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Quantifying the Effects of Foliar-applied Calcium Chloride and Its Contribution to Postharvest Durability of Unrooted Cuttings

Uttara C. Samarakoon[1] and James E. Faust[2]
Department of Agricultural and Environmental Sciences, Clemson University, E143 Poole Agriculture Center, Clemson, SC 29634

John M. Dole
Department of Horticultural Science, North Carolina State University, 120 Kilgore Hall, Raleigh, NC 27695

Abstract. **Vegetatively propagated unrooted cuttings are typically imported to the United States from Central America. Death or damage of cuttings during shipping and propagation can be reduced if cuttings can be made more resistant to external forces, such as physical damage or pathogen infection. However, strategies to develop durable cuttings via treating stock plants have not been previously quantified in controlled studies. During the current study, mechanical strength of leaves and resistance to infection by *Botrytis cinerea* were evaluated after weekly applications of calcium chloride (CaCl$_2$) as a foliar spray to stock plants that delivered calcium (Ca) at the concentrations of 0, 400, or 800 mg·L^{-1}. A texture analyzer quantified the peak force required to fracture the leaf and the work of penetration, or area under the force–displacement curve, and these measurements were indicators of mechanical strength. For poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) cuttings at the time of harvest from the stock plant, work of penetration increased by 10% with the application of 800 mg·L^{-1} Ca compared with the control, whereas peak force by 9%. For zonal geranium (*Pelargonium ×hortorum* Bailey), work of penetration increased 15% with the application of 800 mg·L^{-1} Ca compared with the control. Calcium concentration in the leaves increased from 1.2% to 2.0% in geranium and from 1.0% to 1.6% in poinsettia with increasing application from 0 to 800 mg·L^{-1} Ca. In poinsettia, disease incidence in response to inoculation with *B. cinerea* spores was 55% and 15% less with CaCl$_2$ applications compared with controls with water and surfactant, respectively, whereas CaCl$_2$ application to geranium did not affect disease incidence.**

Vegetatively-propagated herbaceous species are typically started from cuttings harvested from stock plants. For the U.S. market, cuttings are produced at offshore locations and shipped via airfreight. The time from

harvesting the cuttings to delivery at the propagation greenhouse is typically 48–72 h. Delays during shipping or a poor postharvest environment can result in leaf senescence or abscission, delayed root initiation, and fungal infections. When shipments are delayed, propagators are faced with the difficult decision of whether to propagate the cuttings and try to maintain production schedules or wait for replacement cuttings. One approach to reduce the impact of delayed shipping or poor postharvest environments is to improve the preharvest durability of the cuttings by treating the stock plants before cutting removal.

Growers empirically evaluate the cutting quality that affects postharvest durability during shipping and propagation and refer to the desired characteristics as "toning." Toning can be defined as the change in leaf texture required to provide resistance to external forces, such as physical damage or pathogen infection. While growers employ strategies to create well-toned cuttings, these strategies have not been quantified in controlled studies.

Techniques to measure mechanical strength have been used in vegetables and fruits to determine resistance to physical damage with the use of instruments that assess textural properties. In general, methods determining the mechanical strength of leaves are based on puncturing, shearing, tearing, or bending action (Read and Sanson, 2003; Onoda et al., 2011). For example, puncture testing has been used on spinach leaves (*Spinacia oleracea* L.) to assess leaves after nitrogen (N) application (Gutiérrez-Rodríguez et al., 2013). 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.

External factors that influence the mechanical strength of leaves include water availability, humidity, and plant nutrition. In general, drought stress will produce "toned" leaf tissue while excess water will produce "soft" tissue (Toscano et al., 2014). However, drought stress is not an easily managed technique for large-scale stock plant production. Additionally, stock plants are often grown in tropical or sub-tropical locations in greenhouses with limited capacity for implementing humidity control. Thus, water and humidity management have limited practical applicability.

Considering plant nutrition, excess N was reported to increase the susceptibility of plant tissues to pathogens (Dordas, 2009). In contrast, Ca nutrition plays a critical role in tissue development, strength and resistance to external stresses (White and Broadley, 2003). Calcium nutrition has been used to improve postharvest performance in many horticultural products, for example, preharvest foliar Ca applications to fruits were beneficial in the reduction of postharvest brown rot infection in *Prunus persica* L. (peach; Elmer et al., 2007), increased shelf life in *Vitis vinifera* L. (grapes; Marzouk and Kassem, 2011), and increased fruit firmness in *Fragaria ×ananassa* Duchesne (strawberry; Singh et al., 2007). In poinsettia, bracts were less susceptible to bract edge burn and *Botrytis* infection after foliar Ca applications (Jacques et al., 1991), and postharvest longevity of cut roses (*Rosa hybrida* L.) increased with higher Ca fertilization rates (Mortensen et al., 2001; Starkey and Pedersen, 1997). In leafy vegetables, the duration of postharvest storage of lettuce (*Lactuca sativa* L.) increased with Ca (Martin-Diana et al., 2006). The use of Ca as a preharvest treatment has not been evaluated in the context of unrooted cutting production, but has potential as a safe and economical method to improve cutting quality. Foliar application was considered to be a better strategy to provide Ca to new cuttings developing on stock plants because of the limitations of uptake from substrates and remobilization from older tissues (Hongo et al., 2012; White and Broadley, 2003).

