Quantifying the Acidic and Basic Effects of Fifteen Floriculture Species Grown in Peat-based Substrate

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Abstract. Floriculture species differ in their effect on substrate-pH and the resulting substrate micronutrient availability in container production. The objective was to quantify effects of floriculture plant species on substrate-pH. In a growth chamber factorial experiment, 15 floriculture species were grown with 70% peat and 30% perlite substrate and fertilized with nutrient solutions containing 100 mg L⁻¹ N and NH₄⁺-N:NO₃⁻-N nitrogen ratios of 0:100, 20:80, or 40:60. The relationship between substrate-pH and milliequivalents (meq) of acid or base per unit volume of substrate was quantified by titration with hydrated dolomitic lime or HCl. After 33 days, species and solution type effects on substrate-pH and estimated meq of acid or base produced were evaluated. Final substrate-pH ranged from 4.83 for geranium in 40:60 solution to 6.58 for lisianthus in 0:100 solution, compared with an initial substrate-pH of 5.84. This change in substrate-pH corresponded with a net meq of acid or base produced per gram of tissue dry mass gain (NMEQ) ranging across solutions and species from 1.47 of base for coleus in the 40:60 solution to 2.10 of acid for coleus in the 40:60 solution. With the 0:100 solution, geranium produced the greatest NMEQ of acid (0.07), whereas lisianthus in 0:100 solution to 2.10 of acid for coleus in the 40:60 solution. Without the 0:100 solution, geranium produced the greatest NMEQ of acid (0.07), whereas lisianthus produced the greatest NMEQ of base (1.47). Because all N in the 0:100 solution was in the NO₃⁻-anion form, meq of both anions and cations taken up by plant roots could be calculated based on tissue analysis. With the 0:100 solution, species that took up more anions than cations into plant tissue tended to have a more basic effect on substrate-pH, as would be expected to maintain electroneutrality. Data were used to estimate the percent NH₄⁺-N of total N in a nutrient solution that would be neutral (results in no substrate-pH change) for each species. This neutral percent NH₄⁺-N of total N ranged from ~0% (geranium) to 35% (pentas). Species were separated into three clusters using k-means cluster analysis with variables related to NMEQ and anion or cation uptake. Species were clustered into groups that had acidic (geranium and coleus), intermediate (dusty miller, impatiens, marigold, new guinea impatiens, petunia, salvia, snapdragon, and verbena), or basic (lisianthus, pansy, pentas, vinca, and zinnia) effects on substrate-pH. Evaluating the tendency to increase or decrease substrate-pH across a range of floriculture species, and grouping of plants with similar pH effects, could help predict NH₄⁺:NO₃⁻ ratios for a neutral pH effect and assist growers in managing substrate-pH for container production.

Controlling pH in soilless substrate is critical to managing nutrient availability in container production (Peterson, 1981). This can be a challenge considering that several factors interact and affect pH over time, including substrate components, limestone type and rate, applied nutrients and concentration, irrigation water alkalinity, and plant species (Argo and Biernbaum, 1996, 1997; Johnson et al., 2013). Floriculture species also differ in susceptibility to developing iron or manganese toxicity or deficiency symptoms if substrate-pH drifts too low or high during production (Argo and Fisher, 2002). A key grower decision is the selection of a water-soluble fertilizer formulation and concentration that stabilizes pH over time. Fertilizer effects on substrate-pH are dominated by nitrogen form and concentration applied (Argo and Biernbaum, 1996; Barnes et al., 2014; Haynes, 1990; Huang et al., 2001; Johnson et al., 2013; Marschner, 2012). Fertilization with ammonium nitrogen (NH₄⁺-N) produces an acidic reaction that decreases pH as a result of H⁺ efflux from roots during uptake and from nitrification. Nitrification can occur rapidly in container substrate above pH 5.5 and depends on factors that affect microbial activity such as pH, temperature, oxygen, moisture, crop duration, substrate components, nitrogen form, and concentration (Argo and Biernbaum, 1997; Lang and Elliot, 1991). Fertilization with nitrate nitrogen (NO₃⁻-N) usually produces a basic reaction that increases pH, resulting from the efflux of hydroxyl (OH⁻) or bicarbonate (HCO₃⁻) ions from roots (Haynes, 1990; Marschner, 2012). Ammonium typically has a greater impact on substrate-pH compared with NO₃⁻-N when both forms are applied because NH₄⁺-N uptake is energetically favored over NO₃⁻-N uptake (Engels and Marschner, 1995; von Wieren et al., 2001). The effect of urea nitrogen (urea-N) on substrate-pH varies depending on the state of hydrolysis, nitrification, and the subsequent uptake of NH₄⁺-N vs. NO₃⁻-N (Verburg et al., 2003).

