in our previous studies with CaCl_2 was 37% (Samarakoon et al., 2017) as compared with 27% in the current study with Ca-EDTA. A chelating agent can influence Ca uptake as well as other micronutrients (B, Cu, Fe, Zn); therefore, it is possible that the increased concentrations of other nutrients may also have contributed to increased leaf mechanical strength.

Phytotoxicity symptoms following application of Ca-EDTA have been reported in hydroponic tulip production (Nelson and Niedziela, 1998). The phytotoxicity symptoms observed on applications of 160 $\text{mg}\cdot\text{L}^{-1}$ Ca could be due to nutrient imbalances resulting from the treatment applications or leaf burn due to high salt concentration. Our results suggest that 80 $\text{mg}\cdot\text{L}^{-1}$ Ca

Fig. 1. Leaf mechanical strength as indicated by peak force (A), and work-of-penetration (B) for 'Prestige Red' poinsettia leaves. Leaves were sampled from cuttings following weekly foliar spray applications of chelated calcium (Ca-EDTA) at rates of 0 to 160 $\text{mg}\cdot\text{L}^{-1}$ Ca to stock plants during cutting development ($n = 6$); 1 $\text{mg}\cdot\text{L}^{-1} = 1$ ppm, 1 g = 0.0353 oz, 1 g·mm = 0.0014 oz·inch.

from Ca-EDTA would be sufficient to increase mechanical strength without substantial risk of phytotoxicity. Foliar Ca application has been a common technique to control bract edge burn and leaf edge necrosis since the 1990s (Ecke et al., 2004). As evidenced by the increased Ca concentration in the tissues of the Ca-EDTA-treated leaves, Ca-EDTA can be useful for overcoming Ca deficiencies and their associated physiological disorders observed in poinsettia. Based on preliminary investigations, application of Ca to stock plants was not inhibitory to rooting initiation and development during propagation.

Both Ca and SA reduced the moisture loss from cuttings as compared with control. Reduction of evapotranspiration with application of CaCl_2 was observed in big bend bluebonnet [*Lupinus harvardii* (Picchioni et al., 2001)], and improved moisture retention and turgor was achieved with application of Ca in lettuce [*Lactuca sativa* (Martin-Diana et al., 2006)]. Treatment of common bean (*Phaseolus vulgaris*) leaves with SA reduced transpiration rates (Larque-Saavedra, 1979), via

Fig. 2. Concentration of calcium [Ca (A)], iron [Fe (B)], manganese [Mn (C)], zinc [Zn (D)], boron [B (E)], and copper [Cu (F)] measured in 'Prestige Red' poinsettia leaves following weekly foliar applications of chelated Ca (Ca-EDTA) at rates of 0 to 160 mg·L⁻¹ Ca (n = 6); 1 mg·L⁻¹ = 1 ppm, 1 mg·kg⁻¹ = 1 ppm, 1 g = 0.0353 oz.

increased abscisic acid synthesis (Bandurska, 2005). Increased postharvest life of cut roses via regulation of water balance following application of SA was previously reported (Alaey et al., 2011). On the basis of the current study and these other reports, both Ca-EDTA and SA reduce moisture loss during postharvest storage and shipping.

In conclusion, foliar applications of Ca-EDTA can be used to increase cutting quality and postharvest performance. Use of foliar sprays is relatively convenient in commercial facilities

in comparison with alternate methods of strengthened leaf tissue, such as the use of water and/or nitrogen restriction. In this study, Ca-EDTA had a greater impact on leaf mechanical strength than CaCl₂ reported in a previous study (Samarakoon et al., 2017); thus, the application of 80 mg·L⁻¹ Ca from Ca-EDTA provides an effect alternative for improving poinsettia cutting quality. The use of Ca-EDTA for horticultural applications other than stock plant production in poinsettia or for other plant species requires further research.

Fig. 3. Moisture loss percentage during postharvest simulation of 'Prestige Red' poinsettia cuttings following weekly foliar spray applications of chelated calcium (Ca-EDTA) at rates of 0 to 160 mg·L⁻¹ Ca or 0 to 300 mg·L⁻¹ salicylic acid (SA) to stock plants (n = 6); 1 mg·L⁻¹ = 1 ppm.

