**Solution ammonium:nitrate ratio and cation/anion uptake affect acidity or basicity with
floriculture species in hydroponics**

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21 Abstract

22 Floriculture crops affect substrate-pH and resulting solubility of micronutrients in the root zone
 23 during commercial production. Two hydroponic experiments and tissue data from a container
 24 study were analyzed to determine whether (1) cation or anion uptake was related to solution
 25 acidity and basicity in the root zone for three floriculture crops [geranium (*Pelargonium x*
 26 *hortorum*, Bailey. L.H.), petunia (*Petunia x hybrid*, Vilm.-Andr.), and impatiens (*Impatiens*
 27 *wallerana*, Hook. F.)] and whether (2) the proportion of NH_4^+ -N versus NO_3^- -N uptake differed
 28 between these three floriculture species. In the container study, where >96% of nitrogen (N) was
 29 provided primarily as NO_3^- -N, substrate-pH did not change over time when geranium or
 30 impatiens were grown, whereas substrate-pH increased with petunia. Geranium had a higher
 31 cation/anion uptake ratio than petunia based on analysis of tissue nutrient levels, and impatiens
 32 was intermediate. In the hydroponic experiments, the three species were grown in nutrient
 33 solutions that varied in ammonium:nitrate ($\text{NH}_4^+:\text{NO}_3^-$) ratio from 0:100 to 50:50. In the first
 34 hydroponic experiment, there was an approximately 1:1 relationship between net anion minus
 35 cation uptake and net solution acidity or basicity, (milliequivalent (mEq) solution acidity or
 36 basicity = $0.972 \pm 0.195 * (\text{net mEq of anions} - \text{cations taken up}) + 0.140 \pm 0.478$; adjusted- $R^2 =$
 37 0.739) over all species, which indicated a strong correlation between solution-pH change and net
 38 cation or anion uptake. Geranium had the highest cation/anion uptake ratio (1.06) and produced
 39 greater acidity than petunia, which had the lowest cation/anion uptake ratio (0.94). Cation/anion
 40 uptake ratio increased as applied $\text{NH}_4^+:\text{NO}_3^-$ ratio increased. In the second hydroponic
 41 experiment, uptake of NH_4^+ -N versus NO_3^- -N exceeded the ratio that was supplied in the 10:90
 42 and 20:80 $\text{NH}_4^+:\text{NO}_3^-$ solutions for all species, but not in the 50:50 solution. Although some
 43 results were inconsistent between experimental runs, petunia overall had a lower $\text{NH}_4^+:\text{NO}_3^-$
 44 uptake ratio than the other two species. A $\text{NH}_4^+:\text{NO}_3^-$ ratio of 10% NH_4^+ -N for geranium and

45 impatiens and 20% NH_4^+ -N for petunia would be expected to result in a neutral pH response in a
46 hydroponic solution with zero alkalinity. Evaluating cation/anion uptake ratio across a range of
47 $\text{NH}_4^+:\text{NO}_3^-$ ratios could be used to predict the relative acidity or basicity of other plant species to
48 assist in pH management.

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50 **Keywords:** Geranium; Pelargonium; Impatiens; Petunia; pH management

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1.0 Introduction

The solubility of nutrients in a hydroponic solution or soilless growing substrate is affected by pH, particularly for metal micronutrients which become less available for root uptake as pH increases (Lindsay, 1979; Peterson, 1981). Many factors influence pH change over time during soilless plant production, including water alkalinity, addition of mineral or organic acid or base, plant species, nitrogen form, nutrient concentration, acidity and cation exchange capacity of substrate components, and lime source and concentration (Argo and Fisher, 2002; Sonneveld and Voogt, 2009, Ch 13). Both nutrient solutions and soilless substrates have low buffering capacity. Drift in pH is therefore a common occurrence that leads to production losses resulting from nutrient deficiencies or toxicities. A major process by which plants change rhizosphere-pH is an imbalance in root uptake of either cations or anions (Haynes, 1990; Lea-Cox et al., 1996; Marschner, 2012, Ch 14.4; Rengel, 2003). Charge balance is accomplished by a plant taking up equal but oppositely-charged nutrient ions, or by effluxing ions with a charge equal to the net charge taken up by roots (Lea-Cox, 1996; Marschner, 2012; Ch 2). Net cation uptake is compensated for by H^+ efflux from roots, whereas net anion uptake is balanced by OH^-/HCO_3^- efflux (Kirkby and Knight, 1977; Lea-Cox et al., 1996; Marschner, 2012, Ch 2).

Nitrogen uptake plays a pivotal role in the cation/anion balance of the plant. Approximately 70% to 80% of the total cation and anion uptake by plants is estimated to result from the uptake of NH_4^+-N or NO_3^--N (Lea-Cox et al., 1996; Marschner, 2012, Ch 2). Plants have a cation/anion ratio >1 (net cation uptake) when given NH_4^+-N , resulting in an efflux of acids from roots, which lowers substrate-pH. Plants often have a cation/anion ratio <1 (net anion uptake) when given NO_3^--N , resulting efflux of bases, which raises substrate-pH (Marschner, 2012, Ch 2). Fertilizer

effects on substrate-pH with geranium, petunia, and impatiens [*Impatiens wallerana* (Hook. F.)] grown in peat/perlite substrate were closely related to the concentration of nitrate, ammonium, or urea-N (Fisher et al., 2014b; Johnson et al., 2013). In hydroponics, adjustment of the $\text{NH}_4^+ : \text{NO}_3^-$ ratio in the nutrient solution is used as a pH management tool, whereby increasing the applied NH_4^+ -N concentration results in greater cation/anion uptake and more acidic pH response (Conesa et al., 2009; Lea-Cox, 1999; Savvas et al., 2003 and 2006). When NH_4^+ -N is depleted in hydroponic solutions, subsequent NO_3^- -N uptake tends to raise solution-pH (Lea-Cox et al., 1999). In crop culture in substrate, the rapid conversion of NH_4^+ -N to NO_3^- -N through nitrification, and subsequent release of protons into solution, must also be considered when evaluating the influence on $\text{NH}_4^+ : \text{NO}_3^-$ ratio on root zone pH (Argo and Biernbaum, 1997; Lang and Elliot, 1990).