Floriculture species differ in their effect on substrate-pH, even when supplied with the same water-soluble fertilizer (Huang et al., 2001; Johnson et al., 2013). Huang et al. (2001) showed that seedlings of pansy (Viola xwittrockiana Gams.), petunia (Petunia xhybrid Vilm.-Andr.), and vinca (Catharanthus roseus G. Don.) increased the pH whereas celosia (Celosia cristata L.) and zinnia (Zinnia elegans Jacq.) decreased the pH in peat: perlite substrate. In both substrate (Johnson et al., 2013) and hydroponic nutrient solution (Dickson et al., 2016), geranium (Pelargonium xhortorum Bailey L.H.) was acidic and decreased the pH compared with petunia which was basic and increased the pH, whereas impatients (Impatiens wallerana Hook. F.) had intermediate effects to geranium and petunia. The major process by which plants affect root zone pH is through imbalanced uptake of cation and anion nutrients (Haynes, 1990; Lea-Cox et al., 1996; Marschner, 2012; Rengel, 2003). Roots maintain charge balance either by equal uptake of cations and anions or by the efflux of ions equal to the net charge taken up by roots. Net cation uptake is balanced by efflux of H⁺ ions whereas net anion uptake is balanced by efflux of OH⁻ or HCO₃⁻ from roots (Kirkby and Knight, 1977; Lea-Cox et al., 1996; Marschner, 2012).

Some agronomic crop species that are labeled “iron-efficient” acidify the root zone as a strategy to improve iron solubility and uptake when grown in calcareous soil (Marschner, 2012). Geranium is a floriculture species that is referred to as “iron-efficient” because of high susceptibility to iron or manganese toxicity at low pH and also has the tendency to decrease substrate-pH over time (Argo and Fisher, 2002; Johnson et al., 2013). Other floriculture species susceptible to iron or manganese toxicity may also

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fertilized with 100% NO$_3$-N that had greater net anion minus cation uptake would result in greater basicity than species that favored cation uptake. We also hypothesized that floriculture species that are reportedly susceptible to iron or manganese toxicity at low pH may tend to decrease substrate-pH, possibly acidifying the root zone as an iron-efficiency mechanism (Marschner, 2012).

**Materials and Methods**

The experiment was a factorial randomized complete block design with 15 species and three fertilizer NH$_4$:$\text{NO}_3$ nitrogen ratios (0:100, 20:40, and 40:60) from a 0.5x Hoagland's solution (Hoagland and Arnon, 1950), supplied at 100 mg·L$^{-1}$ N, and concentrated in six blocks (benches) with 270 total replicate containers. On 5 Feb. 2015, seedling plugs of 15 floriculture species were transplanted from 144-cell trays (supplied by Knox Nursery, Winter Garden, FL) into four-cell plastic bedding plant containers (FG12047, 88 mL per cell or 350 mL per container) at four plants per container. Each four-cell container was considered as one replicate.

Plants were grown for 33 d on benches in a controlled environment growth chamber located at the University of Florida in Gainesville, FL. Growth chamber lighting was supplied by cool white fluorescent bulbs decrease pH, possibly as a means to increase iron uptake.

Johnson et al. (2013) modeled the interaction between three floriculture species (geranium, impatiens, and petunia) fertilized with 18 water-soluble fertilizers differing in applied nitrogen forms (NH$_4$-N, NO$_3$-N, and urea-N) and concentration. The acidity or basicity of a fertilizer could be manipulated by adjusting nitrogen forms and concentrations to balance species root zone effects and stabilize pH. For example, the model predicted that geranium, impatiens, and petunia would require 0%, 10%, and 31% of total N as NH$_4$-N, respectively, with the remainder of N as NO$_3$-N. This ratio would be expected to maintain a stable pH over time when these species were grown with zero alkalinity irrigation water and without residual lime in the substrate.

Commercial bedding plant operations typically grow a wide range of plant species, often in the same greenhouse, that may differ in effects on substrate-pH. Species diversity is therefore an important consideration in pH management. However, it is also not feasible to provide separate fertilizer regimes to hundreds of cultivars grown at a single location, and grouping of plants is necessary. The objective was to quantify the effects of plant species on substrate-pH for floriculture species fertilized with different NH$_4$:NO$_3$ nitrogen ratios. Floriculture species common in container production were selected, where species also differed in reported susceptibility to iron or manganese deficiency or toxicity and optimum pH (Argo and Fisher, 2002; Whipker et al., 2003).

