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Development of a Hydroponic Growing Protocol for Vegetative Strawberry Production

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Abstract. **Hydroponic growing systems are advantageous for nutrient studies in which root data are important because they alleviate the laborious and time-consuming task of washing roots to remove soilless substrate particulates from them. However, the growing system should be optimized for the crop of interest. Our overall objective was to develop a protocol for hydroponic strawberry (*Fragaria ×ananassa*) production that provided growth equal to or better than soilless substrate. Plants were initially grown in perlite, sand, deep water culture (DWC), or a peat-based soilless substrate. Aboveground plant growth in DWC was similar to that of plants grown in the peat-based substrate and required minimal effort to harvest the entire root system. However, the pH of the DWC nutrient solution decreased to 4.0 ± 0.1 (mean ± *SE*) when plants were provided a modified strawberry (Yamazaki) nutrient solution with a ratio of nitrate (NO_3^-) to ammonium (NH_4^+) of 80:20. As a result, a subsequent trial was conducted to evaluate the buffering capacity of nutrient solutions with NO_3^- to NH_4^+ ratios of 0:100, 20:80, 50:50, 60:40, 80:20, or 100:0, with the addition of potassium bicarbonate ($KHCO_3$). Up to 2.6 mM $KHCO_3$ did not provide adequate buffering in nutrient solutions containing NH_4^+ (0:100 to 80:20 treatments), and nutrient solution pH decreased by ~1.5 units every 2 to 3 days. The 100% NO_3^- nutrient solution, however, maintained a stable pH of 5.9 ± 0.1 when buffered with 0.8 mM $KHCO_3$. Finally, 2(N-Morpholino)ethanesulfonic acid (MES) was evaluated as a potential buffering agent for DWC strawberry production. Plants were grown in a nutrient solution with a 60:40 ratio of NO_3^-:NH_4^+. The buffering capacity of the nutrient solution increased as the MES concentration supplied increased from 1 to 5 mM, and the 5 mM MES treatment maintained a pH of 5.6 ± 0.2. In summary, strawberry plants can successfully be grown hydroponically in DWC, provided that nutrient solution pH is adequately managed. The addition of MES buffer provided better pH stability than $KHCO_3$.**

The US strawberry (*Fragaria ×ananassa*) fruit industry had an estimated value of $3.4 billion in 2021, with a fresh market yield of 21.7 million cwt (1 cwt = 100 pounds) and a processing market yield of 5.0 million cwt (US Department of Agriculture, National Agricultural Statistics Service 2022). The majority of US strawberries are field-grown, although there has been a recent increase in growing strawberries in controlled environments, including high tunnels, greenhouses, and indoor farms. Although greenhouse production of strawberries in the United States is a small fraction of overall berry production, it increased almost 1200% from 1998 to 2014 (Walters et al. 2020). In 2019, greenhouse strawberry production encompassed 61,223 m^2, yielding 532,472 kg of fruit and a retail value of $681,000 (US Department of Agriculture, National Agricultural Statistics Service 2020). Greenhouse strawberry production in containerized systems is also becoming more popular in Europe (van Delm et al. 2016). This increase stems from increased consumer demand for local, year-round production and increased grower interest in exploring new crops and markets for controlled environment production (Flores et al. 2021; Kubota et al. 2016; Lewis and Kubota 2014; Samtani et al. 2019).

The strawberry industry relies primarily on clonal propagation of daughter plants or crown division to generate new plant material for fruit producers. Compared with field production, using controlled environments to produce strawberry daughter plants offers the advantages of reduced disease pressure (especially of soil-borne pathogens), better control of the growing environment, and year-round availability of actively growing starting plant material (Baggio et al. 2021a, 2021b; Hernández-Martínez et al. 2023; Xu and Hernández 2020). These benefits have led to research aimed at improving strategies for growing mother plants in controlled environments to increase the runner number, daughter plant number, and daughter plant quality (Shi et al. 2021; Xu and Hernández 2020). One aspect of optimizing the growing conditions to increase daughter plant production is through nutrient optimization.