The objective of this project was to quantify the effect of preharvest foliar CaCl$_2$ treatments on mechanical strength and resistance to *Botrytis cinerea* infection which

are two important factors that impact cutting durability and survival. Geranium and poinsettia were used as model plants.

Materials and Methods

Three experiments were carried out with poinsettia and geranium to quantify the leaf mechanical strength, nutrient concentration, and resistance to *B. cinerea* of cuttings harvested from stock plants after the application of different concentrations of $CaCl_2$. Experiment 1 examined the responses of harvested cuttings after application of $CaCl_2$ to provide Ca at 0, 400, or 800 mg·L^{-1} to poinsettia stock plants, and Expt. 2 provided the same $CaCl_2$ treatments applied to geranium stock plants. Experiment 3 examined the effect on harvested cuttings after the application of a higher range of $CaCl_2$ treatments (Ca at 0, 800, or 1600 mg·L^{-1}) provided to poinsettia stock plants.

Stock plant management

The stock plants of poinsettia 'Prestige Red' and geranium 'Calliope Dark Red' were grown in a glass greenhouse at Clemson University, Clemson, SC (lat. 35°N), with the environment controlled by climate-control computer (Argus Control Environmental Systems, White Rock, BC, Canada). Heating and ventilation set points during day and night were 22 and 27 °C, respectively. Plants were shaded with retractable curtains [55% photosynthetic photon flux (*PPF*)-reduction] when *PPF* measured outside the greenhouse (LI-200SA pyranometer; LI-COR, Lincoln, NE) exceeded 968 µmol·m^{-2}·s^{-1}. Plants were grown under natural photoperiods until 2 Oct., and then long days were provided through daylength extension lighting with metal halide lamps when *PPF* measured outside was below 387 µmol·m^{-2}·s^{-1} from 1000 to 2400 HR to maintain the poinsettia stock plants in a vegetative state for cutting production. Geranium stock plants were cleaned of mature leaves and flower buds weekly. A peat-based growing medium (Fafard 3B; Conrad Fafard, Inc., Agawam, MA) was used for all experiments. A constant liquid fertigation schedule was used with Peter's Excel (15% N, 2.2% P, 12.5% K, 5% Ca, 2% Mg; Scotts-Sierra, Marysville, OH) to provide 200 mg·L^{-1} N at each watering.

For all three experiments, five containers of stock plants per each $CaCl_2$ treatment were arranged as a randomized complete block design with distance from the evaporative cooling pad as the block effect. An individual container was considered a replicate. For geranium, the experiment was repeated on two different benches in the same greenhouse.

Calcium treatment application to stock plants

For Expts. 1 and 2, $CaCl_2$ (anhydrous 96%; Thermo Fisher Scientific, Waltham, MA) solutions were prepared to provide Ca at 0, 400, or 800 mg·L^{-1} after being dissolved in deionized water. A nonionic surfactant

(CapSil; Aquatrols, Paulsboro, NJ) was added to each solution at a concentration of 0.4 mL·L^{-1} to improve contact with the leaves. A fourth treatment consisted of an additional deionized water control (0 mg·L^{-1} Ca) treatment without the nonionic surfactant. All treatments were applied weekly to poinsettia and geranium stock plants as a foliar spray between 0800 and 1100 HR at a concentration of 203 mL·m^{-2} bench space. Sampling for each analysis mentioned below started 4 and 6 weeks after initiation of treatment applications for geranium and poinsettia, respectively, and continued at regular intervals thereafter as the cuttings reached a harvestable stage for use in different analyses.

For Expt. 3, $CaCl_2$ solutions provided Ca at 0, 800, or 1600 mg·L^{-1} (with the same nonionic surfactant) to poinsettia stock plants as described for Expts. 1 and 2. For Expt. 3, only data on leaf mechanical strength and leaf nutrient concentration were collected.

Harvest and postharvest conditions

For poinsettia, shoot tip cuttings (5 cm stem length with four to five leaves) were harvested between 0800 and 1000 HR. For postharvest simulation, three cuttings were placed in a one zip-seal polyethylene bag (17.7 × 19.5 cm) with 15 round holes (0.65 mm diameter) on each side and two bags per replicate. A paper towel saturated with water was inserted into

each bag to maintain a high humidity environment to prevent desiccation of the cuttings. The cuttings were stored in the dark at 10 °C for 48 h followed by 20 °C for 24 h. Bags with cuttings were placed horizontally on grated shelving so that both sides of the bags were equally well-ventilated.

For geranium, shoot tip cuttings (3 cm stem length with two to three leaves) were harvested between 0800 and 1000 HR. For postharvest simulation, three cuttings were placed in a one zip-seal polyethylene bag (16.5 × 14.9 cm) with 10 round holes (1.7 mm diameter) on each side. Bags were placed horizontally on grated shelving inside a cooler in the dark (two bags of three cuttings per replicate) at 5 °C for 24 h followed by 10 °C for 48 h.