Literature cited

- Alaey, M., M. Babalar, R. Naderi, and M. Kafi. 2011. Effect of pre-and postharvest salicylic acid treatment on physio-chemical attributes in relation to vase-life of rose cut flowers. *Postharvest Biol. Technol.* 61:91-94.
- Alarcón, A.L., R. Madrid, F. Romojaro, and E. Molina. 1998. Calcium forms in leaves of muskmelon plants grown with different calcium compounds. *J. Plant Nutr.* 21:1897-1912.
- Bandurska, H. 2005. The effect of salicylic acid on barley response to water deficit. *Acta Physiol. Plant.* 27:379-386.
- Champa, W.H., M. Gill, B. Mahajan, and N. Arora. 2014. Preharvest salicylic acid treatments to improve quality and postharvest life of table grapes (*Vitis vinifera* L.) cv. Flame Seedless. *J. Food Sci. Technol.* 52:3607-3616.
- Conn, S.J., M. Gilliam, A. Athman, A. W. Schreiber, U. Baumann, I. Moller, N. H. Cheng, M.A. Stancombe, K.D. Hirschi, A.A. Webb, R. Burton, B.N. Kaiser, S.D. Tyerman, and R.A. Leigh. 2011. Cell-specific vacuolar calcium storage mediated by CAX1 regulates apoplastic calcium concentration, gas exchange, and plant productivity in *Arabidopsis*. *Plant Cell* 23:240-257.
- Ecke, P., III., J. Faust, A. Higgins, and J. Williams. 2004. The Ecke poinsettia manual. Ball Publ., West Chicago, IL.
- Gutiérrez-Rodríguez, E., H.J. Lieth, J.A. Jernstedt, J.M. Labavitch, T.V. Suslow, and M.I. Cantwell. 2013. Texture, composition and anatomy of spinach leaves in relation to nitrogen fertilization. *J. Sci. Food Agr.* 93:227-237.

- Hayat, Q., S. Hayat, M. Irfan, and A. Ahmad. 2010. Effect of exogenous salicylic acid under changing environment: A review. *Environ. Expt. Bot.* 68:14–25.
- Hongo, S., K. Sato, R. Yokoyama, and K. Nishitani. 2012. Demethylesterification of the primary wall by pectin methyl-esterase provides mechanical support to the *Arabidopsis* stem. *Plant Cell* 24:2624–2634.
- Larque-Saavedra, A. 1979. Stomatal closure in response to acetylsalicylic acid treatment. *Z. Pflanzenphysiol.* 93:371–375.
- Martin-Diana, A.B., D. Rico, J. Frias, G. Henahan, J. Mulcahy, J. Barat, and C. Barry-Ryan. 2006. Effect of calcium lactate and heat-shock on texture in fresh-cut lettuce during storage. *J. Food Eng.* 77:1069–1077.
- Nelson, M.R. 1988. Index to EPA methods. EPA Circ. 901/3-88-01. U.S. Environ. Protection Agency, Washington, DC.
- Nelson, P. and C. Niedziela. 1998. Effects of calcium source and temperature regime on calcium deficiency during hydroponic forcing of tulip. *Scientia Hort.* 73:137–150.
- Picchioni, G., M. Valenzuela-Vazquez, and S. Armenta-Sanchez. 2001. Calcium-activated root growth and mineral nutrient accumulation of *Lupinus havardii*: Ecophysiological and horticultural significance. *J. Amer. Soc. Hort. Sci.* 126:631–637.
- Rivas-San Vicente, M. and J. Plasencia. 2011. Salicylic acid beyond defence: Its role in plant growth and development. *J. Expt. Bot.* 62:3321–3338.
- Samarakoon, U.C., J.E. Faust, and J.M. Dole. 2017. Quantifying the effects of foliar-applied calcium chloride and its contribution to postharvest durability of unrooted cuttings. *HortScience* 52:1790–1795.
- Shafiee, M., T. Taghavi, and M. Babalar. 2010. Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. *Scientia Hort.* 124:40–45.
- Tang, S., W. Shi, H. Wang, and A. Luo. 2007. Effect of calcium on cyclamen pedicel elongation. *J. Plant Nutr. Soil Sci.* 170:664–668.