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Nutrient Uptake and Management Strategies in Recirculating Hydroponic Systems

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Nutrient Uptake and Management Strategies in Recirculating Hydroponic Systems

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Abstract

Nutrient management in recirculating hydroponic systems requires the periodic replenishment of water and nutrients to the nutrient solution reservoir. Common nutrient management strategies, such as replenishing the reservoir with fresh solution and maintaining a constant solution electrical conductivity (EC), can lead to ion accumulation and nutrient imbalances since nutrients are taken up by roots and depleted from solution at different rates. To avoid nutritional disorders, commercial growers typically dump and replace the hydroponic solution periodically, which is wasteful and has an economic cost. A potential alternative is to specially formulate the nutrient replenishment solution to balance the supply of nutrients with the uptake of nutrients into plant tissues. As a result, nutrients would be consistently replaced in solution at a rate similar to the uptake by plant roots. A range of published nutrient solution formulations for hydroponic leafy greens crops were reviewed and shown to vary considerably in nutrient concentrations, many of which would be expected to oversupply certain nutrients, particularly calcium, magnesium, and sulfur. A study was conducted to quantify nutrient uptake and water use efficiency (WUE) by arugula (*Eruca sativa* L.) and basil (*Ocimum basilicum* L.) and determine if the strategy for replenishing nutrients impacted plant growth and nutrient uptake. A second study evaluated the potential to design a species-specific replenishment solution for arugula and basil to minimize the accumulation of ions in solution over time. Overall, arugula and basil differed in plant growth, uptake of individual nutrients, and transpiration, but were similar in WUE. Nutrient replenishment strategy had minimal to no impacts on plant growth, nutrient uptake, or WUE. Similarly, species-specific replenishment solutions formulated for arugula and basil had minimal effects on plant growth, nutrient uptake into plant tissues, or WUE when compared to nutrient replenishment with a standard hydroponic

solution used commercially. Species-specific replenishment solutions also decreased the accumulation of nutrient ions, particularly calcium, magnesium, and sulfate, compared to a standard hydroponic replenishment solution. For both arugula and basil, solution EC increased when nutrients were replenished with the commercial standard solution, but remained more stable when nutrients were replaced using the species-specific replenishment solutions. Species-specific replenishment solutions may be a strategy for growers to prevent salt accumulation and ion imbalances in recirculating hydroponic systems, minimizing the risk of nutritional disorders and the need to dump and replace solution. Since species-specific replenishment strategies reduced changes in solution EC over time, this approach would improve the practice of managing nutrient supply by maintaining a target EC level. Growers can develop their own species-specific replenishment solutions by monitoring plant uptake of nutrients, growth and yield, and water use during production.

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Table of Contents

Table of Tables

TABLE OF FIGURES

CHAPTER 1 . LITERATURE REVIEW ON FORMULATING NUTRIENT SOLUTIONS FOR RECIRCULATING HYDROPONIC SYSTEMS

Abstract

The objective of this review was to provide a summary of hydroponic nutrient solution formulations and management strategies as well as identify potential challenges in managing nutrients during production. Current nutrient management strategies in recirculating hydroponic systems often result in nutrient imbalances in the root zone, caused by the accumulation of certain ions in solution and/or the uptake and depletion of nutrients by plants. A common strategy in commercial hydroponic production therefore is to periodically discharge and replace the nutrient solution to avoid decreased plant growth and quality. This review explores the potential of formulating species-specific replenishment solutions using mass balance principles as an alternative to current nutrient management strategies. The purpose of species-specific replenishment solutions is to resupply nutrients in proportions and concentrations being removed from solution by plant roots. Examples of species-specific replenishment solutions were developed and compared for three hydroponic leafy greens species using data collected on the accumulation of nutrients in plant tissues, plant growth, transpiration, and calculated water-use efficiency.

Introduction

Modern hydroponic operations recirculate and reuse the nutrient solution to reduce fertilizer costs, improve production efficiencies, and minimize the negative environmental impacts associated with nutrient discharge (Bugbee, 2004; Pardossi et al., 2011; Sonneveld and

Voogt, 2009). Recirculating hydroponic solutions are regularly replenished during production to replace nutrients and water absorbed by roots. However, replenishment of nutrients to maintain optimal growth and yield remains a challenging aspect of closed hydroponic production (Bugbee, 2004; Miller et al., 2020). Nutrients replenished in excess accumulate in the root zone and can result in ion imbalances and toxicity whereas nutrients replenished in insufficient amounts results in nutrient depletion and deficiency. Optimal nutrient replenishment implies nutrients resupplied at a rate similar to root uptake and maintaining nutrient concentrations needed for the greatest plant quality—i.e., balancing nutrient supply with plant demands. This is also referred to steady-state nutrition (Langenfeld, 2021), and is a primary nutrient management goal for growers in commercial practice.

The first objective of this article is to review common nutrient solution characteristics and management practices currently used for hydroponic production. The second objective is to discuss the potential of developing species-specific replenishment solutions to achieve steady-state nutrition in recirculating systems. This article provides a brief review of using mass balance approaches for nutrient replenishment with an example case study of formulating species-specific replenishment solutions for hydroponic leafy greens. We conclude with discussion of potential benefits, limitations, and knowledge gaps for using mass balance principles in nutrient management strategies.

Review of hydroponic nutrient solution characteristics

The hydroponic nutrient solution often supplies the majority of plant essential elements required for growth. Plants require 16 elements to complete their life cycle, including carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca),

magnesium (Mg), sulfur (S), iron (Fe), copper (Cu), boron (B), manganese (Mn), molybdenum (Mo), zinc (Zn), and chloride (Cl) (Hocmuth and Hocmuth, 2018; Marscher, 2012). Plants acquire C, H, and O through photosynthesis and uptake of water, whereas the remaining elements are considered mineral nutrients supplied through fertilization.

Macronutrients are typically supplied in millimolar (mM) concentrations in hydroponic solutions and include N, P, K, Ca, Mg, and S. In contrast, micronutrients are supplied in micromolar (μM) concentrations and include Fe, Mn, Cu, B, Zn, and Mo. Chloride is also considered essential (Marschner, 2012), but is required in small quantities in plant tissues. Sufficient chloride is often supplied in the irrigation water or by fertilizer salts (Sonneveld and Voogt, 2009).

Chloride (Cl) and sodium (Na) are commonly found in nutrient solutions and result from fertilizer impurities and poor water quality (Sonnveld and Voogt, 2009). The accumulation of Na and Cl in recirculating solutions increases root zone salinity and interferes with nutrient uptake (Carmassi et al., 2005; Pardossi et al., 2011). Sodium is non-essential for most greenhouse crops and is toxic at relatively low concentrations. A water quality with Na and Cl concentrations of <2 $mmol \cdot L^{-1}$ is recommended for recirculating solutions to minimize the need to periodically discharge solution (Bar-Yosef, 2007; Sonnveld and Voogt, 2009).

Selection of nutrient concentrations to supply in the hydroponic solution is a key grower decision, and can influence nutrient uptake and fertilizer management (Resh, 2013; Sonneveld et al., 1999; Sonneveld and Voogt, 2009; Winsor and Adams, 1987). Supplied nutrients tend to impact nutrient uptake mainly at low and sub-optimal concentrations in the external solution (Carmassi et al., 2005; Sonneveld et al., 1999), where plants show reduced tissue nutrient levels and growth. In most commercial hydroponic operations, nutrients are supplied in optimal to

luxurious concentrations (Bugbee, 2004; Sonneveld and Straver, 1994; Sonneveld et al., 1999; Walters and Currey, 2015), and in these conditions plants generally absorb nutrients at a relatively constant rate over a wide concentration range (Sonnveld and Voogt, 2009).

Nutrient ratios supplied in the hydroponic solution can have a greater impact on tissue nutrient levels compared to the absolute concentrations of nutrients in solution (Sonneveld and Voogt, 2009). Nutrients with similar ionic charge and valence tend to compete for root uptake. For example, supplying a high concentration of K^+ and a high K^+:Ca^{2+} ratio can suppress the uptake of Ca^{2+} and increase the occurrence of Ca-related physiological disorders such as "blossom-end-rot" in fruiting vegetables and "tip-burn" in lettuce (Houston and Dickson, 2021; Voogt, 2002; Bakker et al., 1989; Marcelis and Ho, 1999). In contrast, supplying a high concentration of Ca^{2+} and a low K^+:Ca^{2+} ratio can cause excess Ca^{2+} uptake and increases the likelihood of disorders such as "gold speck" and "spot" in fruiting vegetables (De Kreij et al., 1992; Voogt, 2002). Examples of other known nutrient and ion antagonisms include NO_3^--N and Cl^-, K^+ and Na^+, and $Fe^{3+/2+}$ and Mn^{2+} (Sonneveld and Voogt, 2009). The appropriate ratio of nutrients to supply in solution depends on the plant species, stage of plant development, and climatic conditions (Resh, 2013; Sonnneveld and Voogt, 2009).

The ratio of supplied N forms, particularly the NH_4^+:NO_3^- ratio, can impact the uptake of total N as well as other nutrients (Bugbee, 2004; Marschner, 2012; Sonneveld and Voogt, 2009). Most plant species exhibit the greatest rate of growth and N uptake when provided a mixed supply of NH_4^+ and NO_3^- (Bugbee, 2004; Marschner, 2012). However, ammonium-N (NH_4^+-N) is known to strongly inhibit the uptake of other macronutrient cations, particularly K^+, Ca^{2+}, Mg^{2+} (Sonneveld, 2002; Sonneveld and Voogt, 2009). It is generally recommended to supply <10% of total N as NH_4^+ for hydroponic fruiting vegetables.

Nutrient solution and root zone pH are managed to ensure the solubility and availability of nutrients for plant uptake, particularly micronutrients. Metal micronutrients (Fe, Mn, Cu, Zn) and B tend to decrease in solubility as pH increases, whereas Mo increases in solubility at high pH (Lindsay, 1979). A slightly acidic pH between 5.5 and 6.0 ensures all nutrients are adequately soluble for root uptake by most plant species (Resh, 2013). Control of nutrient solution pH is often achieved by injection of acid and base chemicals.

Solution electrical conductivity (EC) is monitored and controlled as a method to manage nutrient supply and plant quality (Carmassi et al., 2005; Resh, 2013; Sonneveld et al., 2004; Walters and Currey, 2018). Electrical conductivity refers to the total concentration of dissolved salts, often comprised of nutrient ions from added fertilizers, but also ions found in the irrigation water such as Ca^{2+}, Mg^{2+}, SO_4^{2-}, Na^{+}, and Cl^{-}. Increasing or decreasing EC typically increases or decreases total nutrient concentrations (Domingues et al., 2012; Filgueiras et al., 2002; Sonneveld et al., 2004), respectively, as well as the osmotic potential and uptake of water from the root zone (Sonneveld et al., 2004). Growers can adjust EC by adjusting ion concentrations when formulating hydroponic solutions and aim to maintain target EC values during production to ensure adequate nutrient supply, growth, and plant quality (Resh, 2013; Walter and Currey, 2018).

Sonneveld and Straver (1994) reported optimal solution EC values depend on the specific plant species, which can range between 0.8 and 4.0 $mS \cdot cm^{-1}$ for common hydroponically grown plants. For plant species with tolerance to high salinity, Sonneveld and Straver (1994) and Sonneveld and Welles (1988) report increasing solution EC to between 5.0 and 8.0 $mS \cdot cm^{-1}$ can improve the quality of fruiting vegetables and cut flowers, particularly under low radiation conditions, but also results in reduced yield. Alternatively, certain plants are intolerant to higher

EC, such as hydroponic lettuce, which is susceptible to reduced growth and leaf burn at EC values of approximately 2.5 $mS \cdot cm^{-1}$ and greater.

Survey of published hydroponic nutrient solution formulations

Common hydroponic nutrient solution formulations used by researchers and growers in commercial practice vary considerably in nutrient concentrations and composition, even when designed for the same or similar plant species. To highlight both the range and variability in recommended nutrient concentrations, nutrient data were evaluated from 38 different hydroponic solutions formulated for leafy greens (Tables 1-1 and 1-2). Hydroponic formulations were published in peer-reviewed scientific journals and industry articles, summarized in the Appendix. Expected solution EC was calculated for each solution based on the concentration of individual nutrient ions and using methods described by Sonneveld et al. 1999.

In the surveyed hydroponic formulations, individual nutrients differed in average concentration as shown in Table 1-1. All values in Table 1-1 are reported in $mg \cdot L^{-1}$. Average macronutrient concentrations were greater for N, K, and Ca and lower for P and Mg, with average S concentration being intermediate (Table 1-1). Differences between average macronutrient concentrations in Table 1-1 followed similar trends to general differences observed between macronutrient concentrations found in plant tissues. For example, N is required in larger quantities in most plant tissues compared to P (Bryson and Mills, 2014), and N tended to be supplied in greater concentrations compared to P according to the survey results (Table 1-1). In addition, the majority of N was supplied in the NO_3-N form with relatively low concentrations of NH_4-N. For micronutrients, Fe was typically supplied in the greatest concentration.

Average solution EC was 2.0 $mS \cdot cm^{-1}$, but ranged from 0.9 to 5.8 $mS \cdot cm^{-1}$ (Table 1-1), similar to the range reported by Sonneveld and Straver (1994). Overall, solution EC tended to be greater in surveyed formulations with relatively high concentrations of the divalent ions Ca, Mg, and S (data not shown).

Reported concentrations covered a wide range for most nutrients in Table 1-1. Coefficients of variation (CV) were therefore determined for individual nutrients and solution EC to highlight the variability in reported concentrations. A CV of 1 or greater indicated the standard deviation (in $mg \cdot L^{-1}$) was equal to or greater than the average concentration and therefore had an especially high degree of variation. In contrast, nutrients with a CV of 0.5 or less would indicate the standard deviation was 50% of the average concentration or less for a lower degree of variation, and CV values between 0.5 and 1.0 would be intermediate. Macronutrients with a high degree of variability included Mg and S whereas N and Ca had lower degrees of variability, with P and K being intermediate. The relatively high CV values reported for micronutrients and for NH_4-N were partially a result of the lower concentrations typically supplied in solution.

The nutrient solution concentrations outlined in Table 1-1 have very wide ranges and may even be considered unusual when compared to typical nutrient recommendations. For example, the greatest published concentration of S was 640 $mg \cdot L^{-1}$, while supplying any macronutrient in excess of 350 $mg \cdot L^{-1}$ is generally not recommended for any crop (Pardossi et al., 2011). These values are likely the result of formulations that are not optimized for plant nutrient requirements or formulations that do not take into account the supply of nutrients from the raw irrigation water. Standards of irrigation water classes have been defined by Ontario Ministry of Agriculture, Food and Rural Affairs (OMARFA, 2001). Poor quality water, or water

class 3, can supply anywhere from 200-300 $mg \cdot L^{-1}$ SO_4-S in the raw water. Compared to the published nutrient solution formulations, most adequate and poor quality irrigation sources may provide more than enough S in the raw irrigation water alone. Additionally, K, Ca, Mg can also be found in some sources of raw irrigation water and may be accounted for with lower ranges.