Measurement of cation/anion uptake has been based on depletion of ions from a nutrient solution, analysis of cation and anion levels in plant tissue, or ash alkalinity of plant tissues (Rengel, 2003). When measuring cation/anion uptake, nitrogen is the most complex nutrient given that it is taken up in both cationic and anionic forms, and also because microbial processes including nitrification affect substrate-pH. The complexity of N uptake can be accounted for in hydroponic solutions that have minimal microbial activity (Padgett and Leonard, 1993), or by using labeled N (Taylor et al., 2010).

Species differ in their cation/anion uptake ratio, and subsequent effect on substrate-pH. Rengel (2003) characterized the cation/anion uptake ratio of legumes and cereal crops, and the acid production per kg of dry mass gain. The effect of ammonium (NH_4^+) and nitrate (NO_3^-) on

cation/anion uptake in hydroponic solutions has been studied on lettuce, spinach, tomato, and other crop species (Conesa et al., 2009; Haynes, 1990; Kirkby and Mengel, 1966; Kirkby and Knight, 1977; Lea-Cox et al., 1999; Savvas et al., 2003 and 2006; van Beusichem, 1988;). However, cation/anion uptake ratio has not been characterized across a range of floriculture crops.

Floriculture species differ in tendency to interact with the substrate and raise or lower substrate-pH. In a study with seedlings grown in a peat:perlite substrate, pansy (*Viola x wittrockiana* Gams.), petunia, and vinca (*Catharanthus roseus* G. Don) resulted in raised substrate-pH, whereas celosia (*Celosia cristata* L.), tomato (*Lycopersicon esculentum* Mill.), and zinnia (*Zinnia elegans* Jacq.) had lowered substrate-pH (Huang et al., 2001). Johnson et al. (2013) found that geranium was more acidic and lowered substrate-pH compared to petunia which was more basic and resulted in higher pH, with impatiens being intermediate to geranium and petunia. “Sudden pH drop syndrome” in geranium was described by Taylor et al. (2010) as a rapid decrease in substrate-pH triggered by phosphorus (P) deficiency. Reduced P in soilless container substrate and a lack of P in hydroponic solution were correlated with rapid decreases in root zone pH, the suppression of NO_3^- -N uptake, and a shift towards greater cation/anion uptake compared to plants grown with adequate P (Taylor et al., 2008a, 2008b, 2010). Intense efflux of H^+ from roots can be an adaptive response to solubilize P in mineral soils when tissue levels become deficient (Marschner, 2012, Ch 14.4). However, it remains unclear whether the pH drop observed by Taylor et al. (2010) in geranium under low P conditions was an adaptive response resulting from P deficiency, or was a consequence of greater cation/anion uptake after the supply of a macronutrient anion was limited.

The first objective of this study was to determine if net cation or anion uptake was related to solution acidity and basicity in the root zone for three floriculture crops (geranium, impatiens, and petunia). To address this objective, tissue nutrient and substrate-pH data were analyzed from a container study by Johnson et al. (2013) for three basic fertilizers (low NH_4^+ -N, high NO_3^- -N) applied to geranium, impatiens, and petunia. An experiment was also conducted using hydroponic culture as a model system so that cation/anion uptake over a seven day period could be easily measured by depletion from the nutrient solution. Hoagland's nutrient solutions were modified so that $\text{NH}_4^+:\text{NO}_3^-$ ratios of 0:100, 10:90, and 20:80 could be adjusted with little effect on remaining nutrient concentrations. Acidity and basicity produced in milliequivalents (mEq) was calculated by titrating solutions with strong mineral acid or base and was compared to mEq of nutrient ion uptake.

The second objective was to determine whether the proportion of NH_4^+ versus NO_3^- uptake differed between species. Previous research has found that plant species differ in their "preference" for each N form. For example, species that are adapted to low pH soils have greater NH_4^+ -N uptake whereas species adapted to high pH calcareous soils have greater utilization of NO_3^- -N (Marschner, 2012, Ch 6.1). To measure depletion of NH_4^+ -N versus NO_3^- -N from nutrient solutions, geranium, impatiens, and petunia were grown over a broader range of $\text{NH}_4^+:\text{NO}_3^-$ ratios (0:100, 10:90, 20:80, and 50:50). The experiment was run for two days to ensure that NH_4^+ -N was not completely depleted from the 10 to 50% NH_4^+ -N solutions.

2.0 Materials and Methods

2.1 Johnson et al. (2013) Study (Cation/Anion Balance)

Experimental conditions were described in detail by Johnson et al. (2013). In Spring 2010 at the University of Florida, Gainesville, FL (Latitude: 29.65°N), seedling plugs of geranium ‘Ringo Deep Red’, impatiens ‘Super Elfin Bright Orange’, and petunia ‘Ultra Red’ were transplanted into 6-celled (TJ606, 150mL per cell) containers. The containers held 70%:30% (v:v) peat:perlite substrate mixed with dolomitic hydrated limestone [97% $\text{Ca}(\text{OH})_2 \cdot \text{MgO}$, 92% of which passed through a 45- μm screen, National Lime and Stone, Findlay, Ohio, reported acid neutralizing value of 161% calcium carbonate equivalent (CCE)] at a rate of $2.01 \text{ kg} \cdot \text{m}^{-3}$ to raise substrate pH to approximately 6.0. The peat source was a Canadian sphagnum peat moss (Sun Gro Horticulture, Vancouver, Canada) with long fibers and little dust (von Post scale 1-2; Puustjarvi and Robertson, 1975). At the time of planting, the substrate in each container was moistened with 300mL of a $7.14 \text{ mEq} \cdot \text{L}^{-1}$ N solution of (in % by mass) 17.0N-2.2P-14.2K-4Ca-1Mg-0.085Fe-0.042Mn-0.021B-0.021Cu-0.042Zn-0.008Mo (25 NH_4^+ :75 NO_3^-) commercial fertilizer solution (Greencare Fertilizers, Kankakee, IL) and plants were top-irrigated as needed with $14.28 \text{ mEq} \cdot \text{L}^{-1}$ N of the same formulation for two weeks prior to starting different fertilizer treatments. Saucers placed underneath each container collected leachate and allowed for re-absorption into the substrate. Species were then grown and irrigated with 18 commercial water-soluble fertilizers ranging in potential acidity and basicity at $7.14 \text{ mEq} \cdot \text{L}^{-1}$ N for four weeks after which change in substrate-pH, accumulated dry mass, and change in tissue nutrient analysis were measured.