The objective of this study was to quantify effects of floriculture plant species on substrate-pH. In a factorial experiment conducted in a controlled environment growth chamber, 15 floriculture species were grown in peat:perlite substrate and were irrigated with modified 0.5x Hoagland's nutrient solutions with NH$_4$:NO$_3$ nitrogen ratios of 0:100, 20:80, and 40:60. After 33 d, substrate-pH was measured and meq of acid or base produced per L of substrate was estimated using an acid–base substrate titration (Johnson et al., 2010). Nitrogen is taken up in different forms that vary in their charge (+:NO$_3$–), and without isotope labeling (which was not used in this study), total N level in tissue cannot distinguish between the original fertilizer nutrient form. Dry tissue was harvested from plants irrigated with the 0:100 solution, and tissue nutrient data were used to estimate net uptake of anions minus cations, and cation or anion uptake ratio, assuming that all N was taken up in the anionic NO$_3$ form. Species and solution NH$_4$:$\text{NO}_3$ ratio were evaluated for effects on substrate-pH and meq of acid or base produced per L of substrate. Species were separated into clusters that corresponded to species overall acidic, intermediate, and basic effects on substrate-pH, and linear regression was used to predict species-specific NH$_4$:NO$_3$ ratios expected to result in a stable pH. We hypothesized that species
(32 W, 6500 K). Photosynthetically active radiation was measured at the plant canopy level using a quantum sensor (Apogee Instruments, Inc., Logan, UT), where light intensity averaged 175 μmol·m⁻²·s⁻¹ for a daily light integral of 11.3 mol·m⁻²·d⁻¹ across species. Daily air and substrate temperatures measured during the experiment with external temperature sensors connected to data loggers (Onset Computer Corporation, Bourne, MA) were 22.5 ± 1.5 °C and 23.1 ± 1.6 °C (mean ± SD), respectively.

Floriculture species consisted of geranium (Pelargonium x hortorum Bailey, L.H.) ‘Ringo 2000 Deep Red’, impatiens (Impatiens wallerana Hook. F.) ‘Super Elfin Orange Bright’, petunia (Petunia × hybrid Vilim.-Andr.) ‘Ultra Red’, coleus (Solenostemon scutellarioides L.) ‘Premium Sum Choc olate Covered Cherry’, pentas (Pentas lanceolata Forssk.) ‘Butterfly Red’, snapdragon (Antirrhinum majus L.) ‘Snapshot Yellow’, verbena (Verbena × hybridra L.) ‘Quartz White XP’, vinca (Catharanthus roseus L.) ‘Titan Dark Red’, lisanthus (Eustoma grandiflora Salisb.) Florida Sky Blue, African marigold (Tagetes erecta L.) ‘Taishan Orange’, dusty miller (Senecio cineraria L.) ‘Maritima Silverdust’, pansy (Viola tricolor D.C.) ‘Matrix Clear Yellow’, salvia (Salvia splendens Sellow ex Roem. & Schult.) ‘Vista Red’, New Guinea impatiens (Impatiens hawkeri L.) ‘Divine White Blush’, and zinnia (Zinnia elegans L.) ‘Zahara Double Fire’. Most species had open flowers at the end of the experiment, except for coleus, dusty miller, and lisanthus, which appeared vegetative with no visible flower buds.

The substrate was 70%:30% (v:v) peat:perlite mixed at the University of Florida using Canadian Sphagnum peat (Sun Gro Horticulture, Bellevue, WA) with long fibers and little dust (von Post scale 1–2; Puujustarvi and Robertson, 1975) and wetting agent (0.15 mL·L⁻¹ of substrate; Aquatrols, Paulsboro, NJ). Hydrated dolomitic limestone with 139% neutralizing value (Graymont Western Lime, Inc., Eden, WI) and change in substrate-pH (ΔpH) was measured after the first irrigation at transplant and averaged 5.84 ± 0.04 (mean ± sd) across all NH₄NO₃ treatments. Initial substrate-pH was measured after the first irrigation at transplant and averaged 8.54 ± 0.04 (mean ± sd) across all NH₄NO₃ treatments. Initial substrate EC was 1.98 ± 0.02, 2.14 ± 0.03, and 2.27 ± 0.02 mS·cm⁻¹ for 0:100, 20:80, and 40:60 NH₄NO₃ solutions, respectively. Containers used for initial measurements had the same substrate-EC and substrate-EC was relatively stable in the absence of plants. Final substrate-pH and EC were measured at the end of the experiment for each treatment replicate.