Texture analysis for mechanical properties

The first fully developed leaf from the shoot tip of a cutting was harvested from stock plants in the morning (0800 to 1000 HR), wrapped in a moist paper towel, placed in a sealed polyethylene bag (17.7 × 19.5 cm), loosely packed in a cooler, and kept in a 5 °C room until the start of measurement the following day. Texture analysis was performed within 24–32 h from leaf removal. Six leaves were used per replicate.

For texture analysis measurements made after the previously described 3 d postharvest simulation, the cuttings were kept in a 4 °C
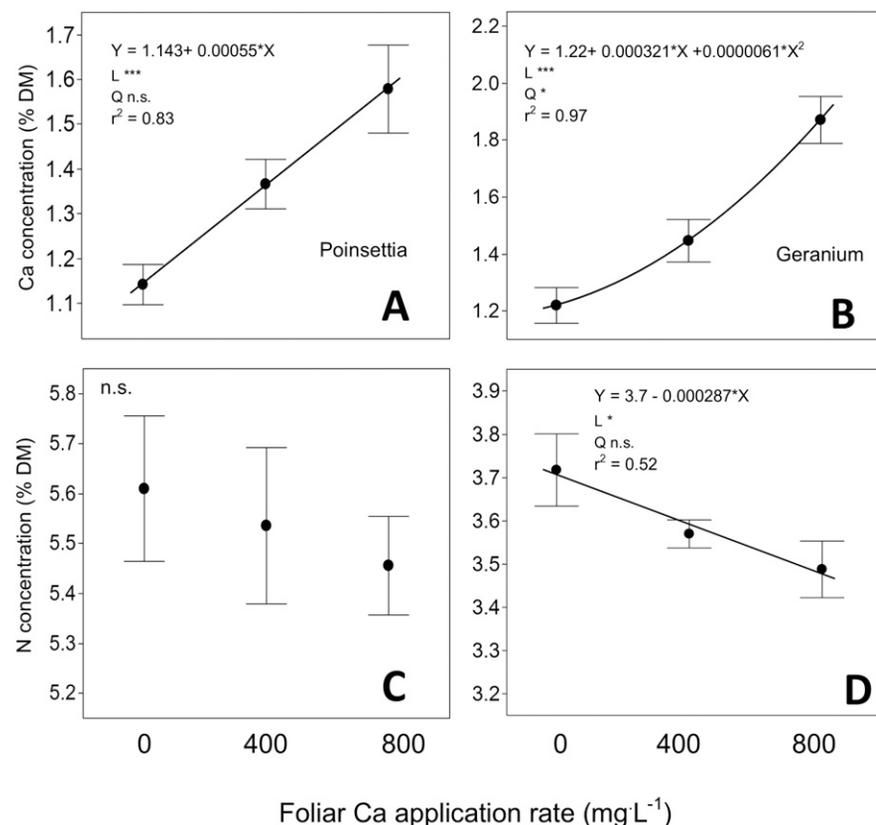
Fig. 1. Concentration of calcium (Ca) (**A** and **B**) and nitrogen (**C** and **D**) as a percentage of leaf dry matter (DM) measured on poinsettia and geranium leaves after weekly foliar applications of Ca in the form of calcium chloride applied to stock plants during cutting development (Expts. 1 and 2). Vertical bars represent ±1 SE (*n* = 5 for poinsettia; *n* = 8 for geranium); L = linear and Q = quadratic contrasts; NS, *, *** represent nonsignificant; or significant at *P* < 0.05 or *P* < 0.0001, respectively.

refrigerator until measurements were made between 1 and 7 h after the removal from postharvest storage. The first fully developed leaf below the shoot tip was collected from stored cuttings for texture analysis.

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 TA-XT Plus Texture Analyzer (Stable Microsystems, Godalming, UK) was used with a TA-1085 small film extension fixture with a 10 mm (78.5 mm^2) opening to hold the leaf and 6.35 mm diameter ball probe (rounded end) to penetrate the leaf. The test specifications were a pretest speed of 2 mm·s^{-1}, test speed of 1 mm·s^{-1}, and posttest speed of 10 mm·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. For poinsettia, 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. For geraniums, the whole leaf was used with the probe penetrating from the adaxial side, avoiding conspicuous veins. For each test, a force–displacement graph was generated, and data were recorded of maximum (peak) force to puncture the leaf (g) and area under the force–displacement curve (g·mm) which is also referred to as the work-of-penetration, hardness, or toughness.

Leaf nutrient analysis

Tissue analysis. The first fully developed leaf, located from the shoot tip of a cutting was collected between 0800 and 1000 HR (six per replicate). Composites of all freshly harvested leaves were washed with distilled water and air-dried at 20 °C for 48 h then placed in an oven at 60 °C until the weight was constant. Oven-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, Schaumburg, IL) and CN analyzer (Vario MICRO cube CHNS; Elementar, Mt. Laurel, NJ) at the United States Department of Agriculture-Agricultural Research Service laboratory in Toledo, OH.