Because nitrification may have changed the $\text{NH}_4^+:\text{NO}_3^-$ ratio in the applied fertilizer that was ultimately taken up by the plant, only data from the three fertilizers with the lowest $\text{NH}_4^+:\text{NO}_3^-$ ratio were used for cation/anion balance and change in substrate-pH (formulations of 12.0N-1.3P-10.0K (1 $\text{NH}_4^+:$ 99 NO_3^-), 13.0N-0.9P-10.8K (4 $\text{NH}_4^+:$ 96 NO_3^-), and 13.0N-1.8P-16.6K (0 $\text{NH}_4^+:$ 100 NO_3^-); Greencare Fertilizers, Kankakee, IL). Each contained 4% or less of total N as NH_4^+ -N with the remainder (96% or more) as NO_3^- -N, and any N taken up into plant tissue was assumed to be in the NO_3^- (anion) form. Change in substrate-pH data from the four-week period was analyzed using analysis of variance (ANOVA) in PROC GLM with SAS 9.2 (SAS Institute, Cary, N.C.) with mean separation using Tukey's honestly significant difference (HSD) at $\alpha=0.05$, and with main and interaction effects of plant species and fertilizer type in a randomized complete block design with six replicates per treatment..

Total dry mass was measured for each of six replicate plants per species at week zero, and six replicate plants per fertilizer and species combination at week four to calculate cation/anion balance. Tissue analyses were determined for one composite sample per species at week zero (3 samples), and one composite sample per fertilizer and species combination at week four (9 samples). Tissue nutrient concentrations were measured by ICP-atomic emission spectrophotometry. Nutrient uptake was calculated by multiplying the average dry mass of plants grown with each fertilizer by the tissue nutrient concentration. The initial nutrient content of the plant at week zero was subtracted from the final nutrient concentration to get the total nutrient uptake over the four weeks of the experiment. Nutrient uptake was then converted to mEq values for calculations.

Cation/anion uptake ratio was calculated by the change in mEq per plant from week zero to week four for cations (K^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , Al^{3+} , Na^+) divided by anions (NO_3^- , $H_2PO_4^-$, SO_4^{2-} , MoO_4^{2-} , Cl^-). After four weeks, samples were taken for each species-fertilizer combination (3 species x 3 fertilizers), with one composite sample per treatment combination. Boron was not included in the cation/anion uptake because boron exists as H_3BO_3 at $pH < 7$ and is taken up as an uncharged molecule (Marschner, 2012, Ch 7.7; Miwa and Fujiwara, 2010). Main effects were tested for species and fertilizers for cation/anion balance, and both main and interaction effects were tested for change in substrate-pH using ANOVA in PROC GLM, with mean separation using Tukey's HSD at $\alpha=0.05$.

2.2 Hydroponic Experiment 1 (Cation/Anion Balance)

The experiment was a completely randomized, 4x3 factorial design with the first factor being plant species (geranium, impatiens, and petunia, along with a no-plant control) and the second factor being fertilizer $NH_4^+ : NO_3^-$ ratio (0:100, 10:90, or 20:80) using a modified 0.5x Hoagland nutrient solutions at $7.14 \text{ mEq} \cdot \text{L}^{-1} \text{ N}$.

In Spring of 2013, 128-cell seedling plugs of 'Ringo 2000 Red Deep' geranium, 206-cell 'Ultra Red' petunia, and 206-cell 'Super Elfin Orange Bright' impatiens (Knox Nursery, Apopka FL) were transplanted into hydroponic systems located on greenhouse benches at the University of Florida, Gainesville, FL. The shoot plus the root dry mass was 4.24 ± 0.6 (mean $\pm$ standard deviation) grams across species.

At transplant, roots were washed with deionized water to remove substrate particles sticking to root surfaces. Seedlings were grown for four weeks in a modified 0.5x Hoagland's solution (Table 1) at $7.14 \text{ mEq} \cdot \text{L}^{-1} \text{ N}$. The solution was mixed with reagent grade salts and zero alkalinity de-ionized water. The solution-pH was adjusted to 6.0 prior to transplant using HCl or NaOH at 0.1 *N*. Solution-pH in each container was monitored every two days and maintained within a range of 5.5 to 6.5 using HCl and NaOH at 0.1 *N*. Every seven days the nutrient solution in each container was emptied and replenished with 4.5 L of fresh solution.

Hydroponic culture vessels were 4.5 L white plastic containers with snap-on plastic lids. The stems of seedlings were supported by black neoprene stoppers (5 cm diameter) held inside plastic baskets which fit into circular holes cut in the container lids. The system was designed so plant roots were submerged in the culture vessel nutrient solution. The neoprene stoppers fit around plant stems but did not constrict growth and reduced evaporation of the nutrient solution. Plastic containers, lids, baskets, and neoprene stoppers were washed in a phosphate-free detergent and rinsed with de-ionized water before use in this experiment. A clear plastic tube fitted with an aquarium air-stone was inserted through a 0.75 cm hole cut into the center of each container lid and provided continuous aeration of the nutrient solution. The plastic containers were painted silver to reduce light transmission to the interior, reduce algae growth in the nutrient solution, and to help maintain a stable solution temperature. Each hydroponic vessel contained geranium, impatiens, or petunia, or no plants, and occupied a 30 cm x 30 cm area of greenhouse bench space.