In controlled environments, strawberry has been evaluated in soilless growing media, including peat-based substrates, pine bark, wood fiber blends, coconut coir, perlite, sand, and rockwool (Bould 1964; Cantliffe et al. 2007; McKean et al. 2020; Palencia et al. 2016; Richardson et al. 2022; Rivera-del Rio et al. 2017; Sarooshi and Cresswell 1994; Taghavi et al. 2017; Tehranifar et al. 2007; Treftz and Omaye 2015; Zucchi et al. 2017). Additionally, strawberry has been grown hydroponically in liquid culture using a nutrient film technique (Darnell and Stutte 2001; Sharma et al. 2018; van Delm et al. 2016) and deep water culture (DWC) (Bagale 2018; Kitazawa et al. 2005; Sakamoto et al. 2016). In most of these studies, plant growth was compared between different types of growing media rather than across growing systems. For example, runner production was similar for greenhouse strawberries grown in pine bark or coconut coir (Cantliffe et al. 2007). Palencia et al. (2016) evaluated agrotextile, coir, perlite, and rockwool, and Richardson et al. (2022) compared a green roof medium, Dutch buckets filled predominantly with perlite, and compost-filled raised beds for strawberries. In both studies, they suggested that all substrates evaluated could be acceptable for strawberry fruit production, depending on the cultivar selected. Taghavi et al. (2017) observed better vegetative growth of container-grown strawberry in peat or bark-based commercial substrate blends as compared with field sand. Tehranifar et al. (2007) observed higher strawberry runner and crown number for plants grown in substrate blends containing peat or coir, relative to sand or perlite exclusively.

Although soilless substrates containing peat, perlite, and/or coconut coir are typically used for controlled environment strawberry production, it is difficult and time-consuming to separate roots from the growing medium at the end of a research study. Therefore, for research applications, growing strawberry plants hydroponically in liquid culture may be a useful system for nutrient-based studies to quantify the impacts of nutrient solution composition or concentration on plant growth and quality, especially roots. A hydroponic system would allow for easy measurement of aboveground

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(e.g., crown, leaves, inflorescences, fruit, stolons, and/or daughter plants) and belowground (roots) biomass, whereas container-grown strawberries are usually restricted to aboveground biomass measurements because of the difficulty in separating roots from the soilless substrate.

However, pH management of nutrient solutions for hydroponic systems can be more challenging because they are often less buffered than peat or bark-based substrates. When roots excrete cations [primarily protons (H^+)] and anions [primarily hydroxyls (OH^-)] to maintain the charge balance in plants during nutrient uptake (Neumann and Römheld 2012; Raven and Smith 1976), this cation or anion exchange can be problematic in poorly buffered substrates or hydroponic systems, and it may cause pH to drift too high or too low for optimal growth. A pH range of 5.5 to 6.4 is recommended for substrate or hydroponic solutions to provide an optimal nutrient solubility (Argo and Biernbaum 1996; Handreck and Black 1994). A pH less than 4 or greater than 7 in the root zone environment is generally detrimental to root growth and health (Baligar et al. 1998). Therefore, an ideal growing system for research applications should provide suitable growth and pH management while balancing the recovery of roots.

The pH in the rhizosphere can fluctuate depending on the nitrogen (N) form supplied in fertilizers or nutrient solutions, as well as the buffering capacity of the substrate or nutrient solution. The N form can have a pronounced impact on the rhizosphere pH, especially when the buffering capacity is low. Ammonium (NH_4^+) will tend to decrease the root zone pH over time, whereas nitrate (NO_3^-) will tend to increase pH over time, as plant roots take up these ions from solution. Adjusting the ratio of NO_3^- to NH_4^+ has been successfully demonstrated for other crops (Lea-Cox et al. 1997; Neumann and Römheld 2012; van Beusichem et al. 1988). Another strategy to minimize pH drift is to incorporate a buffer into the nutrient solution. Carbonates minimize pH drift by neutralizing acids, but they may bind nutrients (e.g., iron) when supplied in high concentrations (Argo and Fisher 2008; Ohyama et al. 2023; Wallace and Abou-Zamzam 1984). Dolomitic limestone, a calcium and magnesium carbonate, is often used in soilless substrates, and potassium bicarbonate ($KHCO_3$) has been shown to be an effective pH buffer in soilless substrates and hydroponic solutions (Fisher et al. 2006; Ohyama et al. 2023; Pancerz and Altland 2020). Another potential buffer is 2(N-Morpholino)ethanesulfonic acid (MES). It readily dissolves in water (high solubility) and has a high molar mass; therefore, it is not likely to be taken up by plants and is less likely to bind nutrients because of its low binding constant (Good et al. 1966). Bugbee and Salisbury (1985) observed adequate buffering with 1 to 3 mM MES added to a nutrient solution with an NO_3^-:NH_4^+ ratio of 8:1.5 for 3 to 4 weeks. However, MES is not as readily used as carbonates because of its higher cost relative to that of other potential buffers.