Energy dispersive X-ray analysis (EDX) for elemental analysis. Whole leaf samples were collected from geranium from stock plants of 0 and 800 mg·L^{-1} Ca treatments and stored as per the sampling procedure for texture analysis and sectioned within 5–20 min. of exposure to laboratory conditions on the following day (24 h). Leaf sections of 0.5 cm^2 were cut from the midcentral region of both halves of the leaves ($\approx$8 samples per treatment) and fixed in a solution containing 1% glutaraldehyde (v/v) in cacodylate buffer at pH 7 for 5 min. After washing the tissues in distilled water, the samples were dehydrated in a graded ethanol solution series (70% to 100%). Hexamethyldisilazene (HMDS; Electron Microscopy Sciences, Fort Washington, PA) was introduced through a graded

ethanol-HMDS series (25% (v/v) HMDS/ 75% (v/v) ethanol; 50% HMDS/50% ethanol; 75% HMDS/25% ethanol; two changes in 100% HMDS). After HMDS removal, the samples were air-dried, fractured, and vertically mounted on scanning electron microscopy (SEM) stubs. Elemental analysis was conducted under variable pressure using EDX spectroscopy on Hitachi SU-6600 analytical variable pressure field-emission SEM (Hitachi High Tech, Dallas, TX) using AZtec energy analysis software (Oxford Instruments, Oxforshire, UK). Measurements were taken of all elements from five points across the cross section of the leaf from adaxial to the abaxial side of geranium (10 mm working distance, 20 s acquisition time, and 5 s processing time). There were five to eight measurements per each of three leaf samples per treatment.

Botrytis resistance

Isolation, maintenance, and preparation of conidial suspension of B. cinerea. Cuttings exhibiting symptoms of *Botrytis* in propagation were collected. Isolation and maintenance of conidia and inoculations were carried out as previously reported (Yourman and Jeffers, 1999). Leaves and/or stems were detached and placed in plastic bags with moistened paper towels on a laboratory bench (22 to 25 °C) until conidia were observed (1–3 d). The mycelium containing visible conidia was placed on a petri dish (100 × 15 mm) with acidified potato dextrose agar (APDA) under sterile conditions. Once visible, conidiophores were transferred to APDA plates and incubated at 25 °C on a 12 h alternating light/dark cycle. After 3–4 d, agar containing mycelium from the leading edge of the colony was transferred to APDA plates and incubated
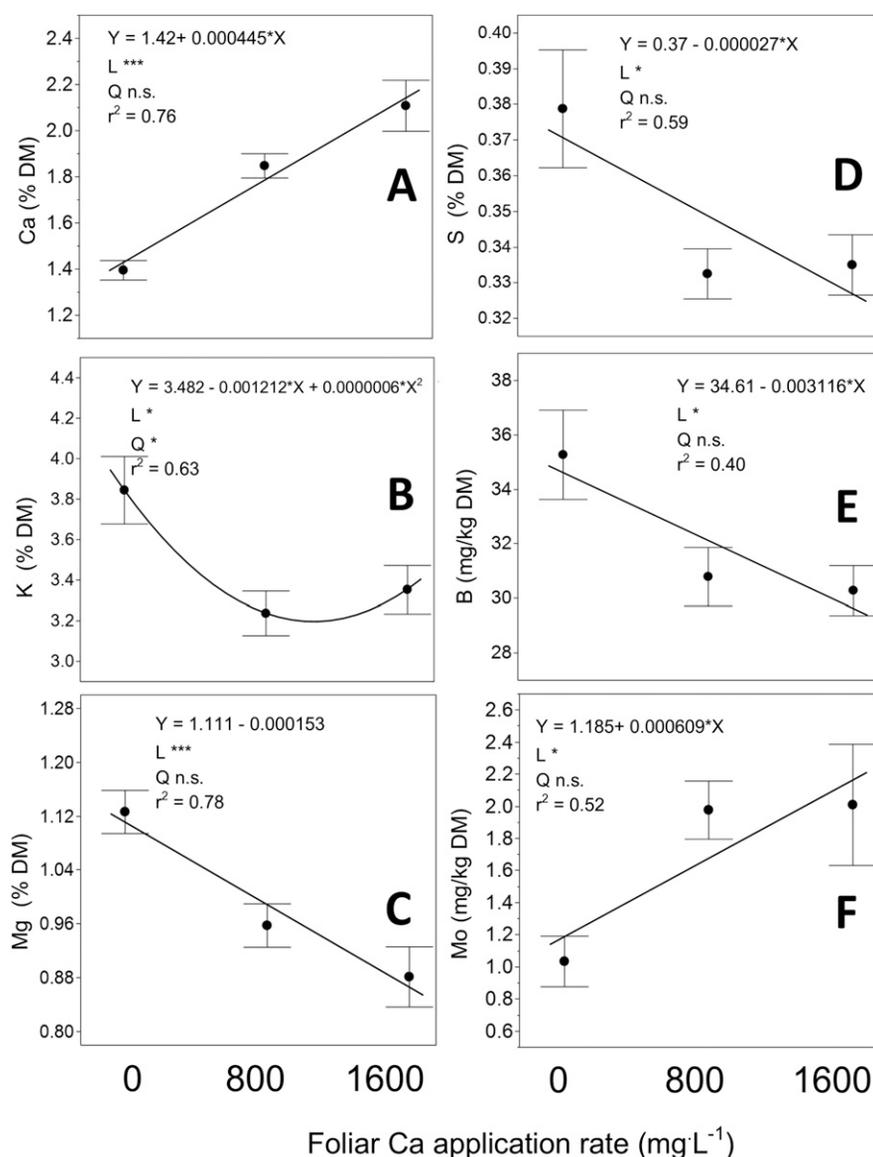
Fig. 2. Concentration of (**A**) calcium (Ca), (**B**) potassium, (**C**) magnesium, (**D**) sulfur, (**E**) boron, and (**F**) molybdenum measured in poinsettia leaves (Expt. 3) after weekly foliar applications of Ca in the form of calcium chloride applied to stock plants during cutting development. Vertical bars represent ±1 SE (n = 5 for poinsettia; n = 8 for geranium); L = linear and Q = quadratic contrasts; NS, *, *** represent nonsignificant or significant at $P < 0.05$ or $P < 0.0001$, respectively.