Conversely, some nutrient solutions listed in Table 1-1 report concentrations of nutrients that are very low or even 0 $mg \cdot L^{-1}$. For example, the average concentration of Cu listed in Table 1-1 is 0 $mg \cdot L^{-1}$ despite Cu being a plant essential element and would therefore be necessary in a hydroponic nutrient solution. This may be because some hydroponic nutrient solution formulations assume leaching of metal micronutrients from metal pipes and fittings and will therefore recommend they be supplied at concentrations that are lower than necessary, or even not at all. Another nutrient listed in Table 1-1 with values of 0 $mg \cdot L^{-1}$ is sulfur. As mentioned previously, this may be caused by certain formulations accounting for sulfur supplied in the raw irrigation water, specifically sources that supply poor quality water. Additionally, 0 $mg \cdot L^{-1}$ NH_4-N can also be recommended in the nutrient solution. This is because 100% N is often supplied by NO_3-N in hydroponics to promote greater uptake of cations and promote quality growth (Sonneveld and Voogt, 2009).

Table 1-2 highlights certain nutrient ratios in the surveyed hydroponic solutions that were reported to influence crop quality as discussed by Sonneveld and Voogt (2009). The ratios in which nutrients are supplied is critical since certain nutrients compete for uptake, particularly macronutrients with similar charge such as cations NH_4-N, K, Ca, and Mg. For example, a solution K:Ca molar ratio of 1:1 (1:1 $mg \cdot L^{-1}$) is recommended to avoid a K-induced Ca deficiency (Sonneveld and Voogt, 2009). The average K:Ca ratio in Table 2 is 1.6:1 with a CV of 0.6 which is close to the ratio recommended by Sonneveld and Voogt. Similarly, 2:1 ratios for

K:Mg (4:1 $mg \cdot L^{-1}$) and Ca:Mg (4:1 $mg \cdot L^{-1}$) are recommended to prevent Mg deficiency (Sonneveld and Voogt, 2009) and the average ratio of Ca:Mg from the surveyed solutions was 4.1:1 with a CV of 1.2. The greater CV value does indicate a wider range of variability, but the average ratio is very close to the recommended ratio. However, these recommendations serve as general guidelines, and the optimal nutrient ratios likely depend on plant species and climate (Resh, 2013; Sonneveld and Straver, 1994). The ratio of NH_4:NO_3 ranges from 0:1 to 0.2:1, which follows the common recommendation that NH_4 should not account for more that 20% of the total N in solution (Sonneveld and Voogt, 2009).

Hydroponic nutrient management practices

Hydroponic leafy greens and herbs are typically produced in either nutrient film technique (NFT) or deep water culture (DWC) recirculating systems (Resh, 2013; Walters and Currey, 2015). These systems are typically substrate free, relying on the structure of the hydroponic system to support the plants as they grow. In NFT systems, seedlings are placed in narrow troughs or gutters in which nutrient solution is injected on one end of the trough and flows through to a drain and is captured and recirculated through the system. This allows the plant roots to be always continually in contact with a thin film of solution while roots rest along the bottom of the gutter. DWC systems are similar in that they also continually recirculate the nutrient solution over time. In contrast to NFT, DWC systems allow plant roots to be in contact with greater volumes of solution at any given time, , with reservoir depths typically between 6 and 8 inches. Due to the greater volume of water, roots are completely submerged and allowed to float freely in the nutrient solution. In both cases, as the nutrient solution is recirculated it needs to be monitored and adjusted over time.

In closed hydroponic systems, such as NFT and DWC, excess nutrient solution supplied to plants is captured, recirculated, and reused. Compared to open systems, in which excess nutrient solution drains to waste, crop production in closed systems is used to minimize the discharge of nutrients and water to the environment, reducing pollution and fertilizer/water costs.

Managing nutrients in recirculating systems continues to be a challenging aspect of hydroponic and controlled-environment production (Bugbee, 2004; Resh, 2013). In closed systems, nutrients supplied in excess of plant demand accumulate and have the potential to reduce yield. Plant stress and reductions in yield may occur as a result of nutrient toxicity, high soluble salt stress, and imbalance of nutrients in the root zone (i.e. non-optimal ratio of nutrient concentrations). In contrast, undersupply of nutrients leads to deficiency and reduced growth.

Nutrient solution pH drift, caused by nutrient uptake by plant roots during production (Resh, 2013), may also result in nutritional problems. Therefore, hydroponic growers regularly monitor solution pH in a recirculating system and inject mineral acid and base chemicals to maintain pH within a desired range for crop growth. Several factors related to the nutrient solution influence pH and acid/base injection, including the supplied N form, water quality, and plant species (Conesa et al., 2009; Dickson and Fisher, 2019; Gerendás, 1997; Lea-Cox et al., 1996; Savvas et al., 2003; Savvas et al., 2006; van Beusichem et al., 1988). The supply and root uptake of NO_3-N, for example, creates root zone basicity and raises pH whereas NH_4-N produces root zone acidity and drops pH (Lea-Cox et al., 1996; Savvas et al., 2003; van Beusichem et al., 1988). High water alkalinity also has a basic effect on solution pH (Argo and Fisher, 2002; Resh, 2013). Therefore, nitrate-based nutrient solutions formulated with high alkalinity water would require greater amounts of acid injection during project to maintain a stable pH. The injection of

mineral acids—typically nitric, phosphoric, or sulfuric acids—has potential to add significant amounts of nutrients and soluble salts.

Hydroponic plant species influence the pH of the nutrient solution through imbalanced uptake of cation and anion nutrients (Dickson and Fisher, 2019; Lea-Cox et al., 1996; Savvas et al., 2003; van Beusichem et al., 1988). Dickson and Fisher (2019) also found plant species tended to interact with solution NH_4:NO_3 ratio to influence pH, and NH_4:NO_3 ratios could be adjusted to stabilize pH for specific plant species. For example, arugula was found to be more basic and was estimated to require 23.3% of total N as NH_4-N (remainder as NO_3-N) to prevent solution pH from increasing over time (Dickson and Fisher, 2019), whereas lettuce was shown to be more acidic and required 6.6% of total N as NH_4-N for a stable pH. Balancing the supplied NH_4:NO_3 ratio with the plant species may be a strategy to stabilize pH and reduce the need for acid injection.

Measuring solution EC is a practical method for estimating the total concentration of nutrients in solution and managing nutrient levels. Electrical conductivity sensor technologies are relatively inexpensive and allow for rapid calibration and real-time measurements (Domingues et al., 2012; Filgueiras et al., 2002). Growers typically increase or decrease EC and nutrient levels by adjusting the rate of fertilizer injection into solution. Although practical for measuring total soluble salts, a limitation of EC is the inability to measure individual nutrient concentrations and determine whether certain ions are accumulating or depleting in recirculating solutions (Houston et al., 2021; Miller et al., 2020).

In addition to controlling nutrient levels, growers also manage solution EC also influences the osmotic potential of the root zone and water uptake and is sometimes managed to influence plant growth rates as well as the quality of harvested crops. For example, growers

increase root zone EC to limit water uptake and prevent the soft and lush growth that can occur under low light conditions. In addition, increasing EC and root zone salinity reduces yield in hydroponic tomato, but can increase soluble sugars and other flavor compounds in the fruit (Sonneveld and Voogt, 2009). Sonneveld and Voogt (2009) reported that target solution EC values should be optimized to ensure both sufficient nutrient supply as well as optimal crop quality.

As plant roots absorb water and nutrient during production, nutrient solution must be replenished in hydroponic systems. A common nutrient replenishment strategy for hydroponic leafy greens and herbs is continually refill the hydroponic reservoir with fresh nutrient solution, replacing nutrients and water absorbed by the plants (Bugbee, 2004; Carmassi et al., 2005; Resh, 2013; Sonneveld and Voogt, 2009; Walters and Currey, 2015). With this strategy, target solution EC values are typically maintained by adjustment of the replenishment solution strength. Solution pH is controlled as previously mentioned by the injection of mineral acid (nitric, sulfuric, and phosphoric acids) or base chemicals (potassium carbonate or bicarbonate) or by adjusting the ammonium:nitrate nitrogen (NH_4:NO_3) ratio. Continually refilling the reservoir with fresh solution represents a simple and common method for nutrient replenishment compared to systems with automatic injection of individual fertilizer salts for precise control of ion concentrations.

Replenishing nutrients to maintain a constant solution EC can still result in root zone nutrient deficiencies and toxicities for reduced yield (Bugbee, 2004; Houston et al., 2021; Miller et al., 2020), and is a common problem in commercial hydroponic production of leafy greens and herbs (Resh, 2013). For example, Miller et al. (2020) showed replenishing nutrients and maintaining a constant EC eventually resulted in depletion of N, P, K, and Fe in solution, causing

reduced yield for lettuce grown in recirculating hydroponics. Similarly, Houston and Dickson (2021) also found at a commercial leafy greens operation that the maintenance of a constant EC while replenishing nutrients resulted in N depletion and excessive accumulation of P, Ca, and Mg. In both scenarios, the concentration and ratio of nutrients replenished in solution were not balanced with nutrients taken up by the plants, and the accumulation of Ca and Mg contributed mostly to solution EC and lead to under-replenishment of other fertilizer nutrients.

Periodic discharge and replacement of the nutrient solution is a common method to prevent the development of root zone nutrient imbalances following nutrient replenishment over time (Resh, 2013). However, this practice is wasteful and increases fertilizer and water costs. Current guidelines on the appropriate time and amount of solution to discharge and replace are also very general and depend on complex interactions between factors such plant species nutritional requirements, developmental stage, and climate conditions (Resh, 2013). Alternatively, formulating nutrient replenishment solutions to balance nutrient supply with plant demand is a potential strategy to achieve more "steady state" nutrition. In a "steady state" nutrition model, closed hydroponic systems are maintained in dynamic equilibrium with the needs of the plants; simplifying fertilizer management and minimizing the need to discharge solution (Bugbee, 2004; Langenfeld, 2021; Sonneveld and Voogt, 2009).

Mass balance approach to designing hydroponic solutions

Principles of "nutrient/water balance" or "mass balance" can be used to determine plant nutrient and water requirements and better formulate hydroponic solutions (Bugbee, 2004; Sonneveld, 1999; Sonneveld and Voogt, 2009). Plant nutrient/water requirements determined using these principles involves measuring supplied nutrients and water (nutrient/water inputs),

nutrient uptake and water absorption by the plants, and discarded or drained solution (nutrient/water outputs). Over the whole growing period, the input of nutrients and water should at least meet the requirements of the plants plus nutrients/water drained from the hydroponic system (Sonneveld and Voogt, 2009), which can be calculated as follows:

$$C_s = (W_u C_u + W_d C_d) \div W_s \quad \text{[Eq. 1]}$$

In Eq. 1, W_s is total supplied water ($L \cdot m^{-2}$), W_u is water absorbed by the plants ($L \cdot m^{-2}$), W_d is water drained to waste ($L \cdot m^{-2}$), C_s is nutrient concentration in the supplied solution ($mg \cdot L^{-1}$), C_u is the nutrient uptake concentration ($mg \cdot L^{-1}$), and C_d is the nutrient concentration in the drainage solution ($mg \cdot L^{-1}$). The nutrient uptake concentration (C_u) is a calculated parameter defined below in Eq. 2.

$$C_u = N_r \div W_r \quad \text{[Eq. 2]}$$

In Eq. 2, N_r is the nutrient uptake rate ($mg \cdot d^{-1} \cdot m^{-2}$) and W_r is the water absorption rate ($L \cdot d^{-1} \cdot m^{-2}$), which can be determined either experimentally or in horticultural practice. In closed recirculating systems with no solution loss to drainage, Eq. 1 can be simplified to the following (Sonneveld and Voogt, 2009):

$$C_s = W_u \times C_u \div W_s \quad \text{[Eq. 3]}$$

In principle, an equilibrium develops between nutrient supply and plant uptake in closed systems (Sonneveld, 1999), and input nutrient concentration (C_s) will equal the uptake concentration (C_u) as in Eq. 4, because water supply (W_s) will also equal water absorbed (W_u) by the plants [evaporation is minimal or zero in hydroponic systems, Sonneveld (1999)].

$$C_s = C_u \quad \text{[Eq. 4]}$$

In closed systems, nutrient uptake concentration (C_u) can be measured as changes in hydroponic solution volume and individual nutrient concentrations. An alternative method to

calculate C_u/C_s is from the accumulation of nutrients in plant tissues and WUE, as described by Bugbee (2004) and shown as follows:

$$C_u = C_T \div WUE \quad \text{[Eq. 5]}$$

In Eq. 5, C_T refers to tissue nutrient concentration on a dry mass basis ($mg \cdot kg^{-1}$), and WUE refers to water transpired per unit of plant growth on a dry mass basis ($L \cdot kg^{-1}$). This approach requires the destructive-sampling of plants to measure plant growth as biomass accumulation, and C_T refers to nutrient concentrations of whole-plant samples to account for nutrients absorbed across all plant tissues (leaves, stems, roots, fruits, flowers). In theory, C_s/C_u calculated from Eqs. 3 and 5 should be identical; differences in practice would likely result from the variability between analytical techniques (nutrients measured in tissue versus solution) and in plant growth measurements.

The concept of nutrient uptake concentrations, which can be determined using mass balance principles, is useful to estimate nutrient supply and concentrations in hydroponic solutions (Bugbee, 2004; Sonneveld, 1999; Sonnveld and Voogt, 2009). Growers can determine uptake concentrations by monitoring transpiration rates and nutrients in solution and adjust nutrient supply using Eqs. 1 and 3, particularly for long-term and/or fruiting plants where destructive-sampling reduces yield and is not practical. In addition, C_u parameters for individual macronutrients have been published for a range of hydroponic vegetable and root crops, and are species dependent.

Nutrient uptake concentration determined from the accumulation of nutrients in plant tissues, as in Eq. 5, may be practical for leafy greens and herbs. These crops are grown in mass, regularly harvested at shorter intervals, and consist only of vegetative plant tissues; plants may therefore be sampled more frequently and easily for tissue analysis and biomass determination.

Parameters for C_u have not been published for many common leafy greens and herb crops grown hydroponically.

It is important to note there is no actual physiological linkage between nutrient uptake and water absorption since these are independent processes in plants. Nutrient uptake and water absorption are both strongly correlated with plant growth and yield (Sonneveld, 1999), which is influenced by climate, particularly global radiation and ambient temperature (Schacht and Schenk, 1990; Paz et al., 2019; Resh, 2013; Sonneveld and Voogt, 2009). However, climate factors tend to have a greater impact on transpiration rates and WUE, whereas nutrient uptake per unit of yield is more constant, therefore affecting nutrient uptake concentrations. Nutrient uptake concentrations can be relatively stable in climate-controlled production (i.e., greenhouses and vertical farming operations), but C_u parameters may still differ between geographical locations and seasons (summer versus winter) depending on the plant species (Sonneveld, 1999).

