Response of *Calceolaria* × *herbeohybrida* Cultivars to Substrate pH and Corresponding Leaf Tissue Nutrient Concentration Effects

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Abstract. *Calceolaria* (*Calceolaria* × *herbeohybrida*) is a flowering potted greenhouse crop that often develops upper-leaf chlorosis, interveinal chlorosis, and marginal and leaf-tip necrosis (death) caused by cultural practices. The objectives of this research were to 1) determine the optimal incorporation rate of dolomitic and/or hydrated lime to increase substrate pH; 2) determine the influence of the liming material on substrate pH, plant growth, and leaf tissue nutrient concentrations; and 3) determine the optimal substrate pH to grow and maintain during calceolaria production. Sphagnum peatmoss was amended with 20% (by volume) perlite and incorporated with pulverized dolomitic carbonate limestone (DL) and/or hydrated limestone (HL) at the following concentrations: 48.1 kg·m⁻³ or 144.2 kg·m⁻³ DL, 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL to achieve a target substrate pH of 4.5, 5.5, 6.5, and 7.5, respectively. *Calceolaria* ‘Orange’, ‘Orange Red Eye’, ‘Yellow’, and ‘Yellow Red Eye’ were grown in each of the prepared substrates. For all cultivars, substrate solution pH increased as limestone incorporation concentration and weeks after transplant (WAT) increased, although to different magnitudes. For example, as limestone incorporation increased from 48.1 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL, substrate solution pH for ‘Orange’ calceolaria increased from 4.1 to 6.9 to 4.8 to 7.2 at 2 and 6 WAT, respectively. Substrate solution electrical conductivity (EC) and growth indices were not influenced by limestone incorporation, but total plant dry mass increased. Few macronutrients and most micronutrients were influenced by limestone incorporation. Leaf tissue iron concentrations for ‘Orange’, ‘Orange Red Eye’, ‘Yellow’, and ‘Yellow Red Eye’ calceolaria decreased by 146%, 91%, 71%, and 84%, respectively, when plants were grown in substrates incorporated with increasing limestone concentrations from 144.2 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL (pH 6.5–6.9). Therefore, incorporating 144.2 kg·m⁻³ DL into peat-based substrates and maintaining a pH <6.5 will avoid high pH-induced Fe deficiency and prevent upper-leaf and interveinal chlorosis.

Soilless substrates used for producing of floriculture crops may be composed of one or a mix of either peatmoss, aged pine bark, coir, or wood fiber, each varying in initial pH. It is a common practice to incorporate limestone into substrates to neutralize substrate

acidity, thereby raising substrate pH to a range of 5.4 to 6.5 for most greenhouse crops (Nelson, 2012). To neutralize substrate acidity, three types of horticultural lime are commonly incorporated at different proportions: calcitic (CaCO₃), DL (CaCO₃·MgCO₃), and HL [Ca(OH)₂·MgO; Owen, 2013]. These liming materials differ in their reactivity, residual effect, chemical composition, and particle size distribution. Reactivity is the magnitude of pH change (ΔpH) over time and is primarily a function of lime particle size, lime chemistry, acid neutralizing value, and initial substrate pH (Fisher et al., 2006). Residual lime is the proportion of unreacted lime remaining after neutralization of substrate pH. In addition, the effectiveness of a lime material to neutralize the acidity of substrates depends on its neutralizing capacity, fineness of grinding (particle size), chemical composition [providing calcium (Ca) and/or magnesium (Mg)], and mineralogy (Barber, 1984). Particle size of the liming material directly influences the dissolution rate and

its effectiveness in neutralizing substrate acidity (Huang et al., 2007). Therefore, substrate manufacturers and greenhouse growers must select the appropriate liming material(s) and proportion(s) for immediate reactivity to initially raise the substrate pH and some residual effect to maintain an optimal pH range throughout the crop cycle.