until conidia developed. This process was repeated until a pure mycelium of *Botrytis* was obtained from two isolates. Conidia were harvested by pipetting 3 mL of a sterile, aqueous solution of 0.01% Tween 80 and the conidia suspensions (15% glycerol) were transferred to 2-mL cryogenic vials (Nalgene Corporation, Rochester, NY) and stored in an ultralow temperature freezer at –80 °C.

To retrieve conidia from the vials stored at –80 °C, a sterile wood applicator was dipped into the conidial suspension and was placed into a microcentrifuge tube containing 25 μL of 0.01% Tween 80 solution. For each isolate, 20 μL of resuspended conidia was placed on a APDA plate and incubated for 10–14 d.

Inoculation and data collection. From each potato dextrose agar plate incubated, colonies were extracted using a sterile wooden applicator after addition of 10 mL of sterile distilled water. Conidial suspensions were prepared to 1:10 dilution and then standardized to $2–3 \times 10^4$ conidia/mL using a haemocytometer.

Cuttings from poinsettia or geranium were placed in plastic, sealed bags with a moist paper towel and stored at 10 °C until used on the following day. For poinsettia, leaf discs from fully opened first leaf of cuttings were cut using a 16.5 mm diameter ring and eight leaf discs were placed in petri dishes containing P5 filter paper (Fisher Scientific, Pittsburgh, PA) soaked with 3 mL of distilled water. One drop of conidia suspension (25 μL) was placed on the center of each leaf disc and incubated at 25 °C for 6 d. For each isolate, there were three petri dishes per treatment and an additional control with leaf discs which were not inoculated. For geranium, whole leaves were used, as the leaf size was too small to take uniform leaf discs. There were three leaves within a petri dish and three petri dishes per treatment. The number of leaf discs with disease incidence was recorded and calculated as a percentage of the total number of leaf discs present within each petri dish.

Data analysis

Data were analyzed using analysis of variance and mean separation by Tukey's Honestly Significant Difference test at $P \leq 0.05$ using JMP Pro 10 (SAS Institute Inc., Cary, NC). Because no significant differences were observed between the two control treatments, for example, with and without surfactant at 0 mg·L^{-1} Ca, data from the two control groups were pooled and orthogonal polynomial contrasts were performed for the leaf nutrient analysis and texture analysis data. In the evaluation of *Botrytis* resistance, data collected from inoculations of the two different isolates were pooled for assessment because the influence of the type of isolate was nonsignificant.

Results

Leaf nutrient concentration. The Ca concentration in leaf tissues of poinsettia in Expt.

1 increased linearly from 1.14% to 1.57% dry matter (DM) with increased application of Ca from 0 to 800 mg·L^{-1} (Fig. 1A). Calcium concentration in geranium leaves in Expt. 2 increased from 1.22% to 1.87% DM with increased application of Ca from 0 to 800 mg·L^{-1} and response was progressively increasing (Fig. 1B). No change in N concentration was observed in poinsettia (Fig. 1C); however, N concentration decreased slightly with the increased concentration of Ca (Fig. 1D), suggesting reduced N uptake in geranium. For both poinsettia and geranium, P, K, Mg, S, B, Fe, Mn, and Mo concentration in leaves did not vary among Ca treatments ($P > 0.05$; data not shown).

For Expt. 3, Ca concentration in poinsettia leaf tissues increased linearly from 1.3% to

2.1% DM with an increased application of Ca from 0 to 1600 mg·L^{-1} (Fig. 2A); however, some macro and micronutrients decreased with increased application of Ca from 0 to 1600 mg·L^{-1} (Fig. 2B–E): By contrast, Mo increased from 1 to 2 mg·kg^{-1} as Ca concentration increased from 0 to 1600 mg·L^{-1} (Fig. 2F). Despite changes in nutrient uptake, the macro and micronutrients other than Ca were within the recommended leaf nutrient concentrations (Mills and Jones, 1996). Phytotoxicity symptoms on poinsettia leaves resulting from the CaCl$_2$ applications were visible as leaf edge burn, yellowing, and abnormal leaf shapes and were more prevalent in treatments with 1600 mg·L^{-1}.