The experiment began after four weeks growth. Each treatment combination was replicated four times, where a replicate consisted of two plants in one culture vessel. Additional containers identical to treatment replicates but with no plants were set up as control replicates. Each container held 4.5 L of nutrient solution. The experiment lasted seven days and average day and night air temperatures were 25.2 ± 1.4 °C and 19.4 ± 0.8 °C, respectively. Average solution temperature was 20 ± 1.4 °C and daily light during the experiment, or daily light integral (DLI), was 14.7 ± 3.8 mol·m⁻²·d⁻¹ of photosynthetically active radiation (PAR).

Solution-EC and solution-pH were monitored every second day until final data collection on day seven, with pH adjusted as previously described. Solution-EC lowered 0.22 ± 0.14 mS/cm across species and solution types after seven days and was not considered in the results. Initial and final solution samples were tested for ion concentration. Prior to chemical analysis, sample storage was in the dark at 5°C and with addition of sufficient H₂SO₄ to lower solution pH to < 3.0 to stabilize the solution. Ammoniacal and NO₃⁻-N analysis was done by semi-automated and automated colorimetry (University of Florida Analytical Laboratory, Gainesville FL). All other ion analysis was done by inductively-coupled plasma (ICP) atomic emission spectrophotometry (Quality Analytical Laboratories, Panama City, FL)

Net acidity-basicity produced in nutrient solutions was calculated from the amount of acid (HCl) or base (NaOH) added during pH adjustments plus the final amount required at day seven to titrate the solution back to pH 6.0 following tissue harvest. A net addition of base or acid during pH adjustment indicated that plant roots produced either acid or base, respectively. Additions were converted to mEq and the mEq base was subtracted from mEq acid, meaning that net

acidity produced by plants (solution acidity) was shown as a negative value and net basicity (solution basicity) was a positive value.

Uptake of individual nutrients was calculated by subtracting the final mEq of nutrients in solutions containing plants from the final average mEq of nutrients in control (no plant) solutions. Net cation or anion uptake was determined by subtracting total cation from total anion uptake (anions minus cations), where negative mEq values indicated net cation uptake and positive values indicated net anion uptake. Assumed nutrient forms taken up by plants were NH_4^+ , K^+ , Ca^{2+} , Mg^{2+} , Na^+ , NO_3^- , H_2PO_4^- , SO_4^{2-} , Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , MoO_4^{2-} , Al^{3+} , Cl^- , and H_3BO_3^0 . The calculated charge difference between these cations and anions measured in the final solution represented 2% of the total milliequivalent concentration of either anions or cations, and resulted from the combination of H^+ , OH^- , carbonates and organic ions not analyzed.

Cation/anion uptake ratios were calculated by dividing the total cation uptake in mEq by total anion uptake for each treatment replicate. Dry mass increase was measured by subtracting the initial species dry mass (in grams) from the final dry mass per treatment replicate after seven days. The mEq of acidity per gram of dry mass increase was determined by dividing the net solution acidity by total dry mass increase in grams for each replicate. The uptake of each nutrient as a percent of total cation or anion uptake was calculated by dividing individual nutrient cation and anion uptake (in mEq) by the total cation or anion uptake for each treatment replicate.

The effects of net cation or anion uptake on solution acidity and basicity was evaluated by analysis of covariance (ANCOVA) using PROC GLM where net ion uptake (calculated as anion minus cation uptake) was the continuous variable, plant species was a class variable, and main

and interaction effects were tested. In addition, the effects of plant species and solution type (as class variables with main and interaction effects) on cation/anion uptake ratio, uptake of each nutrient as a percent of total cation or anion uptake, dry mass increase, net acidity produced, and net acidity per gram of dry mass increase were evaluated using ANOVA in PROC GLM with mean separation by Tukey's HSD at $\alpha=0.05$.

2.3 Hydroponic Experiment 2 ($\text{NH}_4^+:\text{NO}_3^-$ Ratio)

Seeds of the same cultivars in Hydroponic Experiment 1 (Cation/Anion Balance) were germinated in rockwool cubes in Fall of 2013 and irrigated with a commercial water soluble fertilizer solution [in % by mass 17.0N-2.2P-14.2K-4Ca-1Mg-0.085Fe-0.042Mn-0.021B-0.021Cu-0.042Zn-0.008Mo (25 NH_4^+ :75 NO_3^-), GreenCare Fertilizers, Kankakee, IL] at 7.14 mEq $\text{N}\cdot\text{L}^{-1}$ as needed. Eight days after emergence, plants were moved to the same greenhouse as Hydroponic Experiment 1 (Cation/Anion Balance). After four weeks, seedlings were transferred into recirculating hydroponic systems.

Each tank constantly recirculated and aerated 100 L of modified 0.5x strength Hoagland's solution containing 10% of total nitrogen as NH_4^+ -N mixed with reagent grade salts in zero alkalinity de-ionized water. Nutrient concentrations were the same as listed in Table 1 with the exception that iron was supplied at 0.08 mEq $\cdot\text{L}^{-1}$ Fe as FeEDDHA (6.0% FeEDDHA, Akzo-Nobel, The Netherlands) to ensure adequate iron availability at pH greater than 6.0. Each seedling was supported at the stem by a neoprene collar and basket. Baskets were fitted into holes drilled into 1.9 cm thick polystyrene board and the boards were placed over recirculating tanks so that plant roots were submerged in nutrient solution. The dimensions for each tank were

61 cm width x 121.9 cm length x 15.2 cm depth. From day 0 to 14, plants were grown at 64 plants per tank (four tanks and 256 plants) with all tanks containing the same 10% NH_4^+ -N nutrient solution (Table 1). The solution-pH in each tank was maintained between pH 5.5 to 6.5 through additions of HCl and NaOH at 0.1 N, and each tank was completely replenished with fresh solution at day 0 and 7.