During a crop production cycle, substrate pH can drift above or below the recommended range (5.4 to 6.5), affecting plant growth and nutrient availability and uptake, potentially inducing nutritional disorders. Substrate pH drives the chemical reactions determining whether nutrients are either soluble and available for root uptake or insoluble and unavailable (Argo and Fisher, 2002). Phosphorous (P) and most micronutrients, such as manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), and boron (B), are affected by substrate pH. For example, in a study determining the optimal substrate pH of five sedum (*Sedum* sp.) species, Zheng and Clark (2013) reported species-specific effects for plant growth and leaf tissue P, potassium (K), Mg, Ca, Mn, Cu, Fe, Zn, and B concentration of plants grown in peat-based substrates amended with increasing concentrations of HL (pH 4.4–8.2). In another study, Dickson et al. (2016) reported Fe deficiency sensitivity of 24 genotypes of calibrachoa (*Calibrachoa* × *hybrida* Cerv.) grown in peat-based substrates amended with 1.1 (pH 5.4) or 2.0 (pH 7.1) kg·m⁻³ HL. Whereas these studies report the influence of lime/substrate pH on leaf tissue nutrient concentrations, Andrews and Hammer (2006) reported the influence of increasing limestone incorporation concentrations from 0 to 11.9 kg·m⁻³ DL or 11.9 kg·m⁻³ DL + 5.9, 8.3, or 10.7 kg·m⁻³ HL (pH 4.3–7.8) on substrate pH, growth, and leaf color of zonal geraniums (*Pelargonium* × *hortorum* L.H. Bailey ‘Candy Lavender’, ‘Fireball’, and ‘Patriot Red’) and ivy geraniums [*P. pelatum* (L.) L’Hér. Ex Aiton ‘Global Deep Lilac’, ‘Global Salmon Rose’, and ‘Global Soft Pink’]. Similarly, Owen (2013) determined the influence of increasing limestone incorporation concentrations from 0 to 7.1 kg·m⁻³ DL (pH 3.7–6.1) and 0–8.9 kg·m⁻³ DL (pH 4.0–6.4) on substrate solution pH and EC, plant growth, and observed nutrient disorders of garden mum (*Chrysanthemum* × *morifolium* Ramat. ‘Mildred Yellow’) and African marigold (*Tagetes erecta* L. ‘Moonsong Deep Orange’), respectively.

While previous research investigated the effects of lime on substrate pH and growth of popular floriculture bedding plant species, little attention has been given to florist-quality potted flowering crops, which generate \$1.8 billion USD in sales annually (U.S. Department of Agriculture, 2015). *Calceolaria* (*Calceolaria* × *herbeohybrida* Voss.) frequently exhibit high pH-induced Fe deficiency (personal observation). Furthermore, growers using previous literature as recommendations found their crops to exhibit Fe deficiency because optimal substrate pH ranges for calceolaria production were previously reported to be between 6.0 and 6.5

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(White, 1975), then 5.7 and 6.5 (Erwin, 1994), and most recently ranges from 5.8 to 6.2 (Currey, 2017). Therefore, it is unclear the optimal substrate pH to produce high-quality flowering calceolarias. Therefore, the objectives of this research were to determine 1) the optimal incorporation concentration(s) of dolomitic and/or hydrated lime to adjust substrate pH; 2) the influence of the liming material on substrate pH, plant growth, and leaf tissue nutrient concentrations; and 3) the optimal substrate pH to grow and maintain during calceolaria production.

Materials and Methods

Substrate preparation and liming materials.

On 9 Sept. 2017, canadian sphagnum peatmoss (Sun Gro Peat Moss Grower Grade Orange; Sun Gro Horticulture, Agawam, MA) was taken from a compressed bale, loosened, and moistened (by hand) to a moisture content of $\approx 50\%$ (by weight). Moistened peatmoss was amended with 20% (by volume) coarse perlite (PVP Industries Coarse Horticultural Perlite, North Bloomfield, OH), thus formulating (by volume) a 80:20 peat:perlite substrate. Substrate physical properties, including air space [(AS); % volume], total porosity [(TP); % volume], container capacity [(CC); % volume], and bulk density [(BD); $\text{g}\cdot\text{cm}^{-3}$] were determined using three representative substrate samples and analyzed using the NC State University Porometer procedure (Fonteno et al., 1995). Physical properties of the substrate were (by volume) $8.2 \pm 0.8\%$ AS, $86.5 \pm 0.3\%$ TP, $78.3 \pm 0.7\%$ CC, and $0.10 \text{ g}\cdot\text{cm}^{-3}$ BD.