Based on the measurements made at five positions across a leaf section (Fig. 3) of

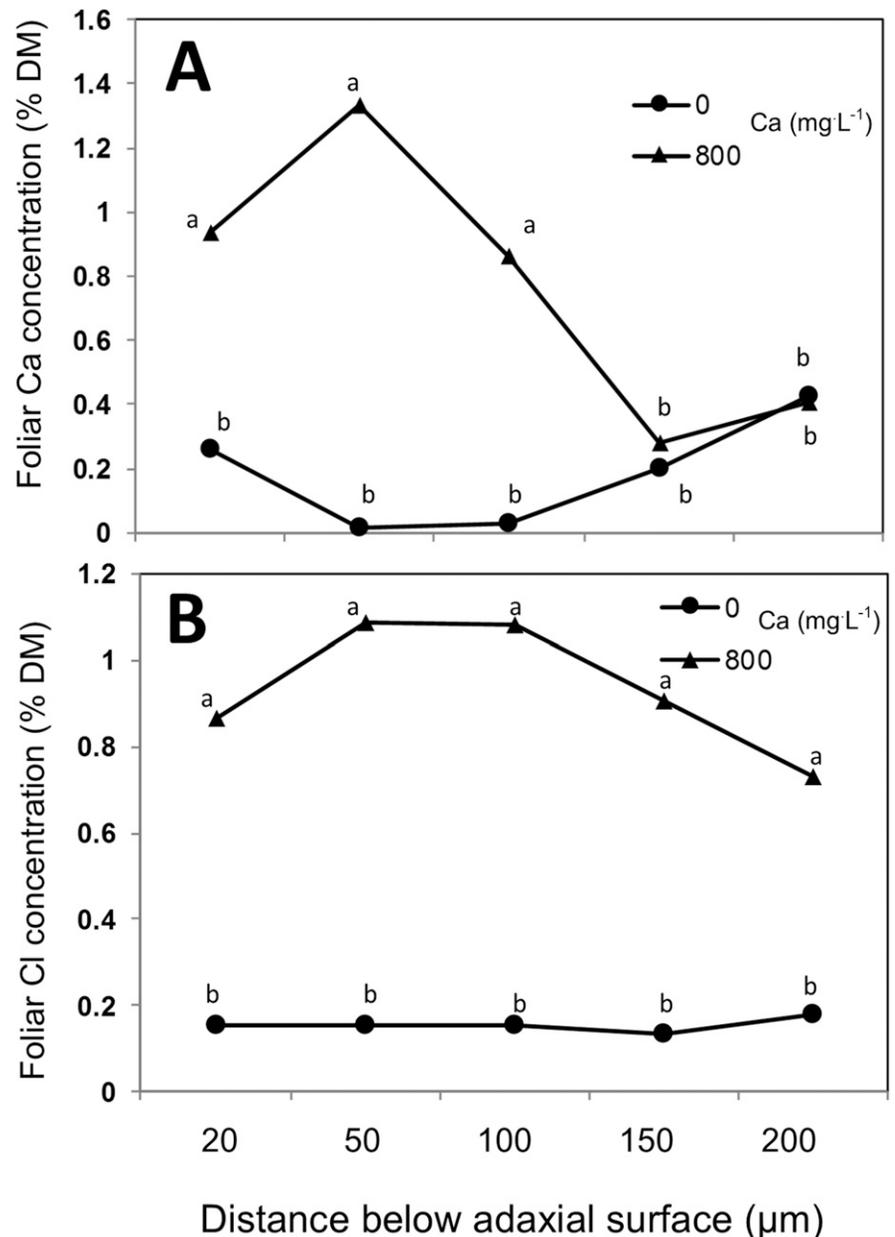Fig. 3. Energy Dispersive X-ray analysis (EDX) of cross sections of geranium leaf samples across points of measurement from adaxil to abxial surface indicating representative concentrations of (**A**) calcium (Ca) and (**B**) chloride after foliar spray application of Ca in the form of calcium chloride (Expt. 2). $n = 11$; Means at each distance followed by different letters were significantly different at $P \leq 0.05$.

geranium, Ca and chlorine (Cl) concentrations were higher in the treatment with 800 mg·L^{-1} Ca as compared with the control. Therefore, the results from the EDX analysis validate the results of Ca concentrations observed in the leaf nutrient analysis of geranium (Fig. 1B). Although Cl concentration was not analyzed by leaf nutrient analysis, data from EDX analysis indicate that Cl was also absorbed and present within tissues (Fig. 3B). Significant differences in Ca ($P = 0.05$) were evident only at 20, 50, and 100 µm from the adaxial surface and not at 150 and 200 µm; therefore, Ca was mainly accumulated from the adaxial to the middle region of the leaf (Fig. 3A). Chlorine concentration was significantly different from the control at all five measurement positions indicating even distribution from the adaxial to the abaxial region ($P = 0.001$; Fig. 3B).