Our overall objective was to identify an effective growing system and develop a protocol that would allow us to easily and effectively monitor both root and shoot growth, daughter plant production and quality, and nutrient uptake and allocation of strawberry. The first study evaluated plant growth and leachate or nutrient solution pH for strawberry grown in different production systems. We evaluated a peat-based substrate, perlite, sand, and DWC. Based on the results of this first study, we identified that the DWC hydroponic system was suitable for aboveground growth, but the use of ultrapure water with minimal buffering capacity allowed pH to fluctuate. Therefore, we conducted two additional studies to address the lack of sufficient buffering capacity in DWC of strawberry plants and improve root growth. The objective of the second experiment was to evaluate different ratios of NO_3^- to NH_4^+ in nutrient solutions buffered with $KHCO_3$. When that was untenable, a second buffering compound, MES, was evaluated for its suitability to buffer nutrient solution pH drift.

Materials and Methods

Mother plant growing conditions. Field-dug, bareroot strawberry 'Albion', 'Fronteras', and 'Monterey' plants were received from Lassen Canyon Nursery, Inc. (Redding, CA, USA) in Oct 2021. They were planted in 19.1-cm-diameter pots filled with a peat-based substrate and grown in a greenhouse (Toledo, OH, USA) maintained at 26/23 °C day/night air temperature with a 16-h photoperiod. Light-emitting diode fixtures (Icarus Ti2; BIOS Lighting, Carlsbad, CA, USA) provided 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (*PPFD*) when ambient light intensities were <300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPFD* at canopy level. Plants were irrigated as needed with a 15N–2.2P–12.5K water-soluble fertilizer (Jack's Professional 15–5–15 Ca-Mg; JR Peters, Inc., Allentown, PA, USA) at 150 $mg \cdot L^{-1}$ N supplemented with 306 $mg \cdot L^{-1}$ magnesium sulfate heptahydrate (Magriculture; Giles Chemical, Waynesville, NC, USA). Substate solution pH and electrical conductivity (EC) were checked monthly. If the EC drifted above 2.0 $mS \cdot cm^{-1}$, then containers were leached to remove excess salts. If pH drifted too low, then 1.04 $L \cdot m^{-3}$ flowable lime (CalOx®; BioSafe Systems, LLC, East Hartford, CT, USA) was applied to increase pH by ∼0.5 units.

Inflorescences were removed weekly. Runners (i.e., stolons) with daughter plants were harvested as needed. Approximately every 2 months, extra runners containing at least one daughter plant were removed to maintain crown vigor yet ensure a continuous supply of daughter plants for propagation. Daughter plants for experiments were cut from the runners ∼1 cm above and below the node and graded into size categories; those that were too big or too small were excluded. Daughter plants with a crown diameter of 5 to 7 mm, visible root nodules present, and two to three unfolded leaves were selected. This corresponded to the small to medium size categories described by Xu and Hernández (2020). Daughter plants were placed in a cooler in the dark at 4 °C for 7 d and then propagated.

Expt. 1: Evaluation of growing systems. Strawberry 'Albion' daughter plants were harvested from mother plants growing in a greenhouse (Toledo, OH, USA). Daughter plants were placed in a 50-cell tray (100-mL cell volume) filled with a peat-based substrate (LM-111AP; Lambert, Rivière-Ouelle, QC, Canada) and propagated for 5 weeks in a reach-in growth chamber (BDR16; Conviron, Winnipeg, MB, Canada) maintained at 24 °C with a 16-h photoperiod and a daily light integral (DLI) of 5.41 $mol \cdot m^{-2} \cdot d^{-1}$. Trays were misted daily and covered with humidity domes for 14 d. They were irrigated as needed with tap water for 7 d, then fertigated with 15N–2.5P–12.5K nutrient solution at 50 to 100 $mg \cdot L^{-1}$ N (Jack's Professional 15–5–15 Ca-Mg).

Rooted strawberry plants were transplanted into four growing systems evaluated as individual treatments. They consisted of three soilless substrate treatments [a peat-based substrate (LM-111AP; Lambert), coarse perlite (P.V.P Industries Inc., North Bloomfield, OH, USA), and sand (0.075 to 9.5-mm; Quikrete Sand & Gravel Products, Toledo, OH, USA)] and one hydroponic liquid culture system (DWC). Roots were washed before transplanting plants into the perlite, sand, or DWC treatments. A 19.1-cm-diameter pot (3.5-L) was used for the peat-based substrate, and 4-L buckets were used for the perlite, sand, and DWC treatments. For the DWC system, a single plant was placed in a net pot (5.1-cm-diameter; Teku®; Pöppelmann GmbH and Co., Lohne, Germany) that was suspended by the container lid just above the nutrient solution. All plants were supplied with a modified strawberry nutrient solution (Yamazaki 1982) with an NO_3^-:NH_4^+ ratio of 80:20 at a total N concentration of 100 $mg \cdot L^{-1}$ (Table 1). Ultrapurified water (18 $M\Omega \cdot cm$) was used to make the nutrient solution.