After formulation of the substrate, initial substrate pH was determined by the 2:1 saturated media method (2 parts deionized water:1 part substrate; Argo and Fisher, 2002) using a handheld pH and EC meter (HI 9813-6; Hanna Instruments, Woonsocket, RI). Average initial substrate pH before lime incorporation was 4.3 ± 0.1 . After determining initial substrate pH, two liming materials, DL [(85% CaCO_3 : MgCO_3); Rockydale Quarries Corporation, Roanoke, VA] and HL [97% $\text{Ca}(\text{OH})_2$: MgO ; Southern Lime, Calera, AL], were used to increase the substrate pH. A screening of the pulverized DL showed 100% of the liming material passed through a 2000- μm (#10) screen, 90% passed through an 841- μm (#20) screen, 60% passed through a 250- μm (#60) screen, and 35% passed through a 149- μm (#100) screen. Pulverized DL and DL+HL were incorporated into substrates measured to 0.02 m^3 at the following concentrations: 48.1 or 144.2 $\text{kg}\cdot\text{m}^{-3}$ DL, 17.6 $\text{kg}\cdot\text{m}^{-3}$ DL + 5.3 $\text{kg}\cdot\text{m}^{-3}$ HL, or 17.6 $\text{kg}\cdot\text{m}^{-3}$ DL + 10.6 $\text{kg}\cdot\text{m}^{-3}$ HL to achieve target substrate pHs of 4.5, 5.5, 6.5, and 7.5, respectively. Additionally, a wetting agent was incorporated at a concentration of 0.6 $\text{kg}\cdot\text{m}^{-3}$ (Aquatrols 2000G, Aquatrols, Paulsboro, NJ). Substrates were sealed in 3-mil black plastic bags ($0.8 \text{ m} \times 1.15 \text{ m}$; Contractor's Choice, Grand Prairie, TX), for moisture retention, and incubated at 20°C for 24 d to allow for lime/pH equilibration. Before transplant, substrate pH was

determined by the 1:2 dilution procedure as previously described and for each target pH, the average pH values were 4.6 ± 0.1 , 5.5 ± 0.1 , 6.6 ± 0.1 , and 7.5 ± 0.1 .

Plant material and culture. On 3 Oct. 2017, 105-cell plug trays (23-mL individual cell volume; Landmark Plastic Corporation, Akron, OH) of 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria were received from a commercial supplier (Ball Tagawa Growers, Arroyo Grande, CA). Young plants of each cultivar with similar heights, stem caliper, and leaf numbers were selected and transplanted one plant per 12.7-cm-diameter container (885-mL volume; ITML Horticultural Products, Middlefield, OH) filled with one of the four premoistened limed substrates. Plants were irrigated to container capacity with water supplemented with 35% sulfuric acid (AutoCraft Battery Acid, Johnson Controls Battery Group, Milwaukee, WI) at $0.16 \text{ mg}\cdot\text{L}^{-1}$ to neutralize alkalinity from 4.0 to 1.6 $\text{meq}\cdot\text{L}^{-1}$ CaCO_3 and reduce pH from 7.3 to a range of 5.8 to 6.0.

Greenhouse environment. Plants were grown in a double polyethylene-covered greenhouse with roll up side curtains [MSU Tollgate Farm and Education Center, Novi, MI (lat. 42°N)] at a constant 20°C air temperature under ambient daylight supplemented with a photosynthetic photon flux density (PPFD) of $32.7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at plant height (as measured with a quantum sensor; LI-190SL; LI-COR Biosciences, Lincoln, NE), delivered from 150-W high-pressure sodium lamps (Sun System HPS 150 Grow Light Fixture; Sunlight Supply, Inc., Vancouver, WA) from 0600 to 2200 hr (16-h photoperiod). On each bench, an enclosed thermocouple recorded air temperature every 30 s and averages were logged every 15 min by a data logger (WatchDog Model 2475 Plant Growth Station; Spectrum Technologies, Inc., Aurora, IL). Line quantum sensors (SQ-316-SS; Apogee Instruments, Inc., Logan, UT) mounted 15 cm above the benchtop measured PPFD every 30 s, and the average of each sensor was logged every 15 min by a data logger (WatchDog 2800 Weather Station; Spectrum Technologies, Inc.). Average daily light integral, air temperature, and relative humidity throughout the 8-week duration of the experiment were $10.1 \pm 3.2 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $20.4 \pm 3.8^\circ\text{C}$, and $65.3 \pm 4.6\%$, respectively. Plants were irrigated with acidified water as previously described and were never allowed to dry out. At each irrigation, plants were fertilized with 17N-1.7P-10K (Jack's Professional Pure Water XL; J.R. Peters Inc., Allentown, PA) containing 3.7% ammoniacal ($\text{NH}_4^+\text{-N}$) and 13.3% nitrate ($\text{NO}_3^-\text{-N}$). Plants received the following ($\text{mg}\cdot\text{L}^{-1}$): 150 N, 10.3 P, 83 K, 17.6 Ca, 8.8 Mg, 0.50 Fe, 0.25 Mn and Zn, 0.10 B, 0.05 Cu, and molybdenum (Mo).