Leaf mechanical properties. In Expt. 1 for poinsettia leaves measured at cutting harvest, peak force and work of penetration increased linearly as the foliar Ca application rate increased from 0 to 800 mg·L^{-1} Ca (Fig. 4A and B). These changes in peak force and work of penetration represent 9% and 12% increases, respectively, at the 800 mg·L^{-1} Ca application rate compared with the control. In poinsettia, the increased mechanical strength due to Ca application was retained after a 3 d postharvest simulation as evident by work of penetration, which increased from 304 to 354 g·mm as Ca applied increased from 0 to 800 mg·L^{-1} (Fig. 4C). The peak force following postharvest simulation was not significantly different with 223 ± 18 g on average (data not shown).

In Expt. 2 for geranium leaves at harvest, peak force was not significantly different with an average of 295 ± 25 g (Fig. 4D). Work of penetration increased linearly by 15% or by 100 g·mm with the application of 800 mg·L^{-1} Ca (Fig. 4E) compared with the control. Neither work of penetration (Fig. 4F) nor peak force was significant for geranium after a 3 d postharvest simulation.

In Expt. 3 with poinsettia, peak force was 7% greater in leaves treated with 800 and 1600 mg·L^{-1} Ca (198.5 ± 1 g) as compared with treatments without Ca (178 ± 4 g: data not shown). No differences were evident between 800 and 1600 mg·L^{-1}, hence leaf mechanical strength was not influenced by increasing Ca beyond 800 mg·L^{-1}. Work of penetration was not significantly different between treatments in Expt. 3 ($P > 0.05$).

Botrytis resistance. Poinsettia leaves were susceptible to infection from two *Botrytis* isolates (data not shown). No disease incidence was recorded on any of the leaf discs of poinsettia in the noninoculated control; however, the inoculated control had 59% disease incidence per petri dish indicating leaf discs were successfully inoculated ($P = 0.0001$). Treatment with 0 mg·L^{-1} Ca with surfactant reduced the disease incidence to 19%. Treatment with 800 mg·L^{-1} Ca reduced the disease incidence down to 4%. The CaCl$_2$ treatments did not affect *Botrytis* infection on geranium.
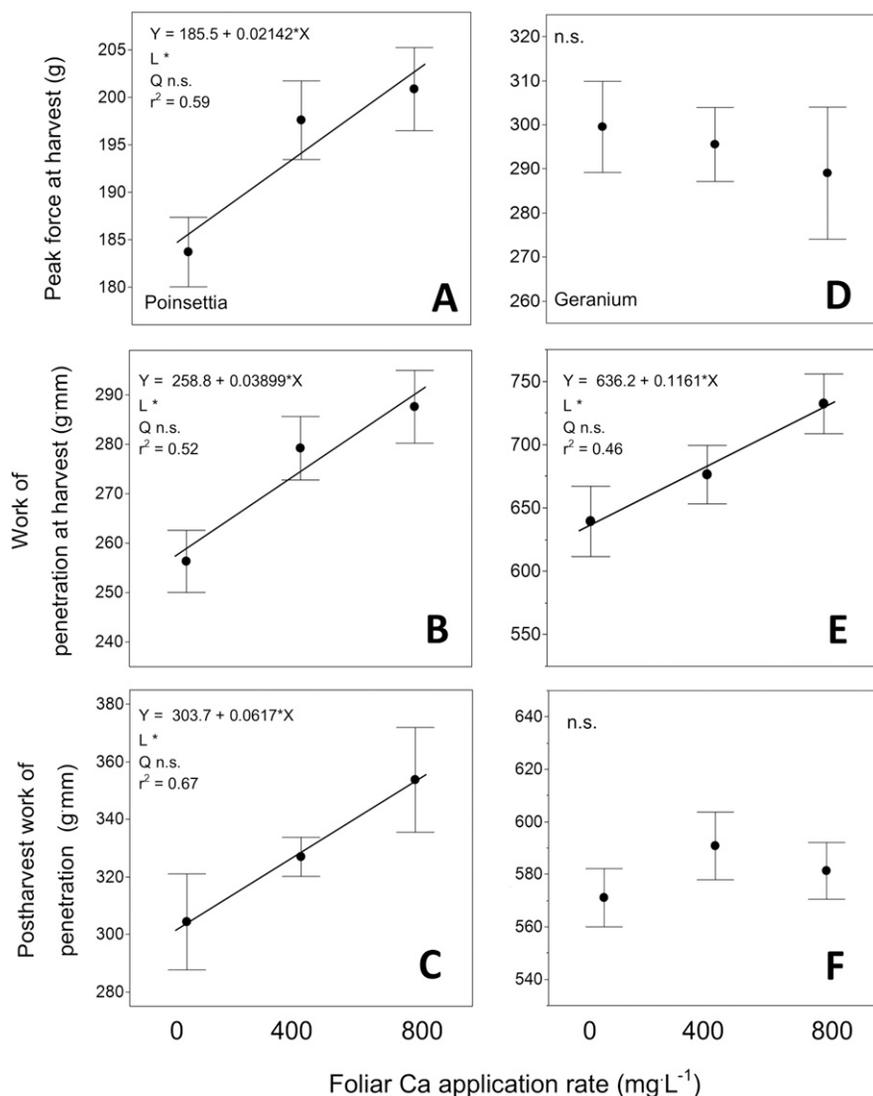

Fig. 4. Leaf mechanical strength as indicated by peak force at harvest, work of penetration at harvest, and work of penetration after a 3 d postharvest simulation for leaves on poinsettia (**A**–**C**) (Expt. 1) and geranium cuttings (**D**–**F**) (Expts. 1 & 2). Leaves were sampled from cuttings after weekly foliar spray applications of calcium in the form of calcium chloride applied to stock plants during cutting development. Vertical bars represent ±1 SE ($n = 5$ for poinsettia; $n = 8$ for geranium); L = linear and Q = quadratic contrasts; NS, *, *** represent nonsignificant or significant at $P < 0.05$ or $P < 0.0001$, respectively.