Species effects on substrate-pH over time were related to meq of acid or base produced per L of substrate using the acid–base titration approach of Johnson et al. (2010). In a laboratory procedure, samples of the same substrate used for the experiment, but not for growing plants, were placed into plastic zip-lock bags at 250 mL of substrate per bag. The substrate in each bag was moistened with 150 mL of 20:80 nutrient solution at 200 mg·L⁻¹. Hydrochloric acid (0.5060 N) was added at 0, 2.5, 5.0, 7.5, 10.0, or 12.5 mL per sample for an equivalent of 0, 10, 20, 30, 40, and 50 meq of acid added per L of substrate. Hydro-\textsuperscript{19l}alized dolomitic lime (139% CCE) was added at 0, 0.18, 0.36, 0.54, 0.72, and 0.90 g per sample for an equivalent of 0, 10, 20, 30, 40, and 50 meq of base added per liter of substrate. There were four replicates for each acid and base titration level, and replicates were allowed to equilibrate for 7 days before measuring substrate-pH. A polynomial curve was fit relating the change in pH units (from initial pH 5.84) to the meq of acid or base added per L of substrate, which was used to estimate the meq of acid or base produced by each species when supplied with each of the three solution types.

The uptake of individual nutrients was measured for plants fertilized with the 0:100 solution. Root and shoot tissue was harvested from seedlings at the beginning of the experiment and from each final replicate that received the 0:100 solution. Tissue was rinsed with HCl (0.1 N) followed by deionized water and was oven-dried for 48 h at 70 °C. Roots were washed with phosphate-free soap before the HCl rinse to remove substrate particles from root surfaces. Plant growth was measured as total dry mass gain (roots and shoots), which was calculated by subtracting the average dry mass for four seedlings from the final dry mass per replicate for each species (four plants per replicate).

Dry root and shoot tissue from plants supplied with 0:100 solution was analyzed for concentration of macronutrient and micronutrient elements by inductively coupled plasma atomic emission spectroscopy (Quality Analytical Laboratories, Panama City, FL). Tissue concentrations of macronutrient and micronutrient elements were within the general sufficiency ranges proposed by Vetalovetz (1996) for bedding plants (data not shown). The concentration of each element in the tissue was multiplied by the dry mass to determine the total nutrient.

**Fig. 2.** Relationship between mineral acid (0.5060 N hydrochloric acid) or base (hydrated dolomitic limestone with 139% neutralizing value) and change in substrate-pH (ΔpH), quantified using the acid-base titration. The polynomial equation milliequivalents (meq) of acid (− value) or base (+ value) per L of substrate = 0.8780 × ΔpH² + 1.3369 × ΔpH + 9.9856 × ΔpH − 0.2569, with P < 0.0001.
Results and Discussion

Final substrate-pH was affected by plant species \((P < 0.0001)\), solution NH\(_4\)^+ : NO\(_3\)^− ratio \((P < 0.0001)\), and their interaction \((P < 0.0001)\), and ranged from 4.83 to 6.58 between species and solution types (Fig. 1). When fertilized with the 0:100 NH\(_4\)^+ : NO\(_3\)^− solution, 13 species resulted in final substrate-pH greater than the initial pH of 5.84. When fertilized with the 40:60 NH\(_4\)^+ : NO\(_3\)^− solution, all 15 species resulted in final substrate-pH lower than the initial pH. Substrate-pH least-square means were 6.24, 5.75, and 5.25 for the 0:100, 20:80, and 40:60 NH\(_4\)^+ : NO\(_3\)^− solutions, respectively.

Species effects on substrate-pH can be compared on the basis of the meq of acidity or basicity produced per gram of plant growth (NMEQ). For example, Rengel (2003) showed that 37 agronomic species differed in NMEQ, ranging from 0.3 to 2.0 meq of acidity produced per gram of shoot dry mass (original reported units were centimoles of acid per kilogram of shoot dry mass). To calculate NMEQ, plant growth was measured as the change in total dry mass over 33 d, which differed by species \((P < 0.0001)\) but not by NH\(_4\)^+ : NO\(_3\)^− ratio \((P = 0.8018)\) (data not shown). In addition, change in substrate-pH over time was converted to units of meq of acid and base produced in the substrate.
Table 1. Species main effects on milliequivalents (meq) net anions minus cations taken up, meq net anions minus cations taken up per gram of dry mass gain, and meq of acid or base produced per gram of dry mass gain when supplied 0:100 solution. Data are species least-square means of six replicates. Mean separation used Tukey’s honestly significant difference at α = 0.05. Percent NH₄⁺-N of total N expected for a stable pH was calculated for each species using linear regression based on species meq of acid or base produced per container volume of substrate (dependent variable) and percent NH₄⁺-N of total N supplied for each of the three nutrient solution (independent variable).