The supply of nutrients in solution can sometimes be influenced by the rate and efficiency at which individual nutrients are taken up (Bugbee, 2004; Ingestad, 1970; Sonneveld, 1999; Voogt, 1992). The relationship between nutrient concentration and uptake by roots varies between nutrient ions. Nutrient ions taken up activity and rapidly in roots (NH_4^+, NO_3^-, $H_2PO_4^-$, K^+) can be therefore supplied at relatively low concentrations in solution. In contrast, nutrient ions taken up passively and at slower rates ($Ca^{2+,}$ Mg^{2+}, SO_4^{2-}) may need supplied at relatively higher concentrations. Low concentrations of NO_3^-, $H_2PO_4^{2-}$, and K^+ measured in the root zone would not necessarily indicate a deficiency in the recirculating solutions, whereas concentrations of Ca^{2+} and Mg^{2+} may need to be greater to ensure adequate uptake.

It is also well-documented plants can take up sufficient nutrients at very low concentrations in the root zone (Clement et al., 1978; Ingestad, 1970; Massey and Winsor, 1980;

Siddiqi et al., 1998; Voogt, 1992; Wild et al., 1987), provided the flow rate of the nutrient solution is high enough to ensure continuous nutrient supply. Achieving an adequate flow rate is often a limiting factor in commercial hydroponic systems (Blok et al., 2017; Sonneveld, 1999), especially substrate systems. Optimal flow rates are more easily achieved in NFT and DFT systems, where nutrient solution constantly flows over plant roots. Increasing the nutrient solution concentration and EC can help compensate for lower flow rates by increasing the quantity of nutrients in the root zone, and for most hydroponic systems, maintaining an adequate EC is critical for optimum crop yield and quality (Sonneveld and Voogt, 2009). Solution EC guidelines have been published for several hydroponic crop species (Pardossi et al, 2011; Sonneveld and Straver, 1994; Sonneveld and Voogt, 2009), although the optimum EC depends on multiple factors including climate, water quality and leaching, production system, and the desired quality of the crop.

The composition of the supplied nutrient solution (nutrient ratios) is sometimes adjusted for crop quality, as discussed previously. However, past research has consistently shown that nutrient solution composition reduces nutrient uptake and growth primarily when nutrients are supplied at low or sub-optimum concentrations (Bugbee, 2004; Carmassi et al., 2005; Sonneveld and Voogt, 2009). With optimum or luxurious supply of nutrients, as is often the case in hydroponic production, nutrient concentrations found in plant tissues are relatively constant over a wide range in solution nutrient concentrations (Carmassi et al., 2005; Sonneveld, 1999), and total nutrient uptake depends mostly on the crop type and overall yield.

Therefore, the composition of the nutrient solution may be formulated similar to the composition of nutrients found in plant tissues to minimize excess supply and accumulation of ions in the root zone as proposed by Bugbee (2004). Eq. 5 can used to determine the nutrient

composition and concentration for a species-specific hydroponic solution. The total supply of nutrients is managed by controlling solution EC to maintain a target level for the crop species, which also helps account for fluctuations in climate, transpiration, and WUE. This species-specific approach is simple in that it assumes a completely closed (no drainage/leaching) recirculating system where all supplied nutrients are either in solution or in plant tissues.

Species-specific hydroponic replenishment solutions

Equation 5 has been proposed as a simple approach for determining the concentration of nutrients needed in a hydroponic replenishment solution to replace nutrients and water absorbed by plants and minimize the accumulation of ions in solution (Bugbee, 2004). To our knowledge, this mass balance method for developing replenishment solutions remains mostly theoretical and has not been widely researched or tested in commercial practice. Also, because plant species differ in WUE and the accumulation of nutrients in plant tissues (Bryson and Mills, 2014; Langenfeld, 2021; Marschner, 2012; Sonneveld and Voogt, 2009), replenishment solutions designed using Eq. 5 would likely need to be species-specific.

As part of this review, we calculated and compared nutrient replenishment solutions determined using Eq. 5 using tissue nutrient, plant growth and plant water use data collected for three leafy greens species during a previous hydroponic study by Dickson and Fisher (2019), but not already published. In this study, seedling transplants of arugula (*Eruca vesicaria* subsp. *sativa* L.), basil (*Ocimum basilicum* L.), and lettuce (*Lactuca sativa* L.) were grown for 35 d in 4.2 L aerated hydroponic culture vessels at five plants per vessel, where each vessel served as one species treatment replicate (n=3). Each vessel contained a half-strength Hoagland's solution at 100 $mg \cdot L^{-1}$ N mixed with reagent-grade salts and de-ionized water, and the solution in each

vessel was completely replaced every 7 d. The experiment was conducted in a controlled-environment growth chamber with cool white fluorescent lighting and a 16-hour photoperiod, with average daily air and solution temperatures (mean ± standard deviation) at 24.1±1.0°C and 24.1±0.9°C, respectively.

After seedlings had acclimated to hydroponic conditions, growth rate was measured over a 6-d period by destructively-sampling two and three plants per replicate at 29 and 35 d, respectively, after which total dry mass gain per plant per replicate was calculated. During the same period, transpiration was measured gravimetrically as the volume of solution depleted per replicate and used to calculate WUE. Percent macronutrient concentrations in plant tissues of whole-plant samples were measured per replicate by oven-drying plants harvested at 35 d, and analyzing dried tissue for N using persulfate digestion (Purcell and King, 1996) and the remaining nutrients using inductively coupled plasma atomic emission spectrophotometry (Quality Analytical Laboratories, Panama City, FL). Percent macronutrient concentrations were converted to $mg \cdot kg^{-1}$ values and used with the calculated WUE data to estimate individual nutrient concentrations in hydroponic replenishment solutions using Eq. 5 for each species.

Analysis of variance was conducted using the GLM procedure in SAS (SAS 9.4: SAS Institute, Cary, NC). Plant species were treated as fixed effects. Replicate (block) was treated as a random effect. Response variables included tissue nutrient concentrations, dry mass gain and transpiration, WUE, and macronutrient concentrations in the calculated replenishment solutions. Any effect found to be significant ($P<0.05$) was investigated further through mean separation using Tukey's honestly significant difference (HSD) multiple comparisons adjustment.

Plant species differed in plant growth, transpiration (plant water use), and WUE over the 6-d measurement period ($P<0.05$; Fig. 1-1). Dry mass gain per replicate was greatest for basil,

lowest for arugula, and intermediate for lettuce (Fig. 1-1B). Fig. 1-1A shows transpiration was also greater for basil and lettuce compared to arugula. There were no statistical differences in WUE values between species (Fig. 1-1C), and all WUE values were within the 200 to 400 $L \cdot kg^{-1}$ dry mass gain range reported by Bugbee (2004) for most crops grown hydroponically. In addition, WUE values were nearly identical for arugula and lettuce at 313.3 and 312.5 $L \cdot kg^{-1}$, respectively. Although water consumption per unit of growth was similar across species in this study when grown under controlled-environment growth chamber conditions, certain crops may be expected to differ in WUE in commercial practice. Langenfeld (2021) reported wheat (*Triticum aestivum*) has a moderate WUE of 3.5 g L^{-1}, lettuce (*Lactuca sativa*) has a lower WUE of 3.3 g L^{-1}, while tomato (*Solanum lycopersicum*) has a higher WUE of 3.8 g L^{-1}.

Species differed in percent nutrient concentrations in dried plant tissues for all macronutrients except phosphorus (Table 1-3). Nutrient concentrations were also within or above the sufficiency ranges recommended by Bryson and Mills (2014) for these species (data not shown), indicating nutrients were supplied in adequate or excess concentrations in the hydroponic solution. Differences in tissue nutrient concentrations were greatest between arugula and lettuce and for Ca (Table 1-3) where tissue Ca was over 2-fold greater for arugula compared to lettuce. Tissue S was also over 5-fold greater in arugula compared to both basil and lettuce. Plant species that are members of the Brassicaceae taxonomic family have been reported to accumulate relatively high concentrations of S (Marschner, 2012), which may explain the greater tissue S levels for arugula in Table 1-3.

Macronutrient concentrations calculated for the species-specific replenishment solutions using Eq. 5 differed between plant species ($P<0.05$, Table 1-4), but only for the nutrients Ca, Mg, and S. Sulfur and Ca concentrations were greatest for arugula (Table 1-4), which followed a

trend similar to that shown in Table 3 where Ca and S were also greatest in tissues. Concentrations for N, P, and K still differed considerably between species despite no statistical differences (Table 1-4). Nutrient concentrations in the species-specific replenishment solutions were greater compared to the supplied Hoagland's solution for N, P, and K, but were lower for Ca and Mg (Table 1-4). The species-specific concentration for S was similar to the amount supplied by the Hoagland's solution for arugula (Table 1-4), whereas calculated S concentrations were lower for basil and lettuce.

Species-specific replenishment solutions were directly compared by standardizing nutrient concentrations to 100 $mg \cdot L^{-1}$ N and evaluating by macronutrient using ANOVA as shown in Table 1-5. When standardized by N, species-specific replenishment solutions had statistically similar Ca and Mg concentrations, but differed in P, K, and S (Table 1-5). Table 1-5 also shows that the concentrations of Ca, Mg, and S supplied by the half-strength Hoagland's solution exceed the concentrations estimated in the species-specific replenishment solutions. Based on results in Table 1-4, the half-strength Hoagland's solution used in this study would be expected to undersupply N, P, and K if used as a replenishment solution for these species. However, Table 1-5 suggests that increasing the strength of the Hoagland's solution to adequately supply N could have the consequence of oversupplying Ca, Mg, and S, resulting in root zone accumulation in closed systems.

Discussion

There are potential benefits to formulating species-specific replenishment solutions for hydroponic leafy greens production. Species-specific replenishment solutions formulated using mass balance principles are designed to help growers achieve a "steady state" nutrition, where

the relationships between nutrient supply, nutrient uptake by plants, and the concentration of nutrients remaining in solution are at equilibrium. Provided the concentrations and ratios of nutrients supplied are balanced with the uptake requirements of the crop, species-specific replenishment solutions would therefore reduce or eliminate the unnecessary accumulation or depletion of ions in the root zone, and the need to periodically discharge and replace the nutrient solution as a management strategy.

Species-specific replenishment solutions would likely improve the common hydroponic management practice of replenishing nutrients to maintain a constant solution EC. As previously mentioned, Miller et al. (2020) and Houston and Dickson (2021) showed the maintenance of a constant solution EC can still result in root zone nutrient imbalances when nutrients in the replenishment solution are not balanced with plant uptake. Combining the use of species-specific replenishment solutions with the common practice of maintaining a target EC may be a simplified strategy for growers to manage nutrients and avoid problems with ion and salt accumulation. Growers would therefore adjust the overall strength of the replenishment solution as needed to control solution EC and meet production goals for plant growth and quality.

It is possible for hydroponic growers to track nutrient and water uptake during production and use mass balance principles to design replenishment solutions specific to their operations using Eqs. 3 and 5. Many growers already work with commercial testing laboratories to analyze nutrient concentrations in plant tissues and in solution (Resh, 2013; Sonneveld and Voogt, 2009). In closed and recirculating hydroponic systems, plant water use can be calculated from the volumes of solution supplied and discharged during production, and measured by installing inline flow meters. For Eq. 5, measurements of plant growth in terms of total dry mass gain is needed for determining WUE, which may be achieved by oven-drying plant material at harvest.

It is likely growers would need to fine-tune the formulation of species-specific replenishment solutions over time to account for fluctuations in nutrient and water uptake, and Bugbee (2004) suggested periodically measuring nutrient concentrations in solution and plant tissue throughout production to better estimate plant nutrient demands over time.

For leafy greens and herbs, it may be possible to estimate total dry mass based on fresh harvested yields and tissue water content. For example, data in Table 1-7 indicate the percent water content of fresh tissue is approximately 91% for basil (9% dry mass) and 95% for lettuce (5% dry mass). Therefore, approximately 9 kg and 5 kg of dry mass may be assumed for every 100 kg of fresh harvested basil and lettuce, respectively. These estimates assume the harvesting of whole plants, whereas in commercial production only the shoot tissues are typically harvested. Therefore, shoot:root ratios may be needed for estimating the total dry mass accumulation from harvested fresh shoot tissue.

A potential limitation of using species-specific replenishment solutions is in production scenarios with multiple plant species. Hydroponic leafy greens and herb species can differ in nutritional requirements, as shown by the differences in tissue nutrient concentrations shown in Table 1-6. It may not be practical or possible to formulate and supply species-specific solutions for every plant species produced. In addition, multiple plant species are sometimes grown in the same hydroponic system and therefore receive the same nutrient solution. One option may be to group species with similar nutritional requirements in the same hydroponic system, and formulate a common replenishment solution that meets the approximate needs of all species. For example, arugula is a member of the Brassicaceae family and has been shown to accumulate greater concentrations of tissue S compared to species such as lettuce (Tables 1-3 and 1-6).

Therefore, a replenishment solution may be formulated with greater S to meet the nutritional requirements for the Brassicas in Table 6 including arugula, bok choi, kale, and mustard greens.

The injection of mineral acids and bases to control solution pH presents a challenge in formulating and using species-specific replenishment solutions, because acid and base injection contributes significantly to the concentration of certain nutrients in solution. Common acids and bases used include nitric acid, phosphoric acid, sulfuric acid, potassium hydroxide, and potassium carbonate, and therefore supply N, P, S, and K. Therefore, the amount of acid/base required in production would need estimated, and the quantities of N, P, S, K subtracted from the replenishment solution. One option is to adjust the NH_4:NO_3 ratio of the replenishment solution to control pH and minimize the need for acid/base injection. However, adjusting NH_4:NO_3 ratio may influence the uptake of other nutrients. As previously mentioned, NH_4 should be limited to 20% or less of the total N supplied as certain plants are susceptible to NH_4 toxicity.

Formulating species-specific replenishment solutions based on the concentration and ratio of nutrients present in plant tissues assumes that plants remove nutrients from the solution in the same ratios that they are supplied. However, excessive or 'luxury' consumption of nutrients by plants is not well understood. Plant tissues with higher concentrations of nutrients than are required by the plant to complete physiological processes may result in the formulation of a replenishment solution that continues to supply those nutrients in excess.

Conclusions

This review outlines the significant amount of variation between published nutrient solution formulations, specifically for leafy green vegetable and herb species. In addition, the current common hydroponic nutrient management strategies lead to an uneven uptake of ions by

plants over time. This uneven removal from solution frequently results in nutritional disorders, making it necessary for nutrient solutions to be replaced. A practical alternative to current management strategies may be formulating replenishment solutions based on mass balance principles. Species-specific replenishment solutions calculated using the accumulation of nutrients in plant tissues may simplify nutrient management and reduce waste caused by frequent solution replacement.