Discussion

Foliar application of CaCl$_2$ to poinsettia resulted in a linear increase in leaf tissue Ca concentration and a quadratic increase in leaf tissue Ca concentration of geranium. These results demonstrate the potential value of foliar Ca applications to enhance fertigation treatments. The penetration of Ca into the leaf tissue was verified with EDX analysis which proved that Ca was not merely accumulating on the leaf surfaces. The observed distribution of Ca within the cells suggests that Ca was mainly accumulating in palisade mesophyll cells compared with the upper and lower epidermal cells. A similar pattern of distribution of vacuolar concentration of Ca from the upper epidermis, palisade mesophyll, spongy mesophyll, and bundle sheath to the lower epidermis was noted in *Arabidopsis* (Conn et al., 2011). In this study, the increased Ca concentration in the leaf tissue was associated with increased mechanical strength and, with poinsettia, increased *Botrytis* resistance. The magnitude of the benefit of foliar Ca applications will likely depend, at least in part, to the amount of Ca supplied through the fertigation treatment. In our study, 66 ppm Ca was provided in the fertigation solution in a constant liquid fertilization program. This is within the accepted range of Ca supplied to poinsettias and geraniums. Tissue analysis guidelines suggest a sufficiency range for Ca of 0.4% to 2.0% DM for poinsettia and 0.8% to 2.4% DM for zonal geraniums (Mills and Jones, 1996). The leaves from our control plants had Ca concentrations ranging from 1.15% to 1.21% DM for poinsettia and geranium, respectively. This demonstrates that the fertigation program used in this study supplied sufficient Ca for normal plant growth, yet foliar Ca applications had additional physiological benefits.

Increased peak force and work of penetration were indications that increasing Ca increased the mechanical resistance of leaves to external force (Fig. 4). Of the two parameters, work of penetration provided a better assessment of mechanical strength as it measured the total force required to break as well as tear through the tissue, providing an indication that tissues with high Ca were more difficult to mechanically damage. The evidence of increased mechanical strength after Ca application was reported in fruits via increased firmness (Ortiz et al., 2011; Sirijariyawat et al., 2012). In addition to being an important component in plant cell walls, Ca could have influenced the binding of cells with middle lamella and membrane integrity. Further research is needed to address that possibility. The reduction of N with the increase in the application of Ca in the leaf tissues may have also contributed to increased mechanical strength (Fig. 1C and D) because N concentration in leaf tissues has been reported to reduce the mechanical strength of spinach leaves (Gutiérrez-Rodríguez et al., 2013).

Reduction of disease incidence to *Botrytis* occurred in poinsettia in response to the application of surfactant and CaCl$_2$, and there was a complementary effect when applied together. The presence of silicon in the surfactant may have caused an increased resistance to *Botrytis* (Fauteux et al., 2005); therefore, the surfactant may have improved the effect of CaCl$_2$ on resistance to *Botrytis*. While this conclusion is primarily based on poinsettia, this approach may also be beneficial on geranium cultivars that are highly susceptible to the fungal pathogen *Botrytis* in propagation because the cultivar 'Calliope Red' used during the current study is known to be relatively resistant to *Botrytis* infection. While it is not possible to separate the effects of Ca and Cl in this study, the reduction of sporulation of *Botrytis* because of increased Ca concentration in the tissue has been previously reported (Yermiyahu et al., 2006).

The use of foliar sprays is relatively convenient in a commercial facility compared with changing humidity or simulating drought to produce high-quality "toned" cuttings. The present study provides evidence for the efficacy of foliar Ca applications to change physiological properties that are important in postharvest and propagation environments. To our knowledge, this is the first evaluation of the potential use of Ca foliar sprays to strengthen the leaves and thereby produce higher quality cuttings and increased disease resistance. Hence, it is possible that the use of Ca foliar application to stock plants may provide a safe and environment-friendly alternative to fungicide application.

Whereas phytotoxicity symptoms with Ca nutrition have not previously been reported in poinsettia (Ecke et al., 2004), according to the present study, increasing the concentration of CaCl$_2$ to provide Ca at 1600 mg·L^{-1} caused phytotoxicity in poinsettia. Further studies are required to determine whether Ca or Cl caused the phytotoxicity. No phytotoxicity was observed on geranium.

In conclusion, our investigation provides evidence for the potential benefit of foliar-applied CaCl$_2$ for improved performance of unrooted cuttings. Calcium chloride sprays can increase the mechanical strength of poinsettia and geranium leaves, and they can increase *Botrytis* resistance in poinsettia leaves.

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