| Plant species         | meq anions minus cations taken up | Cation or anion uptake ratio | meq anions minus cations taken up per gram dry mass gain | meq acid (-) or base (+) per gram dry mass gain | Neutral (%) NH₄⁺-N |
|-----------------------|----------------------------------|-----------------------------|--------------------------------------------------------|-------------------------------------------------|-------------------|
| Coleus                | 0.87 ± 0.04 f                    | 0.75 ab                      | 0.90 abc                                               | 0.47 cde                                         | 4.4               |
| Dusty Miller          | 1.85 ab                          | 0.78 ab                      | 0.59 abc                                               | 0.39 de                                          | 14.0              |
| Geranium              | 0.88 b                           | 0.85 a                       | 0.42 c                                                 | -0.07 f                                          | 0.0'              |
| Impatiens             | 2.20 ab                          | 0.73 ab                      | 0.91 abc                                               | 0.43 de                                          | 9.3               |
| Lisianthus            | 3.47 ab                          | 0.48 c                       | 1.69 a                                                 | 1.47 a                                           | 25.4              |
| Marigold              | 2.71 ab                          | 0.82 ab                      | 0.38 bc                                                | 0.15 ef                                          | 11.2              |
| New Guinea Impatiens  | 2.28 ab                          | 0.76 ab                      | 0.63 ab                                                | 0.30 def                                         | 11.5              |
| Panay                 | 3.44 ab                          | 0.65 abc                     | 1.03 abc                                               | 0.61 cde                                         | 22.9              |
| Pentas                | 1.90 ab                          | 0.67 abc                     | 0.70 abc                                               | 1.16 ab                                          | 35.1              |
| Petunia               | 2.55 ab                          | 0.75 ab                      | 0.90 abc                                               | 0.55 cde                                         | 16.4              |
| Salvia                | 2.50 ab                          | 0.78 ab                      | 0.61 abc                                               | 0.31 def                                         | 15.1              |
| Snapdragon            | 2.44 ab                          | 0.69 abc                     | 0.84 abc                                               | 0.34 de                                          | 13.6              |
| Verbena               | 2.64 ab                          | 0.66 abc                     | 1.51 a                                                 | 0.70 bc                                          | 13.4              |
| Vinca                 | 3.73 a                           | 0.58 bc                      | 1.22 abc                                               | 0.92 bc                                          | 25.9              |
| Zinnia                | 1.51 ab                          | 0.84 a                       | 0.34 bc                                                | 0.46 cde                                         | 24.5              |

Significance level: *** Indicates a significance level of P < 0.0001.
*A negative percent NH₄⁺-N of total N value was predicted for geranium for a neutral pH. Therefore, 0% NH₄⁺-N would be required to minimize acidity.

**Fig. 4. Relationship between milliequivalents (meq) net cation or anion uptake (shown as meq anions minus cations taken up) and meq acid or base produced per gram of dry mass increase for species fertilized with the 0:100 solution. Data represent least-square means of six replicates. The regression equation is meq acid or base produced per gram dry mass increase = 0.725 ± 0.408 * (meq anions minus cations uptake per gram dry mass increase) – 0.066 ± 0.377 with R² = 0.531.**

using an acid-base titration (Johnson et al., 2010). In a laboratory procedure, meq of acid (HCl) and base (hydrated dolomitic limestone) applied to samples of substrate was correlated with change in pH units (from initial pH 5.84). Substrate-pH decreased as much as 3.34 units when titrated with acid and increased as much as 2.58 units when titrated with base (Fig. 2). A polynomial response curve was generated and used to estimate meq of acid (negative values) and base (positive values) produced per L of substrate corresponding to a specific change in pH units. For example, a substrate-pH change of -1 pH units would equal -9.72 meq (acid) using the equation in Fig. 2. Initial substrate-pH (5.84) was subtracted from final values in Fig. 1 to determine change in pH units, which were converted to meq of acid or base produced per L of substrate. Acid or base (meq) per L of substrate was scaled to the volume of substrate per replicate (350 mL) for meq of acid or base produced per container and divided by total dry mass gain for meq of acid or base produced per unit of growth.

There was an interaction between species and pH on NMEQ (P < 0.0001) for all macronutrients, sodium, and chloride (data not shown). Uptake of cations and anions was dominated by NH₄⁺, which contributed between 38.0% and 43.8% of the total (meq) combined cations and anions. As a contribution of total meq of anion uptake, NO₃⁻ (75.9% to 87.3%) was followed by H₂PO₄⁻ (4.4% to 9.2% depending on species), SO₄²⁻ (3.3% to 14.2%), and Cl⁻ (1.5% to 6.7%). Cations taken up were predominantly K⁺ (28.0% to 59.2% of total cations), Ca²⁺ (10.8% to 40.6%), Mg²⁺ (19.6% to 36.6%), and Na⁺ (0.7% to 6.7%). Micronutrients represented <1% of total uptake. When the meq of all cations was subtracted from meq of all anions, the net anion uptake was lowest for zinnia (0.34 meq g⁻¹) and greatest for lisanthus (1.69 meq g⁻¹), and the ratio of cations and anions taken up into plant tissue ranged from 0.48 (lisanthus) to 0.85 (geranium) (Table 1). The observed trend, whereby all plants took up more anions than cations in the 0:100 solution, was consistent with published studies by Kirkby and Knight.
Table 2. Species listed by cluster (numbers one through three) from k-means cluster analysis with cluster main effects on percent NH₄⁺-N of total N for a neutral pH effect, milliequivalents (meq) of acid and base per gram of dry mass gain for each of the three solution types (0:100, 20:80, 40:60), meq anions minus cations taken up per gram of dry mass gain with species supplied 0:100 solution, and cation or anion uptake ratio with species supplied 0:100 solution. Species were clustered based on each species percent estimated NH₄⁺-N of total N for stable pH and mean values ($\alpha = 6$) for each of the remaining variables. Data represent cluster least-square means of two (cluster 1), eight (cluster 2), and five (cluster 3) species. Mean separation used Tukey's honestly significant difference at $\alpha = 0.05$.

| Plant species | Cluster number | Cluster neutral % NH₄⁺-N | meq of acid (–) or base (+) per gram of dry mass gain | meq anions minus cations taken up per gram dry mass gain | Cation/anion uptake ratio |
|---------------|----------------|--------------------------|-------------------------------------------------------|--------------------------------------------------------|--------------------------|
|               |                |                          | 0:100 | 20:80 | 40:60 | 0:100 | 20:80 | 40:60 |                                          |
| Geranium      | 1              | 0% c                     | 0.16 b | −1.10 c | −1.73 b | 0.40 a | 0.80 a | 0.75 a |                                          |
| Coleus        |                | 144.4 c                  | 99.3 bcd |                                          |                                                       |                          |
| Dusty Miller  | 2              | 13.1% b                  | 0.40 b | −0.24 b | −0.80 a | 0.80 a | 0.75 a | 0.64 a |                                          |
| Impatiens     |                | 104.8 c                  | 82.2 cdef |                                          |                                                       |                          |
| Marigold      |                | 476.8 a                  | 67.2 fg |                                          |                                                       |                          |
| New Guinea Impatiens | 117.1 c | 61.1 fg |                     |                                                       |                          |
| Petunia       |                | 88.3 c                   | 80.1 cdef |                                          |                                                       |                          |
| Salvia        |                | 150.1 c                  | 104.7 abc |                                          |                                                       |                          |
| Snapdragon    |                | 89.5 c                   | 68.7 fg |                                          |                                                       |                          |
| Verbena       |                | 156.3 c                  | 76.4 defg |                                          |                                                       |                          |
| Lisianthus    | 3              | 26.8% a                  | 0.92 a | 0.26 a | −0.49 a | 0.99 a | 0.64 a | 0.52 a |                                          |
| Pansy         |                | 147.7 c                  | 52.0 g |                                          |                                                       |                          |
| Pentas        | 87.8 c         | 112.2 ab                 | 73.1 efg |                                          |                                                       |                          |
| Vinca         | 106.3 c        | 75.4 defg                |                     |                                                       |                          |
| Zinnia        |                | 300.0 b                  | 128.8 a |                                          |                                                       |                          |
| Significance level |           | ***                      | *     | ***   | **    | NS    | NS    |                   |

NS, *, **, *** Indicate a significance level of not significant, $P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively.

An average negative percent NH₄⁺-N of total N value ($\geq 1$) was predicted for geranium and coleus (cluster 1). Therefore, 0% NH₄⁺-N would be needed to minimize acidity and provide a near stable pH.

Table 3. Species listed by cluster (numbers one through three) from k-means cluster analysis with species main effects on tissue concentrations of Fe⁺⁺ and Mn⁺⁺ when supplied with 0:100 solution. Data are least-square means of six replicates. Mean separation used Tukey’s honestly significant difference at $\alpha = 0.05$.

| Plant species | Cluster | Tissue Fe (μg·g⁻¹ dry tissue) | Tissue Mn (μg·g⁻¹ dry tissue) |
|---------------|---------|-------------------------------|-------------------------------|
| Geranium      | 1       | 83.2 c                        | 97.0 bcd                      |
| Coleus        |         | 144.4 c                       | 99.3 bcd                      |
| Dusty Miller  | 2       | 172.1 c                       | 83.6 cdef                     |
| Impatiens     |         | 104.8 c                       | 82.2 cdef                     |
| Marigold      |         | 476.8 a                       | 67.2 fg                       |
| New Guinea Impatiens | 117.1 c | 61.1 fg                       |                               |
| Petunia       |         | 88.3 c                        | 80.1 cdef                     |
| Salvia        |         | 150.1 c                       | 104.7 abc                     |
| Snapdragon    |         | 89.5 c                        | 68.7 fg                       |
| Verbena       |         | 156.3 c                       | 76.4 defg                     |
| Lisianthus    | 3       | 147.7 c                       | 52.0 g                        |
| Pansy         |         | 87.8 c                        | 112.2 ab                      |
| Pentas        |         | 127.4 c                       | 73.1 efg                      |
| Vinca         |         | 106.3 c                       | 75.4 defg                     |
| Zinnia        |         | 300.0 b                       | 128.8 a                       |
| Significance level |       | ***                           | ***                           |

**Indicates a significance level of $P < 0.0001$. 

(1977) with hydroponically grown tomatoes in a nutrient solution with 100% NO₃⁻-N. A positive correlation was found between net anion uptake (based on tissue analysis) and NMEQ (based on substrate-pH change) when fertilized with 100% NO₃⁻-N (Fig. 4). Similarly, Rengel (2003) found that meq of acid produced by roots (NMEQ) was correlated with meq of excess cations taken up into plant tissue for 37 agronomic crop species grown in field soil.

The NH₄⁺:NO₃⁻ ratios estimated to result in a neutral pH effect were calculated for each species using linear regression (Table 1). The percent NH₄⁺-N of total N applied in the three nutrient solutions and meq of acid or base per L of substrate over 33 d were independent and dependent variables, respectively. $R^2$ values were above 0.730 and averaged 0.856 for all species (data not shown). With the exception of geranium, species were estimated to require between 4.4% (coleus) and 35.1% (pentas) of total N as NH₄⁺-N with the remainder of N as NO₃⁻-N to balance species and nitrogen effects and result in zero pH change over time (Table 1). Geranium produced meq of acid not different from zero when fertilized with 0:100 solution, resulting in a negative predicted NH₄⁺:NO₃⁻ ratio, and therefore would require fertilization with 100% NO₃⁻-N to minimize acidity. On average, species required greater NH₄⁺:NO₃⁻ ratios for a neutral effect compared with the observations made by Sonneveld and Voogt (2009), who reported that most greenhouse crop species require NH₄⁺-N between 5% and 10% of total N for a stable pH in soilless substrates.

In commercial horticulture production of diverse crops, it is not practical to have a specific fertilizer formulation to stabilize pH for each species. One possibility is to separate species into groups and manage pH for each group separately. Cluster analysis in combination with ANOVA was therefore used to determine grouping of species with similar effects on pH.

Species were separated into three clusters using k-means cluster analysis, using the neutral percent NH₄⁺-N of total N, NMEQ for each of the three solutions, net anion uptake in the 0:100 solution, and cation or anion uptake ratio in the 0:100 solution as dependent variables. The contribution of each variable in terms of separating species into clusters was shown by the cluster analysis $R^2$ values. The $R^2$ equaled 0.871 for the neutral percent NH₄⁺-N of total N, $R^2 = 0.530$ for NMEQ in 0:100, $R^2 = 0.816$ for NMEQ in 20:80, $R^2 = 0.580$ for NMEQ in 40:60, $R^2 = 0.179$ for net anion uptake, and $R^2 = 0.334$ for cation or anion uptake ratio. Therefore, clusters differed more consistently in the neutral percent NH₄⁺-N of total N than in other variables. Clusters were compared by each variable using ANOVA, where clusters differed in percent NH₄⁺-N of total N ($P < 0.0001$) and meq of acid or base per gram of dry mass gain in 0:100 ($P = 0.0108$), 20:80 ($P < 0.0001$), and 40:60 ($P = 0.0055$) solution. There were no statistical differences for meq of anions minus cations taken up ($P = 0.3054$) or cation or anion balance ($P = 0.0869$) in 0:100 solution (Table 2).

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Species are summarized by cluster in Table 2. On average, species in cluster 1 had the greatest mean meq acid per gram of dry mass gain whereas species in cluster 3 had the greatest mean meq of base per gram of dry mass gain for each of the three solution types (Table 2), and overall were acidic and basic in effects on pH. For meq of acid or base (per gram of dry mass gain), species in cluster 2 were not different from cluster 1 in 0:100 solution or cluster 3 in 40:60 solution but differed from both clusters with 20:80 solution and were considered as intermediate in effects on pH. Each cluster differed in species mean percent NH$_4^+$-N expected to stabilize pH, averaging 0%, 13.1%, and 26.8% NH$_4^+$-N of total N for clusters 1, 2, and 3, respectively (Table 2). Supplying species clustered as acidic, intermediate, and basic with ≈0%, 13%, and 27% of total N as NH$_4^+$-N, respectively, would be expected to result in about stable substrate-pH across species.