Growth and nutritional data and calculations. At 2, 4, 6, and 8 WAT, substrate solution was extracted 1 h after irrigation using the pour-through method (Wright, 1986) and analyzed for pH and EC using a handheld pH and EC meter (HI 9813-6; Hanna Instruments). At each sampling week, plant diameter was determined by measuring the widest dimension

and the axis perpendicular to the widest dimension and growth index (GI) was calculated [$\text{GI} = (\text{widest diameter} + \text{perpendicular widest diameter}) \div 2$] for each plant.

At 8 WAT, upon termination of the experiment, recently mature (youngest fully expanded) leaves were removed from each experimental unit (individual plants) from each cultivar grown in each limed substrate. Leaves were washed in a solution of 0.5 N hydrochloric acid for 1 min and rinsed in deionized water before being individually bagged, dried in an oven at 70°C for 1 week. After 7 d, dried tissue was weighed to determine young leaf dry mass (YDM) and ground with a mortar and pestle to pass a $\leq 0.5\text{-mm}$ sieve, placed in 15-mL polypropylene conical centrifuge tubes (Falcon $17 \times 120 \text{ mm}$; Corning, Corning, NY), and analyzed for nutrient concentrations by AgSource Laboratories (Lincoln, NE). Total N was processed by Kjeldahl digestion and determined by flow injection analysis. Extractable K was processed by 2% acetic acid digestion and determined by inductively coupled plasma mass spectrometry (ICP-MS). Total P and all other plant minerals (Ca, Mg, Fe, Mn, Zn, B, and Cu) were processed by nitric acid/hydrogen peroxide digestion and determined by ICP-MS.

The remaining plant tissues were destructively harvested by severing the stem at the substrate surface, individually bagged, and dried in an oven at 70°C . After 1 week, plant dry mass (PDM) was determined. Total plant dry mass [TDM ($\text{TDM} = \text{YDM} + \text{PDM}$)] was calculated for each plant.

Experimental design and statistical analyses. The experiment was laid out in a randomized complete block design with three blocks (replicates) of three experimental units (individual plants) per cultivar per limed substrate. Data were collected on one experimental unit per block for pour-through (pH and EC) data and on all three experimental units for GI and TDM from each block. Data for each cultivar were analyzed independently. Within each block, no significant differences occurred; therefore, data were pooled. Substrate solution pH and leaf tissue nutrient concentration data were analyzed using SAS (version 9.4; SAS Institute, Cary, NC) general linear model (PROC GLM) for analysis of variance, and means were separated using Tukey's honestly significant difference test. For substrate solution EC and GI data, regression analysis over WAT was performed using SAS regression procedure (PROC REG). For all analyses, a $P \leq 0.05$ was used to determine significant effects.

Results and Discussion

Substrate solution pH. For each calceolaria cultivar, limestone incorporation, week, and limestone incorporation $\times$ week interaction ($P < 0.0001$) affected substrate pH. As limestone incorporation increased, average substrate solution pH increased over time, although to different magnitudes for each cultivar (Fig. 1A–D). For example, as limestone

Fig. 1. Substrate solution pH determined by the pour-through method at 2, 4, 6, and 8 WAT for (A) 'Orange', (B) 'Orange Red Eye', (C) 'Yellow', and (D) 'Yellow Red Eye' calceolaria (*Calceolaria xherbeohybrida* Voss.) grown in peat-based substrates amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Each symbol represents a mean of three samples, and error bars represent $\pm$ SE. Means were separated within each week and means sharing uppercase letters are not significantly different by Tukey's honestly significant difference test at $P \leq 0.05$.