The peat-based substrate was initially overhead-irrigated to container capacity, allowed to dry down to ∼60% volumetric water content, and irrigated as needed with 300 mL nutrient solution to container capacity. The coarse perlite and sand treatments were drip-irrigated during the 16-h photoperiod (14-cm-tall stake, 0.5 gpm; Netafim Irrigation, Inc., Fresno, CA, USA) at a frequency of 20 s on/1 h off for days 1 to 3 and 15 s on/1 h and 40 min off until the end of the trial. There were nine irrigation cycles daily that delivered 24.8 mL nutrient solution per 15-s irrigation event and a total of 223 mL of nutrient solution each day over the duration of the study. Each 4-L bucket had a hole in the bottom to drain out leachate as needed. A piece of flexible tubing ran from the drain hole in each bucket to an individual capture reservoir to collect leachates. For the DWC system, an air pump supplied continuous aeration to each bucket (reservoir) through Tygon tubing (0.953-cm outer diameter × 0.635-cm inner diameter; E-3603; Tygon, Akron, OH, USA) with an air stone inserted at the end

Table 1. Components used to constitute the modified strawberry nutrient solution (Yamazaki 1982) recipes mixed to a final concentration of 100 mg·L^{-1} nitrogen (N). Solution pH was adjusted to either 5.7 (Expts. 1 and 2) or 5.8 (Expt. 3). No buffers were added in Expt. 1. In Expt 2, 0.8 to 2.6 mM potassium bicarbonate ($KHCO_3$) was added to help buffer pH. In Expt. 3, 1, 3, or 5 mM of 2(N-Morpholino)ethanesulfonic acid (MES) or 2 mM $KHCO_3$ was added to the nutrient solution as a pH buffer.

	Expt.							
Component	Expt. 1	Expt. 2						Expt. 3
NO_3^-:NH_4^+	80:20	0:100	20:80	50:50	60:40	80:20	100:0	60:40
	Macronutrients (mM)							
NH_4NO_3	0.4	0.0	0.0	0.5	1.0	0.4	0.0	1.0
$(NH_4)_2SO_4$	0.0	3.0	2.3	1.0	0.4	0.0	0.0	0.4
$NH_4H_2PO_4$	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0
$Ca(NO_3)_2$	1.1	0.0	0.0	0.0	0.1	1.1	2.5	0.1
KNO_3	3.0	0.0	1.4	3.0	3.0	3.0	2.0	3.0
$CaCl_2$	1.4	2.0	2.0	2.0	2.4	1.4	0.0	2.4
K_2SO_4	0.0	1.5	0.8	0.0	0.0	0.0	0.0	0.0
KH_2PO_4	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
$MgSO_4$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Micronutrients (μM)							
Na_2Fe EDTA	81.7	81.7	81.7	81.7	81.7	81.7	81.7	81.7
$MnSO_4$	10	10	10	10	10	10	10	10
$ZnSO_4$	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
$CuSO_4$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$(NH_4)_6Mo_7O_{24}$	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085
H_3BO_3	50	50	50	50	50	50	50	50
	Buffer (mM)							
$KHCO_3$	0	2.6[i]	2.3	2.0	1.8	1.6	0.8	0 or 2
MES								1–5
	pH and electrical conductivity (EC; mS·cm^{-1})							
pH	5.7 ± 0.03	5.7 ± 0.1	5.7 ± 0.2	5.7 ± 0.1	5.7 ± 0.2	5.7 ± 0.2	5.6 ± 0.1	5.8 ± 0.02
EC	1.0 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	2.1 ± 0.1

[i] Concentration added to nutrient solution during weeks 4 to 15 of the study.

(Fritware; Bel-Art Products, Pequannock, NJ, USA). Each reservoir was filled with 3.75 L of nutrient solution and topped off with nutrient solution as needed; solutions were replaced weekly. Each pot or bucket was an experimental unit, and there were four replicates per treatment arranged in a completely randomized design.