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Tables and Figures

Table 1-1. Survey results of published nutrient solution formulations for hydroponic leafy greens [mean, standard deviation, coefficient of variation]. Sample size indicates the number of published nutrient solution formulations for individual nutrients. Appendix 1 lists the published sources for nutrient concentrations.

Nutrient	Sample size (n)	Mean concentration ($mg \cdot L^{-1}$)	Survey range ($mg \cdot L^{-1}$)	Std. dev.	Coefficient of variation
Nitrogen (N)	38	172.1	47.0 to 283.5	52.4	0.3
Nitrate N (NO_3-N)	38	162.8	47.0 to 266.0	48.4	0.3
Ammonium N (NH_4-N)	38	9.3	0 to 53.0	14.2	1.5
Phosphorus (P)	38	49.9	4.0 to 117.0	23.1	0.5
Potassium (K)	38	244.0	65.0 to 593.0	116.9	0.5
Calcium (Ca)	38	176.0	38.0 to 340.0	67.6	0.4
Magnesium (Mg)	38	59.0	14.0 to 484.0	75.6	1.3
Sulfate sulfur (SO_4-S)	34	116.5	0 to 640.0	118.1	1.0
Iron (Fe)	25	2.5	1.0 to 8.0	1.9	0.7
Manganese (Mn)	27	0.5	0 to 1.7	0.3	0.7
Zinc (Zn)	25	0.2	0 to 0.6	0.1	0.9
Boron (B)	27	0.4	0.1 to 1.0	0.2	0.5
Copper (Cu)	24	0.0	0 to 0.2	0.0	0.8
Molybdenum (Mo)	23	0.1	0 to 2.5	0.5	3.6
Electrical conductivity[x]	38	2.0	0.9 to 5.8	0.8	0.4

[x]Electrical conductivity values for each published nutrient formulation were calculated from the milli-equivalents of cation and anion nutrients supplied in solution using (Sonneveld, 1999 Eq. 1a).

Table 1-2. Ammonium:total nitrogen (NH_4:N), potassium:nitrogen (K:N), potassium:calcium (K:Ca), calcium:magnesium (Ca:Mg), and iron:manganese (Fe:Mn) ratios determined from survey results for published nutrient solutions for hydroponic leafy greens [mean, standard deviation, coefficient of variation]. Sample size indicates the number of published nutrient solution formulations for individual nutrients. Appendix 1 lists the published sources for nutrient concentrations.

Nutrient	Sample size (n)[z]	Mean[y]	Survey range	Std. dev.	Coefficient of variation
NH_4:N	38	<0.05	0 to 0.2	0.1	1.5
K:N	38	1.5	0.4 to 4.3	0.9	0.6
K:Ca	38	1.6	0.3 to 5.1	0.9	0.6
Ca:Mg	38	4.1	0.4 to 10.2	2.0	0.5
Fe:Mn	26	5.7	0 to 32.0	6.5	1.2

[z]Appendix 1 lists the published sources for nutrient concentrations.
[y]Ratio values indicate proportion data. For example, a value of 5.65 would indicate 5.65 to 1.

Table 1-3. Plant species effects on macronutrient percentages in whole-plant tissue samples. Macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Data represent least-square means of three replicates per species. Means separation used Tukey's honestly significant difference (HSD) at α=0.05.

	Percent macronutrients in dried plant tissues					
Species	N	P	K	Ca	Mg	S
	%					
Arugula	5.7 a	0.6 a	4.7 b	2.6 a	0.3 ab	1.2 a
Basil	4.3 ab	0.8 a	5.0 ab	1.7 ab	0.4 a	0.2 b
Lettuce	4.1 b	0.7 a	6.1 a	1.2 b	0.3 b	0.2 b
Significance[x]	*	NS	*	*	*	**

[x]NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

[y]Tissue percentages can be converted to ppm ($mg \cdot kg^{-1}$) by multiplying percentage values by 10,000.

Table 1-4. Plant species effects on individual macronutrient uptake concentration for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Data represent least-square means of 12 replicates for each species. Letters indicate mean separation using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$. Macronutrient concentrations supplied in 0.5× Hoagland's solution are listed for comparison.

	Nutrient uptake concentration					
Species	N	P	K	Ca	Mg	S
	$mg \cdot L^{-1}$					
Arugula	186.6 a	19.6 a	150.3 a	84.4 a	11.2 ab	37.8 a
Basil	167.4 a	31.8 a	194.6 a	62.9 ab	14.3 a	6.3 b
Lettuce	139.9 a	24.4 a	208.4 a	39.0 b	8.9 b	5.1 b
0.5× Hoagland's solution	100	16	117	100	24	38
Species effects[x]	NS	NS	NS	*	*	***

[x]NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 1-5. Individual macronutrient uptake concentrations for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate sulfur (SO_4-S) when uptake concentrations were standardized to 100 mg·L^{-1} nitrogen (N). Data represent least-square means of 12 replicates per species. Nutrient concentrations supplied in a 0.5× Hoagland's solution are included for comparison. Letters indicate mean separation using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$.

Species	Macronutrient uptake concentration					
	N	P	K	Ca	Mg	SO_4-S
	mg·L^{-1}					
Arugula	100	10.8 b	82.5 b	46.2 a	6.1 a	20.7 a
Basil	100	19.6 a	122.3 ab	41.4 a	9.2 a	3.8 b
Lettuce	100	18.0 a	149.2 a	28.0 a	6.6 a	3.7 b
0.5× Hoagland's solution	100	16	117	100	24	38
Species effects[x]	–[y]	*	*	NS	NS	***

[x]NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.
[y]Species comparisons were not made because solutions were standardized by N.

Table 1-6. Recommended macronutrient leaf tissue concentrations (% of dry weight) for a range of leafy greens and herbs species commonly grown in hydroponic and greenhouse production. Macronutrients included nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Data adapted from Bryson et al. (2014). Data represent average tissue concentrations from 10 to 50 newly emerged leaf samples.

Species	Scientific name	N	P	K	Ca	Mg	S	Production system[z]
		%						
Arugula	*Eruca sativa*	3.4	0.7	5.0	2.4	0.3	0.5	Field
Basil	*Ocimum basilicum (L.)*	5.0	0.8	1.9	1.7	0.8	0.4	Field and garden
Bok choi	*Brassica rapa*	4.0	0.6	4.3	2.3	0.3	0.6	Field
Celery	*Apium graveolens*	1.6	0.5	6.9	0.9	0.5	0.2	Greenhouse
Cilantro	*Cariandrum sativum*	5.0	0.6	4.4	1.1	0.6	0.3	Field and garden
Dill	*Amethrum graveoleus*	5.0	0.4	4.3	1.7	0.3	0.4	Field and garden
Endive	*Cichorium endiva*	3.2	0.3	5.2	1.4	0.3	0.3	Greenhouse
Kale	*Brassica oleracea*	4.3	0.5	3.0	1.9	0.5	0.3	Field
Lettuce	*Lactuca sativa*	4.9	0.7	10.8	1.0	0.5	0.3	Greenhouse
Mint	*Mentha spicata*	3.9	0.3	3.0	0.9	0.6	0.3	Field and garden
Mustard greens	*Brassica juncea*	3.4	0.5	3.8	2.0	0.3	0.6	Field
Oregano	*Origanum vulgare*	3.1	0.2	2.6	0.7	0.5	0.2	Field and garden
Parsley	*Petroselinum crispum*	4.5	0.3	4.0	0.9	0.5	0.2	Field and garden
Rosemary	*Rosmarinus officinalis*	2.3	0.3	2.5	0.6	0.3	0.3	Greenhouse, container
Spinach	*Spinacia oleracea*	4.7	0.6	5.0	1.9	0.6	0.3	Greenhouse
Watercress	*Nasturtium officinale*	5.1	1.0	6.0	1.5	1.5	0.4	Field

[z]Refers to whether leaf tissue samples were collected from field, garden, greenhouse, and container production systems.

Table 1-7. Survey results of published data (mean, standard deviation, coefficient of variation) on total fresh mass, dry mass, percent dry mas, and percent water mass for basil and lettuce. Sample size indicates the number of published biomass data for each species.

Species		Sample size (n)	Mean	Std. dev.	Coefficient of Variation
Basil	Fresh mass	54	77.00	108.39	1.41
	Dry mass	52	7.35	10.43	1.42
	% dry mass	54	0.09	0.02	0.21
	% water mass	54	0.91	0.02	0.02
Lettuce	Fresh mass	34	100.65	69.52	0.69
	Dry mass	32	4.69	2.83	0.60
	% dry mass	32	0.05	0.01	0.27
	% water mass	32	0.95	0.01	0.01

Data from Anderson et al. (2017), Bufalo et al. (2015), Delaide et al. (2016), Ding et al. (2012), Gent (2014), Karimaei et al. (2004), Kiferle et al. (2013), Maggio et al. (2006), Singh et al. (2019), Solis-Toapanta et al. (2020), Walters and Currey (2015), and Yang and Kim (2020)

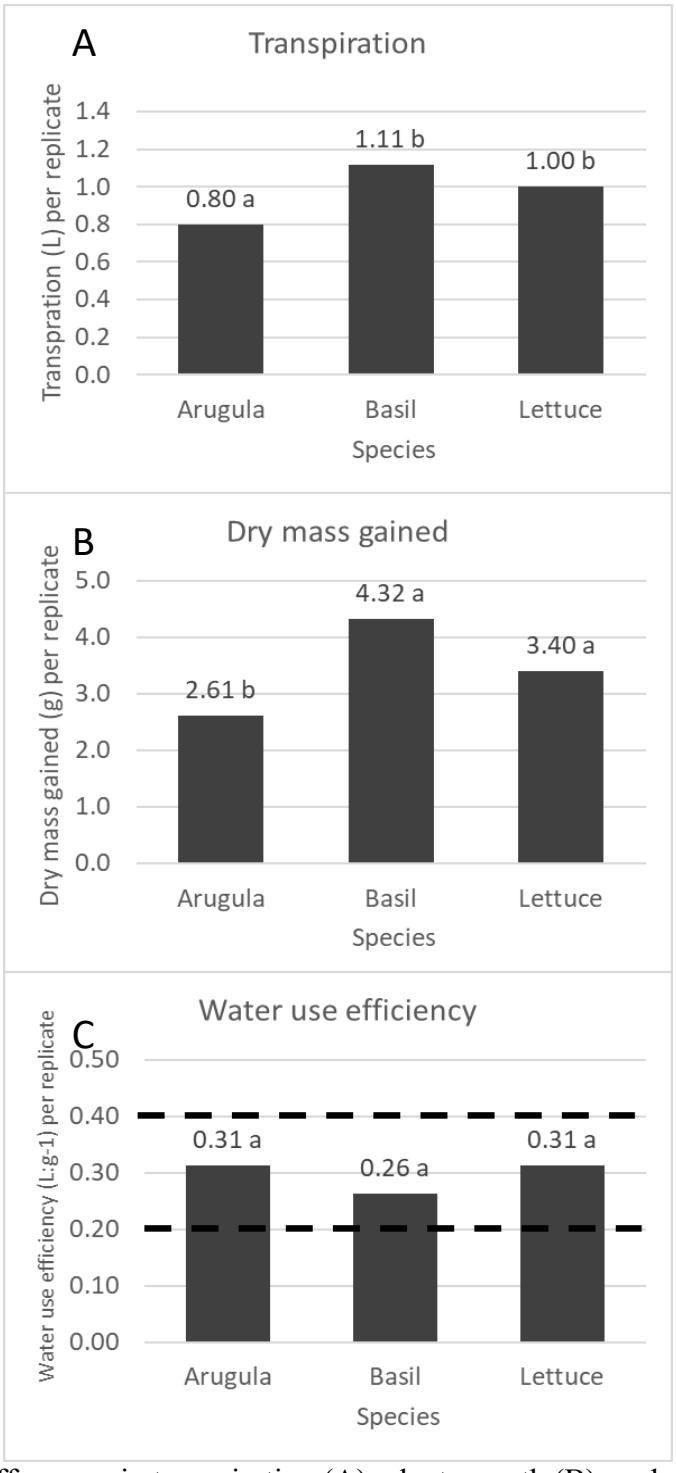

Figure 1-1. Species differences in transpiration (A), plant growth (B), and water use efficiency (C) when grown hydroponically for 6 d. Data represent least-square means of three replicates per treatment with means separation using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection. Horizontal dashed bars represent a typical range of water use efficiency for many hydroponically-grown crops according to Bugbee (2004).

Appendix

Published sources of survey results for individual nutrients in nutrient solution formulations recommended for hydroponic leafy greens.

Source	Listed nutrients
Arizona CEAC	N, NO3, NH4, P, K, Ca, Mg, Fe, Mn, Zn, B, Cu, Mo
Jack's Hydro-feed (16-4-17)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Jacks hydroponic (5-12-26)+CaNO3	N, NO3, NH4, P, K, Ca, Mg, Fe, Mn, Zn, B, Cu, Mo
Modified Sonnveld	N, NO3, NH4, P, K, Ca, Mg, Fe, Mn, Zn, B, Cu, Mo
Hoagland & Arnon (1938)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Hewitt (1966)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Steiner (1984)	N, NO3, NH4, P, K, Ca, Mg, S, Mn, Zn, B, Cu
Knopp (1865)	N, NO3, NH4, P, K, Ca, Mg, S
Hoagland (1919)	N, NO3, NH4, P, K, Ca, Mg, S
Jones & Shive (1921)	N, NO3, NH4, P, K, Ca, Mg, S, Fe
Rothamsted	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, B
Hoagland & Snyder (1993,1938)	N, NO3, NH4, P, K, Ca, Mg, S, Mn, Zn, B, Cu, Mo
Hoagland & Arnon (1938)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Eaton	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, B
Shive & Robbins (1942)	N, NO3, NH4, P, K, Ca, Mg, S, Mn, Zn, B
Robbins (1946)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
White (1943)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Duclos (1957)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
A.J. Abbott	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
E.B. Kidson	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Schwartz (Israel)	N, NO3, NH4, P, K, Ca, Mg, S
Schwartz (California)	N, NO3, NH4, P, K, Ca, Mg, S
Schwartz (New Jersery)	N, NO3, NH4, P, K, Ca, Mg, S
Schwartz (South Africa)	N, NO3, NH4, P, K, Ca, Mg
Saanichton	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
B.C. Canada	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Dr. Pilgrim	N, NO3, NH4, P, K, Ca, Mg, S
Elizabeth	N, NO3, P, K, Ca, Mg, S

N.C., USA	N, NO3, NH4, P, K, Ca, Mg, S
Dr. H.M. Resh	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Dr. H.M. Resh Tropical-Dry	N, NO3, NH4, P, K, ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Dr. H.M. Resh Tropical-Wet Lettuce	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B Cu, Mo
Dr. H.M. Resh Lettuce Florida (1989)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Shive (1915)	N, NO3, NH4, P, K, Ca, Mg, S
Sonneveld and Straver (1994)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Sonneveld and Straver (1994)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Sonneveld and Straver (1994)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo
Sonneveld and Straver (1994)	N, NO3, NH4, P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, Mo

*Nutrient solution recipes sourced from (Mattson and Peters, 2014) and (Resh, 2013)

CHAPTER 2 . EFFECTS OF REPLENISHMENT STRATEGY ON NUTRIENT UPTAKE AND GROWTH OF HYDROPONIC ARUGULA AND BASIL

Abstract

The objective of this study was to evaluate the effects of periodic nutrient replenishment on nutrient uptake and recovery with arugula (*Eruca sativa* L.) and basil (*Ocimum basilicum* L.) grown in small-scale hydroponic systems. Over 28 d, arugula and basil were grown hydroponically and treated with one of two nutrient replenishment strategies. The first replenishment strategy (RS1) consisted of topping off the hydroponic solution every 7 d with a complete water-soluble fertilizer and resupplying nutrients at 100 mg·L^{-1} nitrogen (N), similar to recommended commercial guidelines for hydroponic leafy greens and herbs. The second nutrient replenishment strategy (RS2) consisted of supplying all nutrients at the start of the experiment—in an equivalent amount to RS1—and topping off the solution with de-ionized water every 7 d. Replenishment strategy had no effect on plant growth or accumulation of nutrients in plant tissues for either species at 28 d. However, species differed in uptake of all individual macronutrients. This study emphasized plants can take up nutrients adequately over a wide range of concentrations in the hydroponic solution, and frequent replenishment of nutrients in relatively small quantities resulted in quality plant growth and performance.

Introduction

Achieving “steady-state” nutrition is desired in closed recirculating hydroponic systems to prevent nutrient imbalances in the root zone and the need to discharge and replace solution (Bugbee, 2004; Langenfeld, 2021). Steady-state nutrition implies the supply of nutrients in the

hydroponic solution are in equilibrium with plant uptake, and as a result, optimum plant growth is maintained without excessive ion depletion or accumulation. Formulating nutrient replenishment solutions to match nutrient supply with plant uptake demands can be achieved using mass balance principles (Bugbee, 2004; Langenfeld, 2021; Sonneveld and Voogt, 2009). A mass balance approach to nutrient management in recirculating hydroponic systems assumes all supplied nutrients are either in solution or plant tissues (Bugbee, 2004). Nutrient concentrations needed in a balanced replenishment solution can be determined experimentally or in commercial practice by measuring nutrient and water uptake during production (Bugbee, 2004; Sonneveld and Voogt, 2009).

Nutrients supplied in hydroponic solutions can be impacted by the ability for plant roots to absorb and recover nutrients from solution (Bugbee, 2004). Bugbee (2004) reported the recovery of macronutrients can range from 50% to 85% (of total nutrients supplied) in hydroponic systems, and additional macronutrients may be needed in solution to ensure adequate uptake. In contrast, recovery of certain micronutrients can be over 100% of the amount supplied, particularly zinc and copper which can leach from plastics and metal components used to build hydroponic systems. Few detailed studies have been conducted on nutrient recovery in hydroponics and the potential impacts on formulating replenishment solutions (Bugbee, 2004). Most studies have evaluated nutrient recovery by supplying an initial nutrient charge and measuring the depletion of nutrients from solution, whereas in hydroponic production nutrients are replenished periodically over time.

The objective of this study was to evaluate the effects of periodic nutrient replenishment on nutrient uptake and recovery with arugula (*Eruca sativa* L.) and basil (*Ocimum basilicum* L.) grown in small-scale hydroponic systems. Arugula and basil were grown hydroponically for 28 d

and received two nutrient replenishment strategies. The first replenishment strategy consisted of topping off the hydroponic solution every 7 d with a complete water-soluble fertilizer and resupplying nutrients at 100 $mg \cdot L^{-1}$ nitrogen (N), similar to recommended commercial guidelines for hydroponic leafy greens and herbs (Resh, 2012). The second strategy consisted of supplying all nutrients at the start of the experiment—in an equivalent amount to the first strategy—and topping off the solution with de-ionized water every 7 d. We hypothesized that nutrient uptake and recovery would differ between species, but would not be affected by nutrient replenishment strategy.

Materials and Methods

A factorial experiment was conducted using a randomized complete block design with plant species (arugula, basil) and nutrient replenishment strategy (two strategies) as factors. The experiment was conducted in a polycarbonate controlled-environment greenhouse located at the University of Arkansas in Fayetteville, AR (36.0764° N, 94.1608° W). Average daily temperature over the course of the experiment was (mean ± standard deviation) 25.6±1.6°C, and daily light integral was 15.8±6.9 $mol \cdot m^{-2} \cdot d^{-1}$ of photosynthetically active radiation. Hydroponic solutions were formulated using a 2-part hydroponic recipe consisting of equal parts commercial-grade calcium nitrate (Haifa, Matam-Haifa, Israel) and a 5N-4.8P-21.6K (JR Peters, Allentown PA, United States) water-soluble fertilizer mixed in de-ionized water. When standardized to 100 $mg \cdot L^{-1}$ N, the remaining nutrient concentrations (in $mg \cdot L^{-1}$) were 24 phosphorus (P), 108 potassium (K), 95 calcium (Ca), 30 magnesium (Mg), and 40 sulfur (S). Micronutrients concentrations (in $mg \cdot L^{-1}$) were 1.5 iron (Fe), 0.2 manganese (Mn), 0.1 copper (Cu), 0.5 molybdenum (Mo), 0.1 zinc (Zn), and 0.2 boron (B).

Arugula seed and pelleted compact basil seed were sown in 162-cell rockwool sheets (A/O sheets, Grodan, The Netherlands) at one seed or pellet per cell and germinated in the greenhouse. Rockwool sheets were sub-irrigated with 150 mg·L^{-1} N from a 17N-1.3P-14K (JR Peters; Allenstown, PA) complete water-soluble fertilizer mixed in tap water. Pelleted basil seed contained multiple seeds per pellet and were thinned to one seedling per cell upon the emergence of first true leaves. Seedlings of each species were then transplanted into hydroponic culture vessels at three plants per vessel.

Hydroponic culture vessels were designed following methods described by Dickson et al. (2016). Each hydroponic culture vessel was a 4.5 L white plastic container with a snap-on plastic lid. Each seedling stem was wrapped with a neoprene collar (5 cm diameter), which fit into a black plastic hydroponic net pot (5 cm diameter). Net pots were supported in circular holes cut into the container lid, which allowed roots to be submerged in nutrient solution. The neoprene collar reduced evaporation of the nutrient solution without constricting plant stems. A plastic air tube was inserted into the nutrient solution via a hole in the bucket lid, connected to an aquarium tank air pump, which constantly aerated the nutrient solution. Culture vessels were wrapped in aluminum foil to reduce light transmission to the nutrient solution, prevent algae growth, and to help stabilize solution temperature. Each culture vessel initially contained three plants of either arugula or compact basil. Each culture vessel held 4.0 L nutrient solution. After transplanting into hydroponic culture vessels, all plants received nutrient solution with 100 mg·L^{-1} N and were acclimated to hydroponic conditions for 19 d.

The experiment started on 24 Mar 2020 with the replacement of solution in each culture vessel and initiation of the nutrient replenishment strategies for each species. The first replenishment strategy (RS1) consisted of initially supplying 400 mg·L^{-1} N with no resupply of

nutrients. The second replenishment strategy (RS2) consisted of initially supplying 100 $mg \cdot L^{-1}$ N, and dosing every 7 d with concentrated nutrient solution to resupply nutrients at a rate of 100 $mg \cdot L^{-1}$ N for the remainder of the experiment. De-ionized water was added back to each culture vessel every 2-3 d to replace the solution lost to evapotranspiration. Both RS1 and RS2 supplied an equivalent amount of nutrients and 1.6 g of N over the 28 d experimental period to each culture vessel. Initial solution pH was adjusted to 6. Solution pH was monitored every 2-3 d and maintained between pH 5.5 and 6 using HCl and NaOH at 0.1 *N*.

Treatments consisted of two plant species (arugula, basil) and two replenishment strategies (RS1, RS2) for a total of four treatment combinations arranged using a randomized complete block design with three blocks. Each hydroponic culture vessel was used as one treatment replicate and experimental unit. Replicates (culture vessels) were placed on a greenhouse bench on 1-ft center spacing.

Initial data collection consisted of destructively sampling one plant from every experimental unit, leaving two plants per culture vessel at the start of the experiment (day 0). All treatment combinations were destructively-sampled for data collection 28 d after the start of the experiment.

Data collected at 0 and 28 d on each treatment replicate included leaf SPAD chlorophyll content, shoot and root fresh mass per plant, shoot and root dry mass per plant, solution EC, total tissue (combined roots and shoots) and solution macronutrient concentrations. The mass of accumulated nutrients per plant were calculated from tissue nutrient concentrations and dry mass measurements.

Leaf SPAD chlorophyll content was measured by taking the average of 6 readings per replicate using a Chlorophyll Meter SPAD-502 Plus (Konica Minolta). Fresh mass of shoot and

roots was taken by cutting plant stems at the top of the neoprene collar and trimming the roots away from the rockwool substrate. Shoot cuttings were then weighed immediately after being cut while roots were washed in a dilute acid solution (0.05% HCl) and allowed to air dry before being weighed. After fresh mass had been recorded, plants were then oven dried (60°C) for 72 hr for dry mass determination. Dried tissue samples were then analyzed for macronutrient concentrations. Total N was measured using persulfate digestion (Purcell and King, 1996), and the remaining macronutrients were measured using inductively-coupled plasma atomic emission spectrophotometry (ICP-AES) at the Fayetteville Agricultural Diagnostic Lab (University of Arkansas, Fayetteville AR).

The experiment was designed to be a factorial experiment and was conducted using a randomized complete block design. Analysis of variance was conducted using the PROC GLM procedure in SAS (SAS 9.4: SAS Institute, Cary, NC). Plant species, replenishment strategy and the interaction of plant species with replenishment strategy were treated as fixed effects. Replicate effects were treated as a random effect. Response variables included root and shoot mass, solution and tissue nutrients, and nutrient uptake per plant at 28 d. Any effect found to be significant ($P<0.05$) was investigated further through mean separation using Tukey's honestly significant difference (HSD) multiple comparisons adjustment.

Results and Discussion

Plant species had main effects on fresh and dry biomass accumulation, but there was no significant effect from the replenishment strategy ($P = 0.8050$) or the interaction ($P= 0.9534$). Therefore, means are only reported for the main effect of species in Table 2-1. Total fresh and dry mass per plant was greater for arugula compared to basil at 28 d (Table 2-1).

Leaf SPAD chlorophyll content was not influenced by plant species or replenishment strategy (data not shown), and all plants appeared healthy with no visual symptoms of leaf chlorosis or nutrient deficiency at the end of the experiment. Leaf SPAD values were >35 across all treatment replicates, indicating a dark green foliage color.

Solution EC at 28 d was influenced by species but not by replenishment strategy or the interaction (Table 2-2). Initial solution EC was 3.98 and 1.25 $mS \cdot cm^{-1}$ for RS1 and RS2, respectively, and averaged 1.44 $mS \cdot cm^{-1}$ between replenishment strategies at the end of the experiment. Final solution EC was 0.80 and 2.08 $mS \cdot cm^{-1}$ for arugula and basil, respectively (Table 2-2). The decrease in EC over time for arugula indicated overall nutrient supply and replenishment was lower than nutrient uptake by arugula. In contrast, the increase in EC for basil suggested overall nutrient supply was greater than nutrient uptake.

Plant species significantly influenced the concentration of all nutrients remaining in solution at 28 d (Table 2-2), with the exception of solution K. At 28 d, there were only species differences in solution NO_3-N, P, Ca, Mg, and SO_4-S (Table 2-2), where nutrient concentrations were greater for basil compared to arugula Since macronutrients were supplied in equal amounts between treatments, the similar concentrations of K in solution suggest similar uptake of K between species. Similar uptake of K for basil, despite the lower uptake of other nutrients compared to arugula, might suggest a “luxury consumption” of K which has been reported for certain crop species (Marschner, 2012; van Iersel, 1999).

At 28 d, species significantly differed in percent concentrations of tissue nutrients (Table 2-3), and replenishment strategy only had an effect on Ca concentration with no interactions. Overall, basil resulted in greater concentrations of N, P, and K compared to arugula, whereas arugula resulted in greater concentrations of Ca, Mg, and S (Table 2-3), and tissue Ca

concentrations were slightly greater for RS2 compared to RS1. Overall, plants showed no symptoms of nutrient deficiency, and tissue nutrient concentrations in Table 2-3 remained within acceptable ranges published for arugula and basil by Bryson et al. (2014).

Total accumulation of N, Ca, Mg, and S per plant differed between plant species (Table 2-4), but was not influenced by replenishment strategy or the interaction. However, the total uptake of P and K per plant was not statistically different between arugula and basil at 28 d (Table 2-4). In general, arugula took up greater quantities of individual nutrients compared to basil, likely because of greater growth and dry mass accumulation per plant as shown in Table 2-1. The greatest differences in uptake between species occurred for N, Ca, and S, whereas uptake of P, K and Mg was more similar (Table 2-4).

The percent uptake of individual macronutrients was calculated by dividing the average mg of nutrients recovered for each species and nutrient in Table 2-4 by the total amount of each nutrient supplied. For arugula, percent nutrient uptake was 89.4% for N, 56.8% for P, 87.7% for K, 64.4% for Ca, 57.2% for Mg, and 71.0% for S. For basil, percent uptake was 52.7% for N, 44.6% for P, 72.6% for K, 20.9% for Ca, 19.3% for Mg, and 11.1% for S. Percent uptake of individual nutrients for arugula were similar to the nutrient recovery values reported by Bugbee (2004), but were lower for basil. However, there was no indication of nutrient deficiency, and all nutrients were supplied in adequate or luxurious amounts in this study, and therefore recovery values would likely have been lower compared to nutrient-limiting conditions. Overall, nutrient replenishment strategy had no effect on the uptake and recovery of nutrients in this experiment.

Conclusions

This study confirmed the periodic replenishment of nutrients in closed hydroponic systems did not impact overall nutrient uptake and recovery for arugula and basil grown for a typical crop period (28 d) with commercially recommended nutrient rates. It also emphasized the rate of nutrient uptake remains relatively constant and adequate provided nutrients are supplied within an optimal to luxurious concentration in solution. Based on the results of this study and others, nutrient replenishment strategies are expected to minimally impact the formulation of hydroponic solutions. However, species differed considerably in nutrient uptake requirements, and would therefore influence the formulation of nutrient replenishment solutions using mass balance principles.

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Tables and Figures

Table 2-1. Species (arugula, basil) main effects on final fresh and dry mass (g). Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level. Data represent the least square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Main Effects	Fresh Mass	Dry Mass
	g	
Arugula	181.0 a	25.2 a
Basil	108.4 b	9.8 b
Species[x]	*	**

[x] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 2-2. Species (arugula, basil) and replenishment strategy effects on final electrical conductivity and concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate sulfur (SO_4-S) in solution at 28 d. Replenishment strategy 1 and 2 are denoted as RS1 and RS2, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level. Data represent the least square means of three treatment replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Main Effects	EC	NO_3-N	P	K	Ca	Mg	SO_4-S
	$mS \cdot cm^{-1}$	$mg \cdot L^{-1}$					
Arugula	0.80 b	8.2 b	25.0 b	57.7 a	115.7 b	61.1 b	35.0 b
Basil	2.08 a	168.6 a	49.0 a	89.2 a	301.0 a	101.1 a	131.2 a
RS1	1.37 a	94.3 a	37.8 a	72.9 a	215.1 a	81.2 a	82.5 a
RS2	1.52 a	82.5 a	36.2 a	74.0 a	201.6 a	80.9 a	83.7 a
Species[x]	*	**	**	NS	**	*	*
Replenishment Strategy	NS	NS	NS	NS	NS	NS	NS

[x] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 2-3. Species and replenishment strategy main effects on percent nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) in plant tissues at 28 d. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level. Data represent the least square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Main Effects	N	P	K	Ca	Mg	S
	%					
Arugula	3.1 b	0.48 b	3.3 b	2.1 a	0.6 a	0.9 a
Basil	4.4 a	0.89 a	6.5 a	1.6 b	0.5 b	0.4 b
RS1	3.7 a	0.64 a	5.0 a	1.8 b	0.5 a	0.7 a
RS2	3.8 a	0.73 a	4.8 a	1.9 a	0.5 a	0.7 a
Species[x]	**	**	**	**	**	**
Replenishment Strategy	NS	NS	NS	**	NS	NS

[x] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 2-4. Species main effects on total uptake of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) (mg) by plant tissues. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level. Data represent the least square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Main Effects	N	P	K	Ca	Mg	S
	mg					
Arugula	1429.7 a	218.1 a	1515.7 a	979.4 a	274.7 a	454.2 a
Basil	843.3 b	171.4 a	1254.5 a	316.9 b	92.8 b	71.1 b
Species[x]	*	NS	NS	**	**	**

[x] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively

CHAPTER 3 . EVALUATING THE EFFECTS OF SPECIES-SPECIFIC REPLENISHMENT SOLUTIONS ON PLANT GROWTH AND NUTRIENT UPTAKE

Abstract

Nutrients taken up by plants and depleted from recirculating hydroponic solutions are often replaced by replenishing the recirculating nutrient solution in the reservoir with fresh solution. If nutrients in the replenishment solution are oversupplied, ions accumulate in solution and can lead to nutritional imbalances. This study was designed to evaluate the potential of designing replenishment solutions based on accumulated nutrients in plant tissues in order to balance the replacement of nutrients with plant uptake. Species-specific replenishment solutions were formulated for arugula (*Eruca sativa* L.) and basil (*Ocimum basilicum* L.), where the ratio of macronutrients in solution matched the ratio in dried tissues harvested from each species grown hydroponically. Macronutrient concentrations for the arugula replenishment solution were (in $mg \cdot L^{-1}$) 150N, 13P, 94K, 60Ca, 12Mg, and 22S. Basil concentrations were 150N, 23P, 131K, 44Ca, 10Mg, and 7S. In a greenhouse experiment, arugula and basil were grown for 21 days in 4 L hydroponic culture vessels where the nutrient solution taken up was supplemented with replenishment solution treatments every 3 days. Treatments consisted of refilling the culture vessels with either the species-specific solution or a 20.5N-4.8P-21.6K complete fertilizer solution as a control, mixed at 130 $mg \cdot L^{-1}$ N in deionized water. Electrical conductivity (EC) and replaced solution volume were measured every 7 days. Final data collected included shoot and root mass, shoot and root tissue nutrients, and individual nutrients remaining in solution. During the experiment, there were no significant differences in plant mass, transpiration, water use efficiency (WUE), or nutrient accumulation in plant tissues for either species. In the nutrient

solution, EC increased in the standardized solution and decreased in the species-specific replenishment solution for both species. EC increase in control solutions was caused by the accumulation of calcium, magnesium, and sulfate supplied in excess. Solution N decreased for arugula and basil in both replenishment solutions. This is due to plants removing N from solution more rapidly than it was supplied; however, there were no symptoms of nutrient deficiency and tissue nutrients remained within recommended ranges for optimal growth. Hydroponic growers can formulate species-specific replenishment solutions based on the accumulation of nutrients in harvested plant tissues. This strategy can minimize ion accumulation and the need to periodically dump and replace recirculated solutions to avoid nutritional problems.

Introduction

Developing species-specific replenishment solutions could be an alternative to using generalized nutrient solution formulations. Past research has often used mass balance principles to quantify nutrient and water uptake during nutritional and plant growth studies (Bugbee, 2004; Carmassi et al., 2005; Dickson et al., 2016; Langenfeld, 2021; Pardossi et al., 2011). A mass balance approach may be used to adjust nutrient management in commercial practice. Bugbee (2004) has proposed using a mass balance approach formulated for individual crops based on nutrients accumulated in plant tissue and water use efficiency (WUE). A mass balance approach may allow replenishment solutions to maintain stead-state nutrition, prevent nutritional disorders, and reduce the need to discharge solution. Additionally, if the composition of nutrient solutions were tailored to plant demand, growers may be able to more accurately manage solution based on EC.

The objective of this experiment was to evaluate the effects of species-specific replenishment solutions developed using the principles of mass balance on plant growth and nutrient uptake. Two crop species were treated with either a species-specific replenishment solution or a standardized solution and were evaluated for plant mass, accumulation of individual nutrients in plant tissues, and the change in nutrient concentration in the nutrient solution. We hypothesized that replicates treated with the species-specific replenishment solutions would exhibit similar growth and tissue nutrient accumulation when compared to replicates treated with a standardized solution. We also hypothesized that replicates receiving the standardized solution would have an accumulation of nutrients in the nutrient solution, resulting in a greater solution EC.

Materials and Methods

The experiment was a two-factor experiment with a randomized complete block design, with nutrient replenishment solution formulation and plant species (arugula, basil) as the two factors. The experiment was designed to evaluate the effects of species-specific nutrient replenishment solutions on plant growth, nutrient uptake, and the accumulation/depletion of nutrient in solution compared to replenishing with a commercially standard hydroponic solution. The experiment was conducted in a controlled environment greenhouse at the University of Arkansas in Fayetteville, AR (36.0764° N, 94.1608° W). The average daily temperature (ADT) over the course of the experiment was 23.3 ± 0.8 °C and the average daily light integral (DLI) was 9.38 ± 2.4 mol·m^2·day.

On 22 Sep 2020, arugula (*Eruca sativa*) and pelleted basil (*Ocimum basilicum*) seed were sown in 200-cell rockwool sheets (A/O sheets, Grodan, The Netherlands) at 1 seed or pellet per

cell and germinated in the greenhouse. Rockwool sheets were sub-irrigated with a commercial 17N-1.3P-14K (JR Peters; Allenstown, PA) water-soluble fertilizer solution at 150 $mg \cdot L^{-1}$ N mixed in tap water. Pelleted basil seed contained multiple seeds per pellet, and were thinned to one seedling per cell after the emergence of first true leaves. Sixteen seedlings of each species were transplanted into hydroponic culture vessels on 15 Oct 2020 at two plants per system. Each hydroponic culture vessel was used as one treatment replicate and experimental unit.

Hydroponic culture vessels were designed following methods described by Dickson et al. (2016). Each hydroponic culture vessel was a 4.5 L white plastic container with a snap-on plastic lid. Each seedling stem was wrapped with a neoprene collar (5 cm diameter), which fit into a black plastic hydroponic net pot (5 cm diameter). Net pots were supported in circular holes cut into the container lid, which allowed roots to be submerged in nutrient solution. The neoprene collar reduced evaporation of the nutrient solution without constricting plant stems. A plastic air tube was inserted into the nutrient solution via a hole in the bucket lid, connected to an aquarium tank air pump, which constantly aerated the nutrient solution. Culture vessels were wrapped in aluminum foil to reduce light transmission to the nutrient solution, prevent algae growth, and to help stabilize solution temperature. Each culture vessel contained two plants of either arugula or basil and held 4.0 L nutrient solution.

After transplant into the hydroponic culture vessels, all plants received 4 L of a standard hydroponic nutrient solution supplied at 100 $mg \cdot L^{-1}$ N mixed using equal parts of a 5N-4.8P-21.6K (JR Peters; Allenstown, PA) water-soluble fertilizer and commercial-grade calcium nitrate. Plants were acclimated to hydroponic systems and grown for 15 d (15 Oct 2020 through 30 Oct 2020) prior to the start of the experiment. During this time, basil plants were pinched at 2 nodes to encourage branching.

The experiment began on 30 Oct 2020 with the replacement of the standard hydroponic solution in each culture vessel with 4 L of fresh solution at 130 $mg \cdot L^{-1}$ N. For each species, treatments differed only in the formulation of solution used to replenish nutrients in the culture vessels over time and replace solution absorbed by plant roots. As a control treatment for each species, solution was replenished using a standard hydroponic solution consisting of macronutrients supplied at (in $mg \cdot L^{-1}$) 130 N, 40 phosphorus (P), 219 potassium (K), 141 calcium (Ca), 47 magnesium (Mg), and 55 sulfate (SO_4-S). Separate species-specific replenishment solutions were formulated for arugula and basil to supply 130 $mg \cdot L^{-1}$ N, and the ratio of other macronutrients for each species were calculated from tissue nutrient data collected from Chapter 2 and using mass balance principles described by Eq. 5 in Chapter 1. Individual nutrient concentrations for each replenishment solution are shown in Table 3-1. The pH of fertilizer solutions was adjusted to 6.0 at the beginning of the experiment and the EC of the initial solution was 1.95 $mS \cdot cm^{-1}$. There were two species (arugula, basil) and two nutrient replenishment solutions (standard, species-specific) for a total of four treatment combinations. Each fertilizer formulation and plant species treatment combination was replicated three times, and each treatment replicate consisted of 4 L hydroponic culture vessels with two plants. One treatment replicate was synonymous with one experimental unit used for data collection. Treatments were arranged using a randomized complete block design with three blocks, and one treatment replicate per block.

The volume of solution per treatment replicate was monitored every 2-3 d, at which time the culture vessels were topped off with the appropriate replenishment solution to the initial volume of 4 L. Solution pH and EC were also monitored and pH was maintained between 5.5-6.0 using 1*N* NaOH or HCl. Solution pH and EC were measured using a Thermo Scientific Orion

Versa Star Pro benchtop pH meter (Thermo Fisher Scientific Inc., Singapore). At the end of the experiment, all culture vessels were brought back to 4 L, and the total volume of replenished solution per replicate during the experiment was recorded.

Initial data were collected by destructively sampling two extra culture vessels per plant species (total of four plants per species) prior to the start of the experiment. Shoot and root tissue was collected by cutting plant stems above the neoprene collars and trimming the roots away from the rockwool substrate. Shoot tissue was weighed immediately for fresh mass determination, whereas roots were washed in a dilute acid solution (0.05% HCl acid solution mixed with deionized water) and air-dried prior collecting fresh mass. Tissues were then oven-dried (38°C) for 72 h for dry mass determination. Dried tissue samples were then analyzed for macronutrients using persulfate digestion (Purcell and King, 1996) for N and inductively-coupled plasma atomic emission spectrophotometry (ICP-AES) for the remaining elements (Fayetteville Agricultural Diagnostic Lab, University of Arkansas, Fayetteville AR).

Final data collection occurred on 20 Nov 2020, 21 d after the initiation of the replenishment solution treatments. Final data collected per treatment replicate included shoot and root fresh mass and dry mass, leaf SPAD chlorophyll content, transpired solution, root zone macronutrient concentrations, and macronutrients accumulated into plant tissues at 21 d. Shoot and root mass were collected as previously mentioned. Fresh and dry mass gain was then calculated by subtracting the initial from the final plant mass per replicate. Leaf SPAD chlorophyll content was measured by taking the average of 6 readings per replicate using a Chlorophyll Meter SPAD-502 Plus (Konica Minolta). Nutrient concentrations in both dried plant tissues and solution sampled at 21 d were measured using the methods previously described. Water-use efficiency was calculated by dividing the volume of water replenished by the total

plant dry mass gain per replicate. Since each hydroponic culture vessels started with 4 L of solution and was topped off to 4 L at the end of the experiment, total solution volume replenished was considered equal to evapotranspiration.

Total accumulated macronutrients in plant tissues was calculated by multiplying shoot and root tissue nutrient concentrations by the total dry mass per replicate. Change in nutrient solution EC and individual nutrient concentrations were determined by subtraction the initial nutrient solution values from values of the final nutrient solution samples. Total amounts of individual macronutrients supplied during the 21 d experiment was calculated by multiplying the concentration of individual nutrients supplied by the volume of the initial and replenishment solutions applied to each treatment. All data were adjusted and evaluated on a per plant per treatment replicate basis.

Analysis of variance (ANOVA) using the PROC GLM procedure in SAS (SAS 9.4: SAS Institute, Cary, NC) was used to determine replenishment solution main effects on plant dry mass gain, leaf SPAD chlorophyll content, transpiration and WUE, root zone nutrient concentrations, solution EC, changes in root zone nutrients and EC, and accumulation of nutrients in plant tissues. Means separation for ANOVA used Tukey's honestly significant difference (HSD) at the α=0.05 significance level. Analysis of variance and means separation were evaluated by species since species-specific replenishment solutions were developed separately for arugula and basil, and therefore species were not compared. Treatment variances were heterogeneous, and the residual error for each treatment was studentized in the statistical model by standardizing the residual error by the standard deviation for each species. The data fit assumptions of normality after standardizing the residual error, and therefore transformation of the data was not necessary.

Results and Discussion

Plant growth was measured as shoot and root fresh and dry weights for arugula and basil after 21 d (Fig. 3-1). Replenishment solution had significant effects on the total dry mass of basil (P=0.0416) and the root fresh mass of arugula (P=0.0458) as shown in Fig. 3-1B and Fig. 3-1E, respectively. P-values were near the a=0.05 significance level, and therefore further replication of this study may be needed to increase confidence in these results. Overall, root and shoot growth was not affected by replenishment solution in this experiment.

Total transpiration per plant was calculated by subtracting the volume of water remaining in the hydroponic culture vessel at the end of the experiment from the total volume of solution applied over 21 d (Fig. 3-2A, Table 3-2). The average volume of water transpired plant was not significantly different between replenishment solutions for either arugula (P=0.6756) or basil (P=0.0655). This indicates that arugula and basil plant transpired similar volumes of water regardless of the replenishment solution being supplied.