Tendency to decrease root zone pH is a potential strategy used by some iron-efficient species to increase iron solubility and uptake (Marschner, 2012). Iron solubility is reduced as substrate-pH increases (Lindsay, 1979). Crop species are labeled as iron-efficient if they exhibit root strategies that specifically increase iron uptake, such as acidifying the rhizosphere, efflux of organic acids and chelating molecules, and greater ability to reduce iron at root surfaces (Bienfait, 1988; Marschner, 2012). Albano and Miller (1996) found that marigold responded to low substrate-iron concentration by acidifying the root zone and increasing iron reductase activity, which are iron-efficiency strategies. Albano and Miller (1996) also found that under iron-sufficient and excess conditions, root zone acidification and increased iron reductase activity was not expressed. Floriculture species that have been noted to decrease pH include geranium and marigold (Gibson et al., 2007; Johnson et al., 2013). Floriculture species that are susceptible to iron or manganese toxicity symptoms at low substrate-pH include geranium, marigold, lisanthus, pansy, and New Guinea impatiens (Argo and Fisher, 2002; Harbaugh, 1995; Whipker et al., 2003). However, this group of species sensitive to low substrate-pH differed considerably in this study in their effects on pH (Figs. 1 and 3). For example, lisanthus and pansy had among the most basic pH effects in this study (Fig. 3). Floriculture species sensitive to high pH and iron or manganese deficiency include pansy, petunia, salvia, snapdragon, vinca, and zinnia (Cavins et al., 2000; Gibson et al., 2007; Johnson et al., 2013). Vinca, pansy, and zinnia in this experiment were considerably more basic compared with petunia, salvia, and snapdragon (Fig. 3). Categorizing species response in terms of susceptibility to micronutrient toxicity or deficiency at low or high pH therefore differs from the effect of species on substrate-pH, which was quantified in this study.

Accumulation of Fe$^{2+}$ and Mn$^{2+}$ in tissue was not related to species acid–base cluster (Table 3). As previously mentioned, marigold and geranium are susceptible to iron or manganese toxicity whereas zinnia and pansy are susceptible to iron or manganese deficiency (Albano et al., 1996; Argo and Fisher, 2002; Gibson et al., 2007). However, marigold and zinnia had the greatest tissue concentration of Fe$^{2+}$ whereas geranium and pansy had the lowest concentration. Tissue concentration of Mn$^{2+}$ was also greatest in zinnia and lowest in lisanthus (susceptible to iron or manganese toxicity). Differential uptake of individual macronutrients affects species cation or anion uptake balance and consequently the effect on root zone pH (Bekele et al., 1983; Haynes, 1990; Marschner, 2012). In addition, total tissue iron analysis does not differentiate between biologically active and inactive forms within plant cells, where high proportions of inactive iron have been linked with symptoms of iron deficiency in leaves (Marschner, 2012).

**Conclusions**

The acidic and basic effects of plants on substrate-pH were quantified over a wide range of floriculture container crop species. When supplied with 100% NO$_3^-$-N, species with greater net anion uptake produced greater basicity in the substrate compared with species with greater cation uptake. Commercial growers can adjust the ratio of NH$_4^+$-N and NO$_3^-$-N in the applied water-soluble fertilizer to balance species acidic or basic effects and stabilize pH over time. Percent NH$_4^+$-N of total N supplied estimated to result in a stable pH ranged from 0% (geranium) to 35% (pansy) across species. However, it is not practical to supply a specific fertilizer formulation to stabilize pH for each species in commercial production. Using a k-means clustering and statistical approach, species in this study were separated into groups that corresponded to species about acidic, intermediate, and basic effects on substrate-pH. Supplying 0%, 13%, and 27% of total N as NH$_4^+$-N (remainder as NO$_3^-$-N) would result in about stable pH for acidic, intermediate, and basic species. Growers can group floriculture species by their tendency to increase or decrease substrate-pH and adjust the fertilizer NH$_4^+$:NO$_3^-$ for each group as a strategy to manage pH and reduce the risk of micronutrient disorders.

Floriculture species that are reportedly susceptible to micronutrient toxicity at low pH did not all exhibit the iron-efficiency strategy of acidifying the rhizosphere pH. For example, geranium was acidic and tended to decrease pH, whereas lisanthus and pansy had among the most basic effects on substrate-pH. In addition, accumulation of Fe$^{2+}$ and Mn$^{2+}$ in plant tissue was not related to the measured species effects on substrate-pH or reported susceptibility to micronutrient toxicity or deficiency. For example, marigold (susceptible to iron or manganese toxicity) and zinnia (susceptible to iron or manganese deficiency) had the greatest tissue concentration of Fe$^{2+}$ whereas geranium (susceptible to iron or manganese toxicity) and pansy (susceptible to iron or manganese deficiency) had the lowest concentration.

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