At 14 days, two tanks were refilled with each of the treatment solutions ($\text{NH}_4^+:\text{NO}_3^-$ ratios of 0:100, 10:90, 20:80, and 50:50, detailed in Table 1). A total of eight tanks were used with each treatment solution being supplied in two tanks. Plants were respaced to 30 plants per tank (eight tanks) with 10 plants of each of the three species randomly distributed in each tank. Plants were acclimated to the treatment nutrient solutions for seven days prior to being transferred to 4.5 L culture vessels on December 4th, 2013. There were two plants per culture vessel, and three replicate culture vessels per species and solution combination, organized as a four species (including the control) times four treatment solutions ($\text{NH}_4^+:\text{NO}_3^-$ ratios of 0:100, 10:90, 20:80, and 50:50) factorial randomized complete block design with three blocks. Each block was a section of a greenhouse bench.

The experiment ran for two days in order to ensure that NH_4^+ -N was not completely depleted from the solution. Uptake of NH_4^+ -N versus NO_3^- -N was determined by depletion from solution after two days, where treatment replicate values were subtracted from control replicates to account for possible nitrification, volatilization, microbial growth, or other N loss. The proportion of uptake was evaluated on a percentage basis by dividing mEq of NH_4^+ -N uptake by mEq of total N uptake.

The PAR and average day/night temperatures during the two days of the experiment were $9.6 \pm 1.7 \text{ moles} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ and $23.3 \pm 1.4^{\circ}\text{C} / 19.2 \pm 1.3^{\circ}\text{C}$, respectively. The experiment was repeated on December 16th, 2013. PAR and average day/night temperatures for the repeated experiment were $10.5 \pm 3.1 \text{ moles} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ and $22.8 \pm 1.4^{\circ}\text{C} / 18.1 \pm 1.6^{\circ}\text{C}$, respectively.

Analysis of variance in PROC GLIMMIX was used to evaluate each experimental run in Hydroponic Experiment 2 separately for the effects of plant species and solution type and their interaction on $\text{NH}_4^{+}\text{-N}$ versus $\text{NO}_3^{-}\text{-N}$ uptake, with mean separation using Tukey's HSD at $\alpha=0.05$. Because treatment variances were heterogeneous, the residual error for each treatment was studentized in the statistical model by standardizing the residual error by the standard deviation for each treatment group. The $\text{NH}_4^{+}\text{-N}$ versus $\text{NO}_3^{-}\text{-N}$ proportional uptake data fit the assumptions of normality and therefore data did not require transformation for the ANOVA.

3.0 Results and Discussion

3.1 Johnson et al. (2013) Study (Cation/Anion Balance)

There was a difference in the change in substrate-pH over time between plant species ($P < 0.0001$), but there was no interaction between plant species and fertilizer type on substrate pH, when plants were irrigated with fertilizers that had at least 96% of N supplied as $\text{NO}_3^{-}\text{-N}$. Substrate-pH did not change over four weeks when geranium or impatiens were grown, whereas

pH increased by 0.53 units with petunia (Figure 1B). Geranium, impatiens, and petunia had net anion uptake (cation/anion ratio less than 1) (Figure 1A).

Plant species differed in cation/anion uptake ratio ($P=0.0356$) but there was no effect of fertilizer type. Geranium had a higher cation/anion uptake ratio than petunia, and impatiens was intermediate and did not differ from other species (Figure 1B). Although the cation/anion ratio was less than one for all species, pH only increased over time with petunia. Other factors such as the displacement of protons from substrate exchange sites by cations in the nutrient solution also buffered substrate-pH, and this acidic substrate cation exchange capacity process may have partially balanced the basic effect of net anion uptake by roots. Fisher et al. (2014a) found that addition of Ca^{2+} , more than Mg^{2+} , NH_4^+ , and K^+ , tended to decrease substrate-pH because of cation exchange with a peat/perlite substrate. Fisher et al. (2014b) also found that increasing the concentration of a nitrate-based fertilizer applied to impatiens did not increase substrate-pH, presumably because of cation exchange with a peat/perlite substrate. Differences in cation/anion uptake alone were insufficient to explain the observed difference in substrate-pH, and factors such as cation exchange may have also affected pH change. However, petunia had the lowest cation/anion ratio and was also the only species to increase substrate-pH over time.

3.2 Hydroponic Experiment 1 (Cation/Anion Balance)

One purpose from this experiment was to quantify the relationship between acidity or basicity produced by plants compared with net cation or anion uptake (as in other charge balance studies such as Haynes, 1990; Kirkby, 1977; Lea-Cox, 1996; van Beusichem, 1988). A related purpose

was to evaluate whether this quantitative relationship was consistent between plant species. There was a positive correlation ($P < 0.0001$) between solution acidity or basicity and net cation or anion uptake (shown as anion minus cation uptake), and difference in plant effects in the ANCOVA (Table 2). Net cation uptake (anions minus cations < 0) resulted in solution acidity, whereas net anion uptake (anions minus cations > 0) resulted in basicity (Figure 2). The effect of net cation or anion uptake therefore explained most of the variability in solution acidity or basicity, and all plant species fell along the same regression curve. The regression curve (Figure 2) had a y-intercept not different than zero, indicating that a balanced charge uptake (anions minus cations = 0) did not result in net acidity or basicity. The regression coefficient was 0.972 ± 0.195 (95% confidence intervals around the gradient parameter) indicating net uptake of one mEq of cations would add nearly one mEq of acidity into the solution, or have the opposite basic effect with net uptake of anions. The approximately 1:1 relationship between net ion uptake versus solution acidity is consistent with reports from previous charge balance studies (Haynes, 1990; Kirkby and Knight, 1977; Lea-Cox et al., 1996; Marschner, 2012, Ch 2.6; van Beusichem, 1988).