incorporation increased from 48.1 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL, substrate solution pH of 'Orange' calceolaria increased by 40% (2.8 ΔpH units), 40% (2.8 ΔpH units), 33% (2.4 ΔpH units), and 31% (2.1 ΔpH units) at 2, 4, 6, and 8 WAT, respectively (Fig. 1A). Additionally, for each cultivar, from 6 to 8 WAT, substrate solution pH decreased at each limestone incorporation concentration. For example, from 6 to 8 WAT, substrate solution pH of 'Orange Red Eye' calceolaria grown in substrates limed with 48.1 kg·m⁻³ DL, 144.2 kg·m⁻³ DL, 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL decreased by 6% (0.3 ΔpH units), 9% (0.5 ΔpH units), 3% (0.2 ΔpH units), and 5% (0.4 ΔpH units), respectively. Similarly, Andrews and Hammer (2006) reported pH of substrates incorporated with increasing

concentrations of DL or DL+HL to decrease from 3 to 11 WAT for three cultivars each of zonal and ivy geraniums. They attributed the decline in pH over time to the ability of geraniums to lower substrate pH by root exudation of H⁺ and acidic organic substances to aid in nutrient uptake (Paul and Clark, 1989). In the current study, declining substrate solution pH likely contributed to reduced residual effect or composition (particle size) of the limestone than a plant growth effect. To date, no literature exists determining calceolaria as an Fe-efficient plant, but based on these results, it is speculated that calceolaria is an Fe-inefficient plant.

Furthermore, substrates incorporated with the higher concentration of each limestone (144.2 kg·m⁻³ DL and 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL) resulted in a greater decline in substrate solution pH than those limed with lower concentrations. For example, from 6 to 8 WAT, substrate solution pH of 'Yellow' calceolaria grown in substrates incorporated with 144.2 kg·m⁻³ DL or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL decreased by 7% (0.4 ΔpH units) and 6% (0.4 ΔpH units), respectively, compared with those incorporated with 48.1 kg·m⁻³ DL (4%; 0.2 ΔpH units) or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL (3%; 0.2 ΔpH units). The observed trend occurred for all cultivars and may be a result of reduced solubility of the DL or the fact that HL reacts quickly and is more easily leached out over time (Andrews and Hammer, 2006). Nonetheless, Wiedenfeld and Cox (1988) indicated that the effect of limestone diminishes at high incorporation concentrations, which were observed in the current study.

Substrate solution EC. For each calceolaria cultivar, substrate solution EC was unaffected by limestone incorporation or limestone incorporation $\times$ week interaction but varied by week ($P < 0.0001$). In general, regardless of limestone incorporation, average substrate solution EC resulted in a steady decline from 2 to 6 WAT and increased by 8 WAT (Fig. 2A–D). For example, average substrate solution EC of 'Yellow Red Eye' calceolaria decreased by 117% ($\Delta 0.66$ mS·cm⁻¹) from 2 to 6 WAT and increased by 48% ($\Delta 0.69$ mS·cm⁻¹) 2 weeks later (8 WAT; Fig. 2D). The observed trend of decreasing then increasing substrate solution EC over time interestingly is the inverse trend reported for substrate solution pH. Declining substrate solution EC is often attributed to increasing plant growth, which was observed. However, it is known that N form (NH₄⁺-N lowers substrate pH; NO₃⁻-N raises pH) of the water-soluble fertilizer and N concentration (Owen et al., 2016) can influence substrate pH. Therefore, decreasing substrate solution EC and increasing substrate solution pH may be a result of increased plant growth and the residual limestone contained in the substrate overcame the effect of the water-soluble fertilizer (Argo and Fisher, 2002). During the last 2 weeks of the study (from 6 to 8 WAT), increasing substrate solution EC and decreasing substrate solution pH were results of increased NH₄⁺-N rather than NO₃⁻-N provided by the 3:1 NH₄⁺-N:NO₃⁻-N

water-soluble fertilizer, thus acidifying the rhizosphere.

Growth. For each calceolaria cultivar, GI was unaffected by limestone incorporation or limestone incorporation $\times$ week interaction but varied by week ($P < 0.0001$). Growth index data were pooled and analyzed by week. At 2 WAT, GI of calceolaria cultivars was smallest and ranged from 14.0 to 15.5 cm. From 2 to 6 WAT, GI increased quadratically by 42% (10.1 cm), 38% (8.8 cm), 45% (9.1 cm), and 35% (8.3 cm) for 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria, respectively (Fig. 2E–H). Growth did not differ beyond 6 WAT.