The WUE per plant was calculated by dividing the total volume of water replenished per plant by the plant's total dry mass (Fig. 3-2B). Similar to total transpiration, WUE was not significantly different for arugula (P= 0.4995) and was only marginally insignificant for basil (P=0.0572). The mean WUE for basil treated with the species-specific replenishment solution compared to the standardized solution was 0.16 $L \cdot g^{-1}$ and 0.20 $L \cdot g^{-1}$ respectively. If the main effects of replenishment solution on WUE had been significant it would indicate that a species-specific replenishment solution could allow basil the same plant biomass accumulation as plants supplied a standardized solution with a decrease in the required volume of water supplied.

The values for all other species and replenishment solution treatment combinations fall within the 0.2-0.4 $L \cdot g^{-1}$ reported by Bugbee (2004) as a good estimate for hydroponically grown crops. Similar to total transpiration, WUE was not affected by replenishment solution treatment for either species.

Total mass of individual nutrients supplied by replenishment solutions was determined by multiplying the total transpiration (L) by the concentration of individual nutrients supplied to each replicate ($mg \cdot L^{-1}$) after the initial solution supplied at the start of the experiment. The total mass of individual nutrients (mg) was then divided by 2 to estimate the mass of nutrients supplied to each plant (Table 3-2). Despite a lack of significance, arugula receiving a standard replenishment solution received overall greater quantities of all nutrients except N (Table 3-2). This is due to the standard replenishment solution for arugula supplying greater concentrations of all nutrients except N, and the species-specific treatment received 0.7 L more solution per plant than the standardized treatments (Table 3-2). However, the species-specific replenishment solution resulted in less Ca, Mg, and S supplied than the standardized solution regardless of the arugula species-specific replenishment solution treatments receiving 1.0 L more solution as the standard solution supplies greater concentrations of these nutrients.

Basil treated with a standard replenishment solution also received greater quantities of all nutrients for the same reasons. The total mass of P, K, Ca, Mg, and S were all significantly greater for the standardized solution treatments. As with arugula, basil receiving the standardized replenishment solution required the addition of 1.0 L more solution on average than plants treated with the species-specific replenishment solution.

Final solution EC was influenced by replenishment solution for arugula or basil (Table 3-3). After 21 d, there was a significant difference in solution EC between the two replenishment

solution treatments for both species (Table 3-3). It may be assumed that nutrients have the potential to accumulate over time in greater concentrations in standard solutions than in species-specific replenishment solutions due to both standard solutions having greater EC values over time than the species-specific solutions.

The final concentrations of N, P, and K ($mg \cdot L^{-1}$), were not influenced by replenishment solution for either species (Table 3-3), although solution N for arugula could not be analyzed as it had depleted to below the detection limit of ICP.

Replenishment solution influenced the final solution concentrations of Ca, Mg, and S for both species. The final concentration of Ca, Mg, and S varied between the standardized solution and the species-specific replenishment solution for basil. All 3 nutrients had significantly lower concentrations in the species-specific replenishment solution after 21 d compared to the standardized solution. The final concentration of Ca, Mg, and S also varied between standardized solution and species-specific replenishment solutions for arugula. Similar to basil, Ca, Mg, and S accumulated in greater quantities in the standardized solution.

Species-specific replenishment solutions typically supplied greater concentrations and masses of nutrients over time (Table 3-1, 3-2), and still resulted in significantly less nutrient accumulation in solution over time compared to standardized solution (Table 3-3). This would indicate that the ratios of nutrients to each other have a greater influence on plant uptake than the overall concentration of nutrients in solution. This may be explained by the standardized solution supplying these nutrients in much greater concentrations than the species-specific replenishment solutions for both species (Table 3-1).

Solution N and K were the only nutrients to decrease over time for all treatments (Table 3-4). Changes in solution N ranged from -24 mg· L^{-1} in the standard solution supplied to basil to -130 in the species-specific replenishment solution for arugula. Solution K changes ranged from -167 mg· L^{-1} in the arugula species-specific solution to -217 mg· L^{-1} in the basil standardized replenishment solution. The lack of accumulation of N and K indicates that these nutrients were not oversupplied by either fertilizer treatment.

Over 21 d, the total EC increased for standardized treatments for both species and decreased for both species-specific treatments (Table 3-4). This may be explained by the accumulation of Ca, Mg, and S over time in the standardized solutions for both species. Solution Ca, Mg, and S all showed a general increase over time in standardized solutions (Table 3-4). Solution Ca showed the greatest increase in the standard solution supplied to basil, increasing by 286 mg·L^{-1} in 21 days. The greatest increase in Mg occurred in the standardized solution for arugula, while the greatest increase in S occurred in the standardized solution for basil. This may indicate these nutrients are oversupplied by the standardized replenishment solutions and that a standardized solution would need to be replaced more frequently than a species-specific solution to avoid nutrient imbalances.

Plant shoot and roots typically accumulated similar concentrations of macronutrients when replenished with standard and species-specific solutions for arugula and basil (Table 3-5). Replenishment solution did not have an effect on the concentration of nutrients in plant shoot tissues for either species (Table 3-5). However, there was a marginally significant replenishment solution effect on shoot Mg in basil (P=0.0513). Replenishment solution had an effect on the accumulation of N in the root tissues of basil, however, the effect was just below the significance level (P= 0.0466). Replenishment solution also had an effect on the accumulation of Mg and S in

basil root tissues (Table 3-5). Final tissue nutrient concentrations in Table 5 also remained within the sufficiency ranges recommended for hydroponic arugula and basil by Bryson and Mills (2014), indicating nutrient supply from both replenishment solutions was adequate for healthy plant growth.

Plant growth and tissue macronutrient concentrations were not impacted by replenishment solution in this experiment (Fig. 3-1, Table 3-5); however, final nutrient concentrations and solution EC differed considerably between treatment solutions for both species (Table 3-3). These results suggest nutrient concentrations remained adequate for arugula and basil, and that these species can tolerate a range in supplied nutrient concentrations without developing nutritional disorders. Previous authors have published guidelines on target root zone nutrient concentrations to maintain for achieving optimal yields in hydroponic vegetable crops (Pardossi et al, 2014; Sonneveld and Voogt, 2009). However, similar published guidelines are lacking for hydroponic leafy greens and herbs. Determining the optimal target nutrient concentrations in solution can be difficult, since root zone nutrient concentrations are dynamic and not necessarily correlated with deficiency or toxicity symptoms (Sonneveld, 1999). Bugbee (2004) reports maintaining constant nutrient levels in solution is not necessary, and for some plant species may result in toxicity with certain elements.

Species-specific replenishment solutions designed for arugula and basil using mass balance principles resulted in a lower EC and accumulation of salts compared to the standard replenishment solution (Tables 3-2 and 3-3). These results indicate the potential of formulating replenishment solutions specifically to improve the balance between nutrient supply and plant uptake demand, and to reduce salt accumulation and therefore the need to periodically dump and replace solution. In this study, species-specific replenishment solutions were formulated based

on accumulation of nutrients in plant tissues, water uptake, and total plant growth, which can be easily measured in horticultural practice. Formulating species-specific replenishment solutions may therefore be a strategy for commercial growers to improve nutrient management practices and minimize wasted solution. This experiment served as a proof-of-concept and lasted for 21 d; evaluating species-specific solutions for longer periods and in commercial hydroponic systems is a next step in investigating this approach to nutrient management.

Replenishment solutions in this experiment were standardized by the concentration of supplied N, which resulted in increased or decreased solution EC depending on treatment. In commercial practice, a common management strategy is to adjust the concentration of the replenishment solution to maintain a constant and target solution EC (Miller et al., 2020; Walters and Currey, 2018). Table 3-3 shows replenishment with the standard nutrient solution increased EC for arugula and basil because of the accumulation of ions such as Ca and Mg; replenishing nutrients to maintain a constant EC in this study would have resulted in lower amounts of N replenished over time, eventually leading to reduced growth from N deficiency, which has been observed in both research and commercial practice (Houston and Dickson, 2021; Miller et al., 2020). The occurrence of N deficiency would be less likely when maintaining a constant EC with species-specific replenishment solutions (Bugbee, 2004; Langenfeld, 2021), because the ratio of nutrients supplied to nutrient uptake is closer to 1:1 resulting in near “steady state” nutrition.

Species-specific replenishment solutions may simplify solution management based on EC, but increasing or decreasing solution EC may impact nutrient uptake ratios (Sonneveld and Voogt, 2009). Sonneveld and Voogt (2009) found that increasing solution EC resulted in increased K uptake and accumulation in lettuce leaf tissues, and subsequently decreased uptake and accumulation of Ca and Mg. Luxury consumption of K has been reported in certain

agronomic crop species (Marschner, 2012), but excess uptake of K is not common to all plant species in controlled environments (van Iersel et al., 1998; Sonneveld and Voogt, 2009). Walters and Currey (2018) showed that hydroponic basil can tolerate a wide range in solution EC values, from 0.5 and 4.0 $mS \cdot cm^{-1}$, without affecting yield or tissue macronutrient concentrations. Nutrient concentrations and ratios found in plant tissues are only slightly affected by increasing the supplied solution EC for many crops grown in soilless culture (Sonneveld and Voogt, 2009; van Iersel et al., 1998), and therefore the ratio of nutrients supplied in species-specific replenishment solutions would likely require minimal to no adjustment if EC was increased or decreased to meet crop nutrient demands.

Injection of mineral acids to control solution pH can contribute significant quantities of N, P, or S during hydroponic production (Bugbee, 2004; Resh, 2012), which would need to be accounted for when formulating specifies-specific replenishment solutions. For example, Bugbee (2004) reported half the N requirement for a crop can be supplied from nitric acid when used to control solution pH. The quantity of acid needed for injection would depend on several interacting factors including the mineral acid (nitric, phosphoric, and/or sulfuric) and concentration, substrate components, fertilizer N forms, irrigation water alkalinity, and plant species. Therefore, determining the quantity of acid needed during production requires grower experience or the use of complex predictive models.

Dickson and Fisher (2016) proposed adjusting the ammonium:nitrate (NH_4:NO_3) ratio in the supplied solution as a strategy to stabilize pH and reduce acid injection with hydroponic leafy greens and herbs. Research from the same authors found 23.3% and 11.4% of total N would need supplied as NH_4-N (remainder as NO_3-N) to stabilize pH for hydroponic arugula and basil, respectively, while 46.0% and 13.9% NH_4-N would be needed to stabilize pH for the same

species grown in soilless substrates (Dickson and Fisher, 2016). Increasing NH_4-N in solution can inhibit uptake of other nutrient cations such as K, Ca, and Mg, reducing plant quality and promoting nutritional disorders such as “tip burn” in lettuce and “blossom-end-rot" in vegetable species (Houston et al., 2021; Marcelis and Ho, 1988). The potential of adjusting NH_4:NO_3 ratios as a strategy to control pH in species-specific replenishment solutions needs further investigation.

Conclusions

This study highlights that unless replenishment solutions are tailored to a crop species, there is the potential for nutrients to accumulate or deplete in hydroponic systems. Balancing nutrient supply with plant demand using species-specific replenishment solutions has the potential to reduce or eliminate the accumulation of macronutrients such as Ca, Mg, and S in solution, reducing the need to replace the solution. The formulation of replenishment solutions to meet the nutritional requirements for individual crop species may be an effective strategy to mitigate ion accumulation and root zone nutrient imbalances in closed hydroponic systems without compromising plant quality and yield. Further study is needed to evaluate the potential for species-specific replenishment solutions that also stabilize EC and to determine if managing a solution by EC using a balanced solution is a viable option for commercial practice.

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Tables and Figures

Table 3-1. Initial target electrical conductivity ($mS \times cm^{-1}$) and concentrations of nitrate-nitrogen (NO_3-N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate (SO_4-S) in solution ($mg \cdot L^{-1}$) for standardized nutrient solution and species-specific designed replenishment solutions.

Species	Fertilizer	EC	NO_3-N	P	K	Ca	Mg	SO_4-S
		$mS \cdot cm^{-1}$	$mg \cdot L^{-1}$					
Arugula	Standard	1.95	130	40	219	141	47	55
	Species-specific	1.85	130	26	175	79	24	34
Basil	Standard	1.95	130	40	219	141	47	55
	Species-specific	2.08	130	34	191	65	15	10

Table 3-2. Total volume of water replenished per plant (L) and total mass of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate (SO_4-S) supplied per plant (mg) during the 21-day experiment. Data are least-square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Species	Replenishment solution	Volume of replenished solution[x]	N[y]	P	K	Ca	Mg	S
		——L——	——————————mg——————————					
Arugula	Standard	7.0 a	906 a	277 a	1526 a	982 a	328 a	383 a
	Species-specific	7.7 a	997 a	200 a	1344 a	607 a	183 a	262 a
	Solution effects[z]	NS	NS	NS	NS	NS	NS	NS
Basil	Standard	5.5 a	715 a	218 a	1203 a	775 a	259 a	302 a
	Species-specific	4.5 a	589 a	153 b	867 b	295 b	67 b	43 b
	Solution effects	NS	NS	*	*	**	**	**

[x]Volume of replenished solution per plant was calculated by subtracting the volume of solution remaining in each hydroponic culture vessel at the time of final data collection from the volume of solution supplied over 21 days per replicate, divided by 2 plants per replicate

[y]Mass of individual nutrients supplied per plant as calculated as the volume of water replenished per plant (L) multiplied by the concentration of each nutrient supplied by the respective replenishment solutions ($mg \cdot L^{-1}$) to obtain total mg of each nutrient supplied with initial mass of nutrients supplied subtracted

[z] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 3-3. Final electrical conductivity ($mS \cdot cm^{-1}$) and concentrations of nitrate-nitrogen (NO_3-N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate (SO_4-S) in solution ($mg \cdot L^{-1}$) for standardized nutrient solution and species-specific designed replenishment solutions. Data are least-square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level, statistical analysis was completed on species individually, species were not compared to each other.