A relationship was also found between species ($P = 0.0058$) and solution $\text{NH}_4^+:\text{NO}_3^-$ ($P < 0.0001$) effects on cation/anion uptake ratio (Figures 3A and 3B). Geranium, petunia, and impatiens had cation/anion uptake ratios greater than 1, less than 1, and not different than 1, respectively (Figure 3A). Increasing solution $\text{NH}_4^+:\text{NO}_3^-$ also increased cation/anion uptake (Figure 3B), whereby the 0:100 solution had a cation/anion uptake ratio less than 1, the 10:90 had a ratio not different than 1, and the 20:80 solution had a ratio greater than 1.

Previous research has found that nitrogen is generally taken up in greater molar quantities than other ions, plants supplied with 100% NO_3^- -N usually have greater net anion uptake. Increasing NH_4^+ -N usually results in greater net cation uptake (Haynes, 1990; Marschner, 2012, Ch 2.6). This sensitivity to nitrogen form was also found in the Hydroponic Experiment 1 (Cation/anion Balance), where supplying 20% of total N as NH_4^+ -N was sufficient to achieve a cation/anion uptake ratio greater than one with all three species.

One way to compare pH influence between species is on the basis of the amount of acidity or basicity produced per unit of growth. For example, Rengel (2003) showed that 37 legume and cereal crop species differed in soil acidification, ranging from 0.3 to 2.0 mEq acidity produced per gram of shoot dry mass (original reported units were in centimoles of acidity per kilogram shoot dry mass).

Differences in acidity may be linked to the geographical origin of plant species, where the legume and cereal species that originated from humid climates and acid soils showed no appreciable acidification of the rhizosphere. In comparison, those species originating from temperate climates showed moderate rhizosphere acidification, and those from semiarid climates showed strong acidification (Rengel, 2003). Species adapted to high pH soil, or are efficient at taking up iron from the soil, may produce more acidity because of preference for greater cation/anion uptake compared to species that are inefficient at taking up iron or are adapted to low pH soils (Marschner, 1995, Ch 17.5). Other causes for species acidity include root responses to iron and phosphorus deficiency and the release of organic acids into the soil (Marschner, 2012, Chs 14.4 and 17.5)

418
419 In Hydroponic Experiment 1 (Cation/Anion Balance), species differed in total dry mass increase
420 after seven days ($P < 0.0001$), but there were no solution main or interaction effects (Figure 4A).
421 Impatiens had lower dry mass accumulation (3.64 grams) compared with geranium (6.99 grams)
422 and petunia (5.70 grams).

423
424 Net solution acidity and basicity (Figure 4B) was affected by plant species ($P < 0.0001$), solution
425 $\text{NH}_4^+:\text{NO}_3^-$ ($P < 0.0001$), and their interaction ($P = 0.0169$). In the 0:100 solution, all species had a
426 basic effect, with petunia resulting in greater basicity than the other two species. In the 10:90
427 solution, geranium and impatiens resulted in a neutral effect, whereas petunia had a basic effect.
428 In the 20:80 solution, geranium and impatiens had an acidic effect but solution acidity with
429 petunia was not significantly different from neutral. These results emphasize that although
430 fertilizers are often labeled as “acidic, basic, or neutral” based on CCE calculations for field
431 application (Pierre, 1933), the net pH change is the result of multiple factors such as fertilizer
432 formulation, plant species, and water alkalinity (Fisher et al., 2014b; Johnson et al., 2013).

433
434 Based on Hignett’s (1985) adaptation of Pierre’s method to calculate fertilizer CCE, all fertilizer
435 solutions tested in Hydroponic Experiment 1, from 0 to 20% NH_4^+ , would be predicted to have a
436 basic reaction. The Nitrogen CCE method (Johnson et al., 2013) did correctly predict greater
437 acidity with increasing NH_4^+ concentration, and in the order from petunia to impatiens to
438 geranium. However, using the Nitrogen CCE method, the 0% NH_4^+ solution was predicted to
439 have an acidic reaction for geranium, and basic for impatiens and petunia, whereas the 20%
440 NH_4^+ solution was predicted to be acidic for geranium and impatiens, but not petunia. The

observed pH change in the hydroponic solutions differed greatly from the Pierre (1933) calculation which ignores plant species effects, and the Nitrogen CCE method over-predicted the acidity of the solutions. Pierre's method is based on field soils, and the Nitrogen CCE method is calibrated for a peat-based substrate. There is therefore a need to develop and calibrate a CCE model under hydroponic conditions. The implications for fertilizer strategy in a zero alkalinity hydroponic solution are an $\text{NH}_4^+:\text{NO}_3^-$ ratio that would result in a neutral pH reaction would be between 0:100 and 10:90 for geranium, 10:90 for impatiens, or 20:80 for petunia.

The pH effect (mEq of acidity or basicity) per unit growth (gram of dry mass gain) was influenced by both plant species ($P=0.0013$) and solution $\text{NH}_4^+:\text{NO}_3^-$ ratio ($P=0.0035$) effects. Impatiens and petunia varied in acidity and basicity with nutrient solution whereas geranium had no effect on acidity or basicity across all solution types (Figure 4C). Impatiens had the broadest range of acidity or basicity per gram of growth with the three fertilizer solutions. The acidic reaction by impatiens in the 20:80 solution was within the range of acidification described by Rengel (2003) for 37 legume and cereal species. Petunia had a basic reaction for the 0:100 and 10:90 solutions, and a neutral reaction with the 20:80 solution.

The contribution of individual nutrients as a percentage of either total cation or anion uptake was analyzed, by nutrient, with ANOVA. There were differences between species in the percent uptake of macronutrient cations and anions other than NH_4^+-N , Na^+ , SO_4^{2-} and Cl^- (Figure 5). Solutions also differed except for Na^+ and Cl^- . With NH_4^+-N uptake, geraniums grown in the 10:90 solution showed highest NH_4^+-N uptake (22.8% of total cations), followed by both petunia

(15.8%) and impatiens (13.9%). There were no differences between species in the 20:80 solution, with NH_4^+ -N accounting for an average of 22.5% of total cation uptake across species.

Among cations, K^+ accounted for the greatest mEq uptake, followed by Mg^{2+} , Ca^{2+} , and NH_4^+ -N (Fig 5A, C). As NH_4^+ -N concentration increased in the solution, the proportional contribution of K^+ , Mg^{2+} , and Ca^{2+} all decreased (Fig. 5C). Nitrate dominated anion contribution (Fig. 5B, D), with other anions accounting for 21.6% or less.

The contribution of NH_4^+ -N and NO_3^- -N were added together and analyzed using ANOVA, as a proportion of the maximum 200% of cations plus anions that would affect charge balance. There were solution ($P < 0.0001$) and species by solution ($P = 0.0272$) effects. N from the combined forms accounted for approximately half of the total ion contribution, ranging from 83.9% for impatiens with the 0:100 solution to 107.5% for geraniums with 10:90. The lowest total N contribution to total anion and cation uptake occurred with the 0:100 solution for all three species, meaning that adding NH_4^+ -N increased the overall effect of N on charge balance.

3.3 Hydroponic Experiment 2 ($\text{NH}_4^+:\text{NO}_3^-$ Ratio)

Uptake of NH_4^+ -N versus NO_3^- -N, expressed in terms of NH_4^+ -N as a percent of total N taken up, was compared with supplied $\text{NH}_4^+:\text{NO}_3^-$ ratio from 0:100 to 50:50 in order to evaluate differences in “preference” of each N form between species. Data from the two experimental runs were not analyzed in the same ANOVA, because an F-test found that the variances between experimental runs were not equal. However, if plants had taken up NH_4^+ -N and NO_3^- -N in the

same ratio as it was supplied in the nutrient solution, treatment means would equal 0, 10, 20, or 50% for the four solutions (shown as dashed lines on the charts) (Figure 6). In the first experimental run (Figure 6A), however, geranium, impatiens, and petunia all had a higher percentage of NH_4^+ -N uptake than was supplied in the 10:90 and 20:80 solutions. In the 50:50 solution, geranium and impatiens had NH_4^+ -N uptake percentages of (least-square mean $\pm$ 95% confidence limit) $45.7 \pm 3.5\%$ and $45.8 \pm 3.5\%$, respectively, just below the NH_4^+ -N percentage supplied, whereas petunia had lower NH_4^+ -N uptake at $30.8 \pm 3.5\%$. Overall, the greatest NH_4^+ -N uptake occurred in the 50:50 solution and both geranium and impatiens had greater NH_4^+ -N versus NO_3^- -N uptake than petunia ($P < 0.0001$).

In the second experimental run, species trends in NH_4^+ -N uptake were similar to the first experimental run, where both geranium and impatiens had a greater percentage of NH_4^+ -N uptake than petunia ($P = 0.0095$). Individual species preferences for NH_4^+ -N versus NO_3^- -N uptake across solution types were inconsistent with the first experimental run, likely because of the greater variability observed in the data compared to the first experimental run. Therefore, only solution type differences ($P < 0.0001$) are shown in Figure 6B. There is a greater percentage of NH_4^+ -N uptake than was supplied in the 10:90 and 20:80 solutions, similar to the first experimental run. In the 50:50 solution, uptake did not differ from the supplied $\text{NH}_4^+:\text{NO}_3^-$ ratio. A consistent trend in both experiments was that more uptake of NH_4^+ -N over NO_3^- -N occurred over all species in solutions with less than 50:50 $\text{NH}_4^+:\text{NO}_3^-$.

4.0 Conclusions

Differences in cation/anion uptake by roots were consistent with the observed acidity or basicity for the three species, and cation/anion uptake also increased as $\text{NH}_4^+:\text{NO}_3^-$ ratio increased. Petunia had the lowest cation/anion ratio based on plant tissue analysis, and also resulted in the highest substrate-pH when geranium, impatiens, and petunia were grown for four weeks in peat/perlite substrate and were fertigated with NO_3^- -N. In hydroponic solutions, there was an approximately 1:1 relationship between net cation or anion uptake by roots versus solution acidity or basicity. Across solutions ranging in $\text{NH}_4^+:\text{NO}_3^-$ ratio from 0:100 to 20:80, mean cation/anion uptake was not different than 1 in geranium, equal to 1 in impatiens, and less than 1 in petunia. Species differences in solution acidity and basicity were therefore consistent with differences in cation/anion uptake.

Cation/anion uptake also increased as solution $\text{NH}_4^+:\text{NO}_3^-$ ratios increased, whereby uptake ratios across species were less than 1 in the 0:100 solution, equal to 1 in the 10:90 solution, and greater than 1 in the 20:80 solution. Nitrate dominated anion uptake, and the combined NH_4^+ -N and NO_3^- -N accounted for approximately half of the mEq of total cations and anions taken up. As $\text{NH}_4^+:\text{NO}_3^-$ ratio increased from 0:100 to 20:80, uptake of NH_4^+ -N increased and all species took up NH_4^+ -N at a higher concentration than was supplied in the 10:90 and 20:80 solutions. Although the results were not consistent between all experiments, petunia overall took up a lower ratio of $\text{NH}_4^+:\text{NO}_3^-$ than the other two species. For pH control in horticultural management, adjusting only the $\text{NH}_4^+:\text{NO}_3^-$ ratio to supply 10% NH_4^+ -N for geranium and

530 impatiens and 20% NH_4^+ -N for petunia would be expected to result in a neutral pH effect in
531 hydroponic solution with zero alkalinity.

532

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534

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539

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Table 1. Ion concentrations supplied ($\text{mEq}\cdot\text{L}^{-1}$) by fertilizers used in the hydronic experiments. Three nutrient solution $\text{NH}_4^+:\text{NO}_3^-$ ratios (0:100, 10:90, and 20:80) were used in the Hydroponic Experiment 1 (Cation/Anion Balance) and four solution ratios (0:100, 10:90, 20:80, and 50:50) were used in the Hydroponic Experiment 2 ($\text{NH}_4^+:\text{NO}_3^-$ Ratio), respectively.^z

Solution $\text{NH}_4^+:\text{NO}_3^-$ ratio	Total N	NH_4^+	NO_3^-	H_2PO_4^-	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	Na^+
0:100	7.14	0	7.14	0.52	2.99	4.99	1.98	2.37	0	0.131
10:90	7.14	0.71	6.43	0.52	2.99	4.99	1.98	3.43	0.31	0.131
20:80	7.14	1.43	5.71	0.52	2.99	4.99	1.98	4.17	1.02	0.131
50:50	7.14	3.57	3.57	0.52	2.99	4.99	1.98	8.04	1.41	0.131

^zMicronutrients were supplied in all nutrient solutions at (in $\text{mEq}\cdot\text{L}^{-1}$) 0.04 Fe, 0.02 Mn, 0.003 Cu, 0.008 Zn, and 0.0004 Mo. Boron was considered an uncharged molecule (see 2.1 and 2.2 Materials and Methods) and was supplied at $0.5\text{ mg}\cdot\text{L}^{-1}$. All nutrient solutions contained $0.023\text{ mEq}\cdot\text{L}^{-1}$ Al. The fertilizer was derived from $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, FeEDTA, H_3BO_3 , $\text{MnSO}_4\cdot\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{MoO}_7\cdot 2\text{H}_2\text{O}$ and $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$. In Hydroponic Experiment 2 ($\text{NH}_4^+:\text{NO}_3^-$ Ratio), Fe was supplied at $0.08\text{ mEq}\cdot\text{L}^{-1}$ as FeEDDHA.

Table 2. Results of analysis of covariance (ANCOVA) evaluating plant species as class variables and net ion uptake (mEq anion minus cation uptake) as the continuous variable on solution acidity or basicity for Hydroponic Experiment 1 (Cation/Anion Balance).

	df	Type III SS	Mean Square	F Value	P-value
Species	2	3.33	1.67	1.07	0.3550
mEq Anion minus Cation Uptake	1	83.34	83.34	53.59	<.0001
Interaction (Species*mEq Anion minus Cation Uptake)	2	1.6	0.8	0.51	0.6037

List of Figures

Figure 1. Cation/anion uptake ratio (A) and change in substrate-pH (B) from the Johnson et al. (2013) Study (Cation/Anion Balance). Three floriculture species (geranium, impatiens and petunia) were grown for four weeks in a peat/perlite substrate. Each irrigation contained 100 mg·L⁻¹ N from three water-soluble fertilizers (12.0N-1.3P-10.0K, 13.0N-0.9P-10.8K, and 13.0N-1.8P-16.6K; Greencare Fertilizers, Kankakee, IL) with 4% of less of total nitrogen as NH₄⁺-N and the remainder as NO₃⁻-N. Figures 1A and 1B consisted of 18 and three replicates, respectively, per plant species and bars represent the least-square means ± 95% confidence intervals.

Figure 2. Net ion uptake (mEq anion minus cation uptake versus solution acidity for Hydroponic Experiment 1 (Cation/Anion Balance) with three floricultural species. Symbols represent individual replicate containers. Regression equation is solution acidity or basicity = $0.972 \pm 0.195 * (\text{anion minus cation uptake}) + 0.140 \pm 0.478$.

Figure 3. The effects of geranium, impatiens, and petunia (A) and solution NH₄⁺:NO₃⁻ ratios 0:100, 10:90, and 20:80 (B) on cation/anion uptake ratio (mEq of cation uptake divided by mEq of anion uptake) after plants were grown for seven days in hydroponic solutions in the Hydroponic Experiment 1 (Cation/Anion Balance). The dashed lines represent an equal amount of cation and anion uptake (cation/anion ratio equal to 1). Data represent least-square means of 12 replicates per treatment ± 95% confidences intervals.

Figure 4. Comparison of solution $\text{NH}_4^+:\text{NO}_3^-$ and plant species effects in the Hydroponic Experiment 1 (Cation/Anion Balance) on total plant dry mass increase after seven days (A), net solution acidity and basicity (mEq) per hydroponic container after seven days (B), and calculated solution acidity and basicity (mEq) per gram of plant dry mass increase over seven days (C). Data represent the least-square mean of four replicates per species and solution combination $\pm$ 95% confidence intervals.

Figure 5. Contribution of different nutrients to total cation or anion uptake in the Hydroponic Experiment 1 (Cation/Anion Balance), comparing main effects in ANOVA by species (A) and (B), or nutrient solution (C) and (D), and means with the same letter were not different using Tukey's HSD at the $\alpha=0.05$ level. Micronutrients (and also Na^+) contributed less than 1% of total cation or anion uptake, and are not included in the chart for simplicity of presentation.

Figure 6. Interaction between solution $\text{NH}_4^+:\text{NO}_3^-$ ratio and plant species effects on NH_4^+ -N uptake as a percentage of total N uptake for the first experimental run (A) and main solution $\text{NH}_4^+:\text{NO}_3^-$ effects on NH_4^+ -N uptake as a percentage of total N uptake in the second experimental run (B) in the Hydroponic Experiment 2 ($\text{NH}_4^+:\text{NO}_3^-$ Ratio). Data represent the least-square mean of three (A) or nine (B) replicates $\pm$ 95% confidence intervals. Dashed lines indicate the percent NH_4^+ of total N supplied in the 0:100, 10:90, 20:80, and 50:50 $\text{NH}_4^+:\text{NO}_3^-$ solutions.

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