Limestone incorporation affected TDM for all cultivars, though to different magnitudes (Fig. 3). Total dry mass was smallest for plants grown in substrates incorporated with 48.1 kg·m⁻³ DL and ranged from 5.6 to 7.4 g. For each cultivar, TDM increased with increasing limestone incorporation up to 144.2 kg·m⁻³ DL. For example, TDM of 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria grown in substrates limed with 48.1 kg·m⁻³ DL to 144.2 kg·m⁻³ DL increased by 15% (1.3 g), 41% (4.2 g), 13% (1.1 g), and 24% (1.8 g), respectively. It is important to note that TDM decreased by 7% (0.4 g) for 'Yellow Red Eye' calceolaria grown in substrates incorporated with 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Less dry mass may likely be attributed to higher substrate solution pH and thus limited nutrient availability and uptake. By 8 WAT, all cultivars grown in substrates incorporated with DL+HL exhibited chlorosis (yellowing) and interveinal chlorosis, which is speculated to be high pH-induced Fe deficiency (Fig. 4). Furthermore, less dry mass may be the result of increased substrate solution EC, for example, increased NH₄⁺-N because calceolaria is more sensitive to NH₄⁺-N than NO₃⁻-N (Erwin, 1994).

Leaf tissue nutrient composition. Differences in leaf tissue contents among limed substrates were observed for some macronutrients (Total N, Ca, and Mg) and micronutrients (Cu and Fe) for each cultivar. To date, no leaf tissue nutrient sufficiency ranges exist for calceolaria; therefore, general plant sufficiency ranges reported by Bryson and Mills (2014) for N (1% to 6% N), Ca (0.5% to 1.5% Ca), Mg (0.15% to 0.40% Mg), Cu (2–20 mg·kg⁻¹ Cu), and Fe (50–75 mg·kg⁻¹ Fe) will be referenced along with substrate solution pH values at 8 WAT.

Total nitrogen. Limestone incorporation affected total N for 'Orange Red Eye' and 'Yellow' but not for 'Orange' or 'Yellow Red Eye' calceolaria. Leaf tissue total N concentrations of 'Orange Red Eye' and 'Yellow' calceolaria increased by 14% ($\Delta 0.7\%$ N) and 9% ($\Delta 0.4\%$ N), respectively, when plants were grown in substrates incorporated with 48.1 kg·m⁻³ DL to 144.2 kg·m⁻³ DL resulting in a ΔpH of 0.6 (pH 4.5 to 5.1) and 0.8 (pH 4.5 to 5.3) pH units, respectively (data not shown). Overall, total N concentrations of 'Orange Red Eye' and 'Yellow' calceolaria grown in the

Fig. 2. Substrate solution electrical conductivity (EC; A–D) determined by the pour-through method and growth index (GI; E–H) at 2, 4, 6, and 8 weeks after transplant of calceolaria (*Calceolaria × herbeohybrida* Voss. 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye') grown in a peat-based substrate amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+ hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Within each week, symbols represent a mean of 12 samples for EC and 36 samples for GI, and error bars represent ±SE. Regression lines are presented with corresponding R²; *, **, ***significant at $P \leq 0.05$, 0.001, or 0.0001, respectively.

Fig. 3. Total dry mass of calceolaria (*Calceolaria × herbeohybrida* Voss. 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye') at 8 weeks after transplant into a peat-based substrates amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+ hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Each bar represents a mean of nine samples, and error bars represent ±SE. Means within a cultivar with the same uppercase letter are not different by Tukey's honestly significant difference test at $P \leq 0.05$.

substrate incorporated with 144.2 kg·m⁻³ DL were 4.6 and 4.3% total N, respectively, and within the general plant sufficiency range reported by Bryson and Mills (2014).

Calcium. Leaf tissue Ca concentrations for 'Yellow' ($P = 0.0324$) and 'Yellow Red Eye' ($P = 0.0115$) calceolaria increased with limestone incorporation. The greatest leaf tissue Ca concentrations were observed in 'Yellow' and 'Yellow Red Eye' calceolaria plants grown in substrates incorporated with the 144.2 kg·m⁻³ DL limestone incorporation

concentration and were 0.87 (pH 5.3) and 1.11% Ca (pH 5.2), respectively (data not shown). Zheng and Clark (2013) reported sedum [*Sedum sexangulare* (L.)] leaf tissue Ca concentration to be highest in plants grown in substrates amended with HL at a substrate pH of 5.4 than at 4.4, 6.4, 7.2, or 8.2. This is consistent with the current study, in which the highest leaf tissue Ca concentration occurred when plants were grown in substrates incorporated with 144.2 kg·m⁻³ DL and the average substrate solution pH at

8 WAT was 5.3 ± 0.1 . Leaf tissue Ca concentrations for 'Yellow' and 'Yellow Red Eye' calceolaria cultivars were within the general plant sufficiency range reported by Bryson and Mills (2014).

Magnesium. Leaf tissue Mg concentrations for 'Orange' ($P = 0.0098$), 'Orange Red Eye' ($P = 0.0207$), and 'Yellow' ($P = 0.0069$) calceolaria were significantly influenced by limestone incorporation. For all three cultivars, the highest Mg concentrations were observed in plants grown in substrates amended with 144.2 kg·m⁻³ DL. Leaf tissue Mg concentration of 'Orange', 'Orange Red Eye', and 'Yellow' calceolaria grown in substrates incorporated with 144.2 kg·m⁻³ DL were 0.48, 0.68, and 0.54% Mg, respectively (data not shown). These results were inconsistent with Zheng and Clark (2013), who found Mg concentration of five sedum species to be higher in plants grown in substrates with a pH of 7.2 to 8.2 than at 4.4 to 5.4. They suggested Mg deficiency occurred in plants grown in substrates with a pH of 4.4 to 5.4. However, in the current study, lowest leaf tissue Mg concentrations were observed for 'Orange', 'Orange Red Eye', and 'Yellow' calceolaria grown in substrates incorporated with 48.1 kg·m⁻³ DL and were 0.39%, 0.41%, and 0.44% Mg, respectively (data not shown). Regardless of leaf tissue Mg concentration, no visible Mg deficiency symptoms were observed when 'Orange', 'Orange Red Eye', and 'Yellow' calceolaria were grown in substrates incorporated with 48.1 or 144.2 kg·m⁻³ DL with an average substrate solution pH at 8 WAT of 4.5 to 4.7 and 5.1 to 5.6, respectively. Furthermore, Bryson and Mills (2014) indicated that not only pH but also the use of DL can influence Mg leaf tissue concentration because it is high in Mg content. This supports the current findings in which the highest Mg leaf tissue concentration occurred in plants at the highest DL incorporation concentration. It is

Fig. 4. Depiction of (A) 'Orange', (B) 'Orange Red Eye', and (C) 'Yellow' calceolaria (*Calceolaria × herbeohybrida* Voss.) at 8 weeks after transplant (WAT) into peat-based substrates amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. For each cultivar, substrate solution pH represents the average at 8 WAT.

also speculated that the lowest DL incorporation concentration provided enough Mg to plants to prevent development of deficiency symptomology. Furthermore, irrigation water alkalinity may have provided some supplemental Mg. Overall, for 'Orange', 'Orange Red Eye', and 'Yellow' cultivars, leaf tissue Mg concentration were within the general plant sufficiency range reported by Bryson and Mills (2014).

Copper. Leaf tissue Cu concentrations for all calceolaria cultivars were influenced by limestone incorporation ($P < 0.001$). The lowest leaf tissue Cu concentrations of 5.8, 5.9, 6.3, and 7.0 mg·kg⁻¹ Cu were determined for 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria plants, respectively, grown in substrates incorporated with 48.1 kg·m⁻³ DL (Fig. 5) resulting in a pH 5.6, 5.1, 5.3, and 5.0. In general, as limestone incorporation concentration increased from 48.1 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL, leaf tissue

Fig. 5. Leaf tissue copper (Cu) concentration of calceolaria (*Calceolaria × herbeohybrida* Voss. 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye') at 8 weeks after transplant (WAT) into a peat-based substrates amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Each bar represents a mean of nine samples, and error bars represent ±SE. Means within a cultivar with the same uppercase letter are not different by Tukey's honestly significant difference test at $P \leq 0.05$.

Cu concentration for all calceolaria cultivars increased. For example, leaf tissue Cu concentration of 'Yellow Red Eye' calceolaria increased by 66% (13.3 mg·kg⁻¹ Cu) when plants were grown in substrates incorporated with 48.1 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL resulting in a ΔpH of 2.2 (pH 4.4 to 6.6) pH units. Conversely, Zheng and Clark (2013) reported leaf tissue Cu concentration to decrease from 12.6 to 6.2 mg·kg⁻¹ Cu for 'Immergrunchen' sedum [*S. hybridum* (L.) 't. Hart'] grown in substrates incorporated with HL only. Although in the current study, leaf tissue Cu concentrations were significantly influenced by limestone incorporation, the Cu concentrations were within the general plant sufficiency range reported by Bryson and Mills (2014) with the exception of 'Yellow Red Eye' calceolaria, which was 1.5% (0.3 mg·kg⁻¹) above the general Cu sufficiency range for most plants.

Iron. Leaf tissue Fe concentrations for 'Orange' ($P < 0.01$), 'Orange Red Eye' ($P < 0.0001$), 'Yellow' ($P = 0.0147$), and 'Yellow Red Eye' ($P < 0.01$) calceolaria were influenced by limestone incorporation, though to different magnitudes (Fig. 6). In general, leaf tissue Fe concentrations increased as limestone incorporation increased from 48.1 kg·m⁻³ DL to 144.2 kg·m⁻³ DL. Leaf tissue Fe concentration for 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria increased by 16% (13.8 mg·kg⁻¹ Fe), 40% (49.6 mg·kg⁻¹ Fe), 16% (22.7 mg·kg⁻¹ Fe), and 39% (61.4 mg·kg⁻¹ Fe), respectively, when plants were grown in substrates incorporated with increasing concentrations of DL (48.1 to 144.2 kg·m⁻³ DL), resulting in a ΔpH of 0.9 (pH 4.7 to 5.6), 0.6 (pH 4.5 to 5.1), 0.8 (pH 4.5 to

5.3), 0.6 (pH 4.4 to 5.0) units, respectively. When substrates were incorporated with increasing limestone from 144.2 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL, leaf tissue Fe concentration decreased. For example, leaf tissue Fe concentration for 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye' calceolaria decreased by 146% (50.9 mg·kg⁻¹ Fe), 91% (58.4 mg·kg⁻¹ Fe), 71% (57.4 mg·kg⁻¹ Fe), and 84% (71.9 mg·kg⁻¹ Fe), respectively when plants were grown in substrates incorporated with increasing concentrations 144.2 kg·m⁻³ DL to 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL (Fig. 6), resulting in a ΔpH of 1.3 (pH 5.6 to 6.9), 1.5 (pH 5.1 to 6.7), 1.5 (pH 5.3 to 5.8), 1.6 (pH 5.0 to 6.6) units, respectively. Bryson and Mills (2014) indicated liming materials containing Ca, when incorporated at excessively high concentrations and the accompanying anions, carbonate oxide, or hydroxide, increases pH and reduces the solubility of Fe compounds. In fact, this supports the findings of the current study where substrate solution pH (≥ 6.5) was found to be higher in substrates incorporated with DL+HL, thus inhibiting the availability and uptake of Fe, and resulting in lower leaf tissue Fe concentrations and exhibiting chlorosis and interveinal chlorosis. Furthermore, the substrate solution pH data support the reduced leaf tissue Fe concentration and deficiency symptoms observed.

Conclusion

Results from this study demonstrate the effects of liming material on substrate pH, plant growth and on some macro- and micronutrient concentrations, with the greatest influence on leaf tissue Cu and Fe concentrations of the four

Fig. 6. Leaf tissue iron (Fe) concentration of calceolaria (*Calceolaria × herbeohybrida* Voss. 'Orange', 'Orange Red Eye', 'Yellow', and 'Yellow Red Eye') at 8 weeks after transplant (WAT) into a peat-based substrates amended with (by volume) 20% perlite and incorporated with pulverized dolomitic limestone (DL) or DL+hydrated lime (HL) at 48.1 or 144.2 kg·m⁻³ DL, or 17.6 kg·m⁻³ DL + 5.3 kg·m⁻³ HL, or 17.6 kg·m⁻³ DL + 10.6 kg·m⁻³ HL. Each bar represents a mean of nine samples, and error bars represent ±SE. Means within a cultivar with the same uppercase letter are not different by Tukey's honestly significant difference test at $P \leq 0.05$.

calceolaria cultivars trialed. Overall, as limestone incorporation increased from DL to DL+HL, substrate solution pH and plant growth increased, whereas Fe tissue concentration decreased. When the substrate solution, growth, and leaf tissue concentration data of calceolaria are taken together, it is recommended to incorporate peat-based substrates with 144.2 kg·m⁻³ DL to achieve and maintain a substrate pH ≤ 6.5 , thereby preventing the likelihood of a high substrate pH-induced Fe deficiency, prevent chlorosis, and produce high-quality plants. Indeed, all cultivars exhibited high pH-induced Fe deficiency symptomatology, but 'Orange' calceolaria appeared to be more susceptible. Collectively, these results expand liming requirements, plant growth effects, nutritional monitoring, some leaf tissue nutrients that were not previously

published, and the general understanding of high pH-induced Fe deficiency of calceolaria. This work will further benefit greenhouse growers by providing more precise substrate pH recommendations for calceolaria production.

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