Species	Replenishment solution	EC	NO_3-N	P	K	Ca	Mg	SO_4-S
		$mS \cdot cm^{-1}$	$mg \cdot L^{-1}$					
Arugula	Standard	2.75 a	1.9	41.2 a	10.4 a	258.3 b	219.3 a	98.7 a
	Species-specific	0.69 b	N/A	41.9 a	52.2 a	31.0 b	38.3 b	4.5 a
Basil	Standard	2.71 a	105.5 a	27.7 a	1.4 a	426.7 a	144.3 a	202.3 a
	Species-specific	1.19 b	58.5 a	16.7 a	49.9 a	97.8 b	19.7 b	42.1 b

Table 3-4. Change in solution electrical conductivity ($mS \times cm^{-1}$) and concentrations of nitrate-nitrogen (NO_3-N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfate (SO_4-S) in ($mg \cdot L^{-1}$) over 21 days between a standard replenishment solution and a species-specific replenishment solution. Negative values indicate a decrease in solution concentration while positive values indicate an accumulation over time. Change in concentrations calculated by subtracting the initial nutrient solution concentrations from the final solution concentration. Data are least-square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

Species	Replenishment Solution	EC	NO_3-N	P	K	Ca	Mg	SO_4-S
		$mS \cdot cm^{-1}$	$mg \cdot L^{-1}$					
Arugula	Standard	0.80 a	-128.1 a	1.5 a	-208.3 a	117.5 a	172.3 a	43.8 a
	Species-specific	-1.26 b	-129.9 a	2.3 a	-166.6 a	-109.8 b	-8.7 b	-50.4 a
	Solution effects[x]							
Basil	Standard	0.76 a	-24.5 a	-12.0 a	-217.3 a	285.9 a	97.3 a	147.4 a
	Species-specific	-0.76 b	-71.5 a	-23.0 a	-168.9 a	-43.0 b	-27.3 b	-12.8 b
	Solution effects							

[x] NS, *,**,*** Nonsignificant or significant at $P < 0.05$, 0.01, 0.0001, respectively.

Table 3-5. Effects of standard and species-specific replenishment solutions on tissue nutrient concentrations of concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) (values shown are percentage of nutrients in plant tissues) for arugula and basil after 21 days. Data are least-square means of 3 replicates. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection. Mean separation for ANOVA used Tukey's HSD at the α=0.05 significance level, statistical analysis was completed on species individually, species were not compared to each other. Data analyzed separately for shoot and root tissues.

Species	Replenishment solution	N	P	K	Ca	Mg	S
		Shoot tissue					
		%					
Arugula	Standard	5.16 a	0.50 a	4.39 a	3.13 a	0.83 a	1.30 a
	Species-specific	4.89 a	0.51 a	4.25 a	2.83 a	0.71 a	1.03 a
Basil	Standard	4.42 a	1.08 a	5.14 a	2.50 a	0.63 a	0.37 a
	Species-specific	4.30 a	0.93 a	5.12 a	2.40 a	0.51 a	0.37 a
		Root tissue					
		%					
Arugula	Standard	3.87 a	2.63 a	3.31 a	3.88 a	0.72 a	1.27 a
	Species-specific	4.32 a	1.04 a	3.75 a	0.70 a	0.43 a	1.04 a
Basil	Standard	5.23 b	1.10 a	4.22 a	0.60 a	2.09 a	1.20 a
	Species-specific	5.50 a	1.03 a	4.31 a	0.61 a	1.52 b	0.79 b

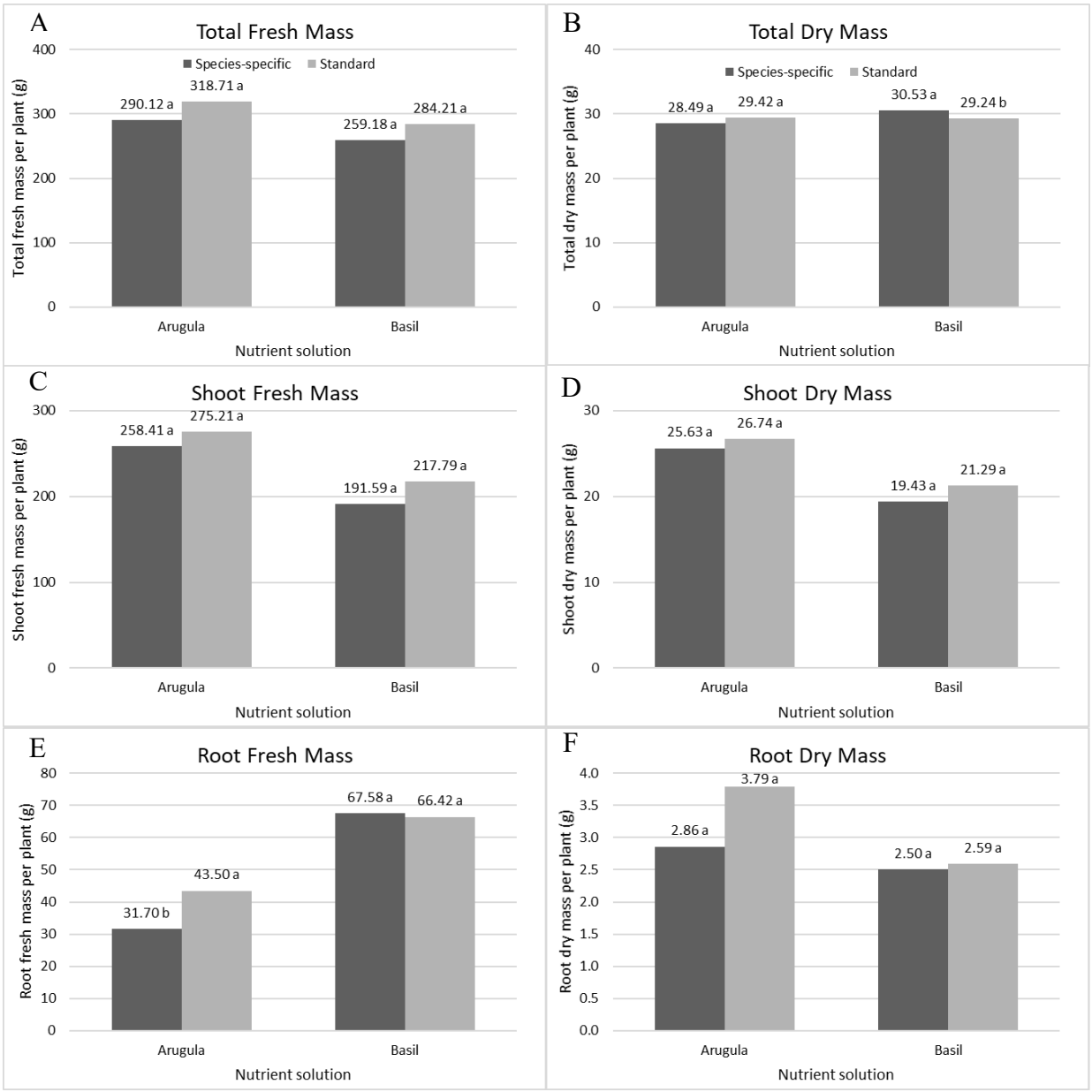

Figure 3-1. Effects of standard and species-specific replenishment solutions (per plant) on total plant fresh mass (a), total plant dry mass (b), shoot fresh mass (c), shoot dry mass (d), root fresh mass (e), and root dry mass (f) for arugula and basil after 21 days. Values are averages of 3 replications. Each treatment replicate consisted of a 4L hydroponic culture vessel with two plants, and one replicate was synonymous with one experimental unit used for data collection.

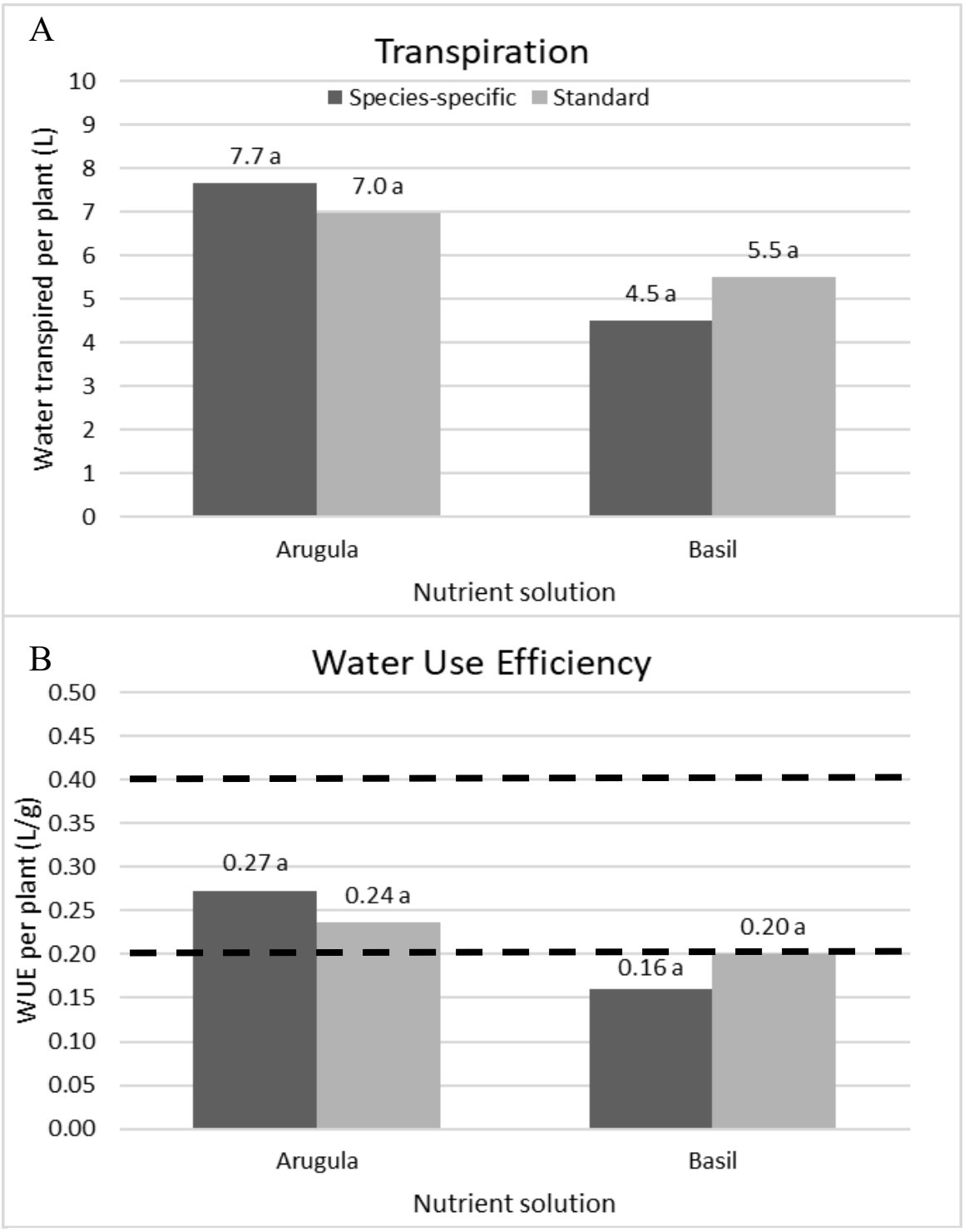

Figure 3-2. Total transpiration of solution (A) and water use efficiency (B), per plant, for arugula and basil replenished with standard and specifies-specific solutions. Data represent least-square means of three replicates per treatment. Each treatment replicate was synonymous with one experimental unit used for data collection; a 4L hydroponic culture vessel with two plants. Means separation used Tukey's honestly significance difference (hsd) at the 0.05 significance level

CHAPTER 4 . CONCLUSION

In hydroponic leafy greens production, replenishing the hydroponic reservoir with nutrient solutions designed to balance nutrient supply with plant uptake demand can reduce salt accumulation and ion imbalances in the root zone, and minimize the need to dump and replace solution. Formulating balanced replenishment solutions requires quantifying nutrient uptake during production, which can be achieved from measuring the depletion of nutrients from solution or the accumulation of nutrients in plant tissues over time. The ratio of nutrients to supply in a replenishment solution (i.e., solution composition) can be determined from the ratio of nutrients taken up, and the concentration of the nutrient solution (i.e., solution strength) can be calculated from the crop water-use efficiency (WUE). Growers can quantify nutrient uptake by analyzing hydroponic solutions and/or plant tissues for nutrient content, which is already common in commercial practice. Growers measure yield and often install flow meters to measure water supply/consumption, which can then be used to calculate WUE. Therefore, growers can use data collected in-house to better formulate hydroponic replenishment solutions to improve nutrient management in recirculating systems.

The formulation of balanced hydroponic replenishment solutions likely needs to be specific to the plant species or type of crop produced. Leafy greens and herb species evaluated in this study (arugula, basil, and lettuce) differed in the uptake of individual nutrients, growth, and total solution transpired, which influenced the expected composition and concentration of nutrients in a replenishment solution. Water-use efficiency was similar for arugula, basil, and lettuce in the first controlled-environment experiment (Chapter 1), averaging 0.3 $L \cdot g^{-1}$ dry mass gain across species; however, WUE appeared greater for basil (0.2 $L \cdot g^{-1}$ dry mass gain) compared to arugula (0.3 $L \cdot g^{-1}$ dry mass gain) in a later greenhouse experiment (Chapter 3).

Water-use efficiency is also influenced by environmental factors including light, temperature, relative humidity, and carbon dioxide level. Therefore, nutrient concentrations and the strength of species-specific replenishment solution may need increased or decreased depending on season and climate.

Formulations of hydroponic solutions commonly-used in research and commercial practice were shown to differ considerably in individual nutrient concentrations and have potential to oversupply nutrients leafy greens and herbs, particularly calcium (Ca), magnesium (Mg), and sulfur (S). In this study, specially-formulated and species-specific replenishment solutions designed for arugula and basil reduced the accumulation of Ca, Mg, and S in solution without compromising yield or plant quality, showing promise this approach could be used to minimize the need to dump and replace solution.

Formulating species-specific replenishment solutions may be a step towards achieving "steady state" nutrient management. "Steady state" management is a strategy that accounts for, and balances nutrients supplied from all sources, including fertilizers, mineral acids and bases, and the raw irrigation water in with plant demand. Improving nutrient management in this way allows for reduced solution management over time and could potentially allow growers to achieve lower maintenance costs.

The formulation of replenishment solutions is crucial to maintaining a balanced hydroponic solution. Numerous factors influence the uptake of nutrients by plants and therefore the concentrations of nutrients required in a replenishment solution. For example, the injection of mineral acids in solution to combat water alkalinity or to control pH will add nutrients to the solution. Additionally, overall alkalinity and pH of the solution will impact plants' ability to remove nutrients from the solution. There is also the possibility of controlling pH by altering the

NH_4:NO_3 ratio in solution, however the addition of a greater concentration of NH_4 may impact the uptake of other cations. Plant nutrient uptake is also influenced by climate and plant development stage. For example, high light levels can cause an increase in the volume of water transpired by plants. Under these circumstances, the EC of the nutrient solution would need to be lower than under low light conditions to account for the greater volume of water being transpired and avoid salt stress. The transition of a plant from a vegetative to a reproductive stage may also cause a shift in the plant's nutrient requirements. When formulating a replenishment solution for a fruiting crop, the various development stages may require different replenishment solutions.

The possibility for growers to formulate species-specific replenishment solutions based on plants WUE and accumulation of tissue nutrients may help to reduce variability and waste caused by the constant replacement of water and nutrients. Species-specific replenishment solutions need to be tested in larger, commercial hydroponic systems for a greater to further validate the findings of these studies.