Plants were grown in a walk-in growth chamber (GR48; Environmental Growth Chambers, Chagrin Falls, OH, USA) for 6 weeks. Light was supplied by high-pressure sodium lamps (150 W) to provide a 16-h photoperiod. Air temperature, relative humidity (RH), and *PPFD* were measured using an aspirated thermocouple, RH sensor, and quantum sensor, respectively, and values were recorded every 10 min using a data logger (WatchDog 2475; Spectrum Technologies, Aurora, IL, USA). The mean daily air temperature was 23.6 ± 0.2 °C (mean ± *SD*), RH was 78.4% ± 2.7%, and the *PPFD* increased weekly from 200 to 307 μmol·m^{-2}·s^{-1} (at canopy level) over the duration of the study to provide a DLI of 11.5 to 17.7 mol·m^{-2}·d^{-1}.

The pour-through method (LeBude and Bilderback 2009) was conducted 3 weeks after transplanting to evaluate the leachate pH and EC of the peat-based substrate. Leachate samples from the two drip irrigation treatments (coarse perlite and sand) were measured to determine the pH and EC every 2 weeks. The pH and EC of the DWC reservoirs were checked weekly, and the pH was adjusted to 5.7 as needed with potassium hydroxide (KOH; 1 M). All pH and EC measurements were performed using a handheld meter (HANNA HI9814 GroPro; Hanna Instruments, Woonsocket, RI, USA).

Runner and inflorescence numbers were recorded weekly, and inflorescences were removed after counting. After 6 weeks, plants were harvested and separated into the mother plant, runners, and roots. Each portion was individually dipped into 0.1 M hydrochloric acid (HCl) acidified water, rinsed in 18 MΩ water, placed in a paper bag, dried in a forced air oven at 60 °C for at least 3 d, and weighed for dry mass.

Data were analyzed using SAS (SAS version 9.4; SAS Institute, Cary, NC, USA) and a one-way analysis of variance (ANOVA) using PROC GLM. When $P < 0.05$, Tukey's honest significant difference (HSD) test was conducted using $\alpha = 0.05$.

Expt. 2: Impact of NO_3^-:NH_4^+ on DWC nutrient solution pH. In Expt. 1, the DWC nutrient solution pH decreased over time when supplied at an NO_3^-:NH_4^+ ratio of 80:20. Expt. 2 evaluated the buffering capacity of six NO_3^-:NH_4^+ ratios supplemented with $KHCO_3$. Strawberry 'Fronteras' daughter plants were transferred to net pots filled with expanded clay pebbles (Grow!t™; Hydrofarm, Petaluma, CA, USA), placed in a tray, and grown in a reach-in growth chamber (BDR16) for 7 d. A shallow layer of water was maintained in the tray to keep the clay pebbles moist. Chamber set points provided a high RH (>80%), constant air temperature of 24 °C, 16-h photoperiod, and DLI of 5.41 mol·m^{-2}·d^{-1}. Plants were irrigated with tap water until the roots began to elongate (3 d); then, they were irrigated with a 15N–2.2P–12.5K fertilizer solution at 50 mg·L^{-1} N. After 7 d, plants were transferred to the walk-in growth chamber (GR48) used in Expt. 1 and maintained at the same environmental set points. Plants were grown in a DWC system, as described in Expt. 1. The daily mean air temperature was 24.7 ± 0.6 °C (mean ± *SD*) and RH was 70.1% ± 4.0%. The initial *PPFD* at the canopy level was 267 μmol·m^{-2}·s^{-1}, and it was increased at 2-week intervals during the first 8 weeks of growth. For a 16-h photoperiod, this corresponded to an increase in DLI from 15.4 to 18.9 mol·m^{-2}·d^{-1}. During subsequent weeks, instantaneous *PPFD* was maintained at 347 μmol·m^{-2}·s^{-1} (DLI = 20.0 mol·m^{-2}·d^{-1}).

To enable adequate plant establishment, the initial nutrient solution was the modified strawberry nutrient solution described in Expt. 1, with an 80:20 ratio of NO_3^-:NH_4^+ and a total N concentration of 100 mg·L^{-1}. After 2 weeks, the initial nutrient solution in each reservoir was replaced with a treatment solution. The treatment solutions were modified to provide six different NO_3^-:NH_4^+ ratios (0:100, 20:80, 50:50, 60:40, 80:20, or 100:0) (Table 1) at an N concentration of 100 mg·L^{-1}. Plants were grown for 15 weeks with these modified nutrient solutions. Each reservoir was an experimental unit, and there were four replicates per treatment arranged in a completely randomized design. Reservoirs were replenished with nutrient solution as needed and replaced weekly. During weeks 1 and 2, 1.5 mM $KHCO_3$ was added to all the nutrient solutions. During subsequent weeks, the amount of $KHCO_3$ that was needed to buffer the nutrient solutions increased, especially in those with higher percentages of NH_4^+. During week 3, 2.3, 2.0, 1.7, 1.5, 1.3, and 0.8 mM $KHCO_3$ were added to nutrient

solutions with NO_3^-:NH_4^+ ratios of 0:100, 20:80, 50:50, 60:40, 80:20, and 100:0, respectively. During week 4, the $KHCO_3$ added to the nutrient solutions was increased to 2.6, 2.3, 2.0, 1.8, 1.6, and 0.8 mM, respectively; it was not further increased during the trial. The pH of solutions in each reservoir was monitored and adjusted back to 5.7 every 2 to 3 d using 1 M KOH or 1 M HCl.

Runner and inflorescence numbers were monitored weekly, and inflorescences were removed after counting. After 15 weeks of treatment, plants were harvested as described in Expt. 1 to attain the dry mass of the mother plants. Data were analyzed as described in Expt. 1.

Expt. 3: Comparison of the effect of MES or $KHCO_3$ on the buffering capacity of strawberry grown in DWC. To assess the effectiveness of different buffering agents, strawberry 'Monterey' was grown in DWC. Daughter plants were placed in a phenolic foam cube (24.6 cm^3; Smithers-Oasis, Kent, OH, USA). The cube was placed within a net pot, the net pots were placed in a tray, and the plants were propagated in a reach-in growth chamber (BDR16) for 9 d. A shallow layer of water was maintained in the tray to keep the phenolic foam cubes moist. Environmental conditions and irrigation were as described for Expt. 2. Plants were transferred to a walk-in growth chamber (GR48) and grown in a DWC system, as described in Expt. 1. Plants received a 16-h photoperiod. The mean daily air temperature was 25.2 ± 1.6 °C (mean ± *SD*), and the mean RH was 64.8% ± 7.1%. The DLI was gradually increased as plants grew, with values of 11.9, 14.9, 15.6, and 18.5 $mol \cdot m^{-2} \cdot d^{-1}$ during weeks 1, 2, 3, and 4 to 6, respectively.

To enable adequate plant establishment, the initial nutrient solution was the modified strawberry solution described in Expt. 2, with an NO_3^-:NH_4^+ ratio of 80:20 and a total N concentration of 100 $mg \cdot L^{-1}$. After 2 weeks, the nutrient solution was adjusted to an NO_3^-:NH_4^+ ratio of 60:40 described in Expt. 2 (Table 1). Plants were grown for an additional 4 weeks with the 60:40 ratio of NO_3^-:NH_4^+ nutrient solution, which was modified to provide different buffers. There were five buffering treatments. Three treatments consisted of different concentrations of MES (1, 3, or 5 mM) added to the nutrient solution, and two treatments consisted of 2 mM $KHCO_3$ added to the nutrient solution. The initial solution pH was adjusted to 5.8. The pH of the nutrient solution in each reservoir was measured every 2 to 3 d and adjusted back to 5.8 as needed. The pH of the MES-buffered treatments was adjusted with 1 M KOH. In the $KHCO_3$-buffered treatments, the pH of one was adjusted with 1 M KOH, and that of the other was adjusted with 2 mM $KHCO_3$. Reservoirs were filled with 3.3 L of nutrient solution and replenished as needed with nutrient solution; solutions were replaced weekly. Each reservoir was an experimental unit, and there were four replicates per treatment arranged in a completely randomized design.

Runner and inflorescence numbers were counted weekly, recorded, and removed from plants. After 4 weeks of treatment, plant height, plant width (mean of the widest width and the corresponding width perpendicular to it), and dry mass (partitioned into aboveground and belowground fractions) were assessed. Data were analyzed as described in Expt. 1.

Results and Discussion

Expt. 1: Evaluation of growing systems. The pH of the nutrient solutions or leachates differed across the four growing systems evaluated (Table 2). The nutrient solution or leachate pH was not compared statistically because the collection timepoints did not align. However, our data indicated an increase in the pH of the perlite and sand drain solutions, whereas the pH of the DWC nutrient solution decreased. Although the starting pH of the nutrient solution was 5.7, the pH of the drain solutions from the drip-irrigated coarse perlite and sand substrates increased to 7.2 ± 0.1 and 7.3 ± 0.1, respectively. Likewise, the pH of the peat-based substrate solution increased to 6.1 ± 0.1, but the pH of the DWC system decreased to 4.0 ± 0.1 in the absence of any pH adjustment.

The observed increase in the drain solution pH relative to the applied nutrient solution pH for perlite and sand growing systems is consistent with their physical and chemical properties. Perlite (potassium sodium aluminum silicate) and sand are inert, have a neutral pH of 7.0 to 7.5, and have a lower water-holding capacity and buffering capacity than peat (Maucieri et al. 2020; Olympios 1992). Similar to our observation, Yang et al. (2023) noted a higher leachate pH from perlite than from organic substrates (peat, bark, coir, or wood fiber) during the vegetative growth stage of cucumber (*Cucumis sativus*). The pH of the peat-based substrate solution in our study remained within the suggested range of 5.5 to 6.5 for soilless cultivation of strawberry (Hancock et al. 1991; Hosseinzadeh et al. 2017). This is likely because of the addition of liming materials by the commercial substrate producer to increase the starting pH of the peat-based substrate and increase the buffering capacity (Fisher et al. 2006; Handreck and Black 1994; Pancerz and Altland 2020). However, the lack of buffering capacity in the DWC system allowed the nutrient solution pH to drift, predominantly based on the root nutrient uptake and corresponding ion exchange to maintain the charge balance in the plant.

Despite the low nutrient solution pH, plants grown in DWC produced more total inflorescences (3 ± 0.3) than those grown in coarse perlite (1 ± 0.3) ($P = 0.02$; Table 2). Runner number and aboveground biomass were similar across all four growing treatments ($P = 0.32$ and $P = 0.63$, respectively). Root dry mass was greatest in the peat-based treatment (6.3 g ± 0.4; $P < 0.0001$), and it ranged from 0.7 (DWC) to 1.7 g (perlite) in the other treatments.

The greater root growth in the peat-based substrate, compared with that of the other treatments, is likely attributable to the chemical and physical properties of the substrate. The pH of the peat-based substrate remained within the recommended range for ideal production. The root growth of some species are sensitive to the nutrient solution pH. Gillespie et al. (2021) observed a decrease in spinach (*Spinacea oleracea* 'Corvair') root fresh and dry mass as the DWC nutrient solution pH decreased from 5.5 to 4.0. Likewise, 20-d-old bean plants grown in sandy loam soil had stunted root growth (shorter and lower mass) when grown at a rhizosphere pH of 4.5 compared with 5.6 to 6.6 (Thomson et al. 1993). However, other crops are less sensitive. For example, the root fresh and dry mass of basil (*Ocimum basilicum* 'Dolce

Table 2. Plant growth and pH of strawberry (*Fragaria ×ananassa* 'Albion') in four growing systems [deep water culture (DWC), a peat-based soilless substrate, coarse perlite, or sand] for 6 weeks. The pH values of the reservoir nutrient solution (DWC; measured weekly), leachate (peat-based substrate; measured 3 weeks after transplant), or drain solutions (perlite and sand; measured 2 and 4 weeks after transplant) are mean values across the duration of the study. Plant growth data (mean ± *SE*) were analyzed by a one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) at $\alpha = 0.05$ for significant sources of variation (n = 4). Within a column, means followed by a different letter are statistically different.

System	pH	Inflorescence (no.)	Runner (no.)	Mother plant dry mass (g)	Root dry mass (g)
Deep water culture	4.0 ± 0.1	3 ± 0.3 a	5 ± 0.5	7.3 ± 0.9	0.7 ± 0.1 b
Peat-based substrate	6.1 ± 0.1	1 ± 0.5 ab	4 ± 0.3	5.8 ± 0.8	6.3 ± 0.4 a
Perlite	7.2 ± 0.1	1 ± 0.3 b	4 ± 0.6	5.4 ± 0.7	1.7 ± 1.2 b
Sand	7.3 ± 0.1	2 ± 0.5 ab	6 ± 1.2	6.4 ± 1.6	0.8 ± 0.4 b
ANOVA	[i]	0.02	0.32	0.63	<0.0001
HSD		1.6			2.3

[i] pH was not compared statistically because timepoints did not align.

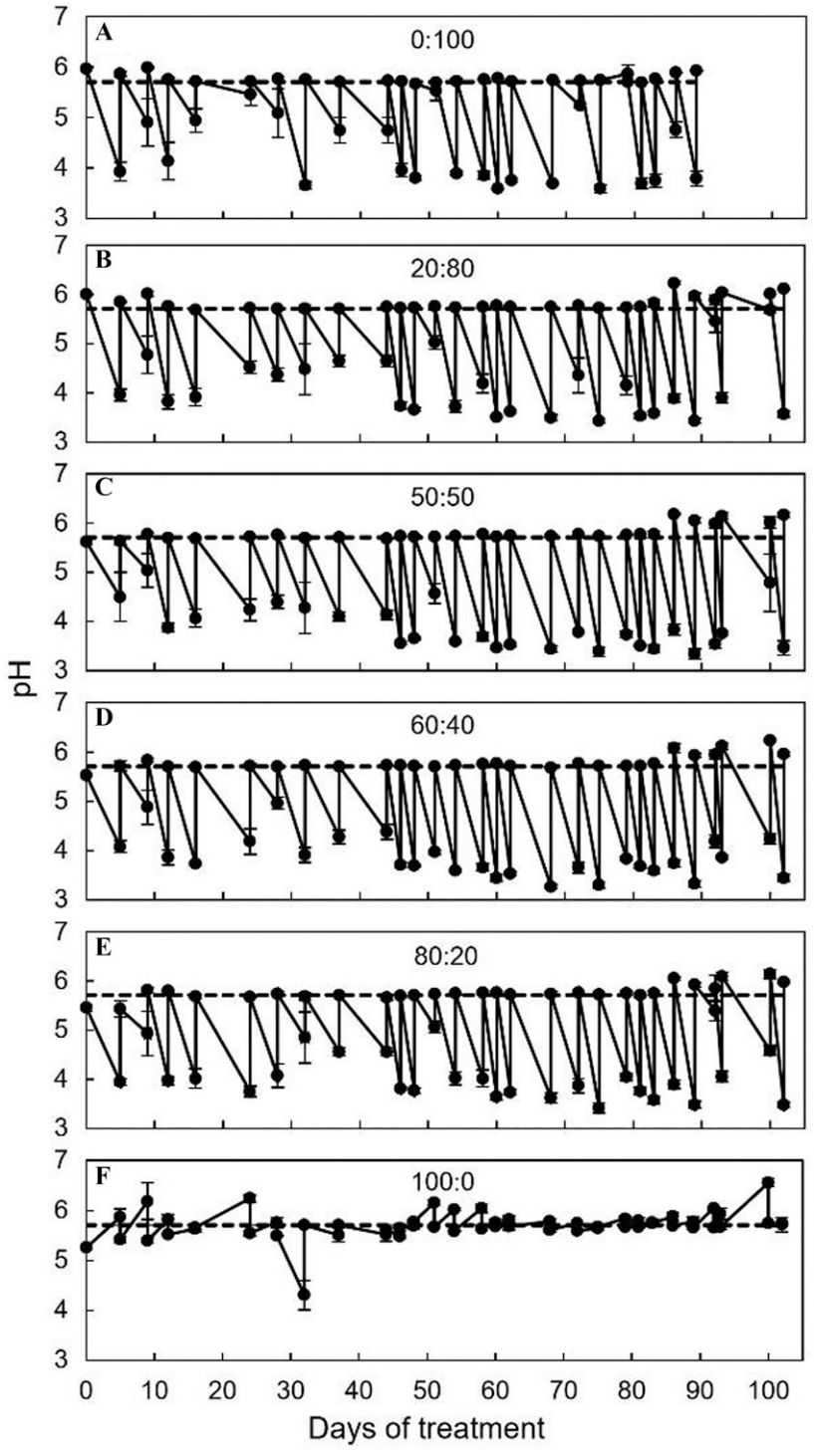

Fig. 1. The effect of potassium bicarbonate ($KHCO_3$) and ratio of nitrate (NO_3^-) to NH_4^+ (ammonium) on the buffering capacity of nutrient solutions on strawberry (*Fragaria ×ananassa* 'Fronteras') grown in deep water culture (n = 4). Solution NO_3^-:NH_4^+ ratios were 0:100 (**A**), 20:80 (**B**), 50:50 (**C**), 60:40 (**D**), 80:20 (**E**), and 100:0 (**F**). The amounts of $KHCO_3$ added to each nutrient solution were 2.6, 2.3, 2.0, 1.8, 1.6, and 0.8 mM, respectively. The initial nutrient solution pH was 5.7 (dotted line), and it was readjusted back to 5.7 every 2 to 3 d using potassium hydroxide. Data points are means ± *SE*.

Fresca' and 'Nufar') grown in DWC were similar at nutrient solution pH values of 4.0 and 5.5 (Gillespie et al. 2020). It appeared that strawberry root growth is sensitive to sub-optimal pH. Additionally, the roots from the plants grown in the peat-based substrate were thicker and had better secondary branching, relative to the other treatments. Plants grown in the perlite and sand treatments had very fine roots, which may be the result of the pulse irrigation, which maintained a high moisture content in these substrates.

For research applications, the main advantages of growing in coarse perlite or sand instead of a peat-based substrate would be easier separation of roots from the substrate, loss of fewer roots during the cleaning process (leading to more accurate root mass values), and cleaner roots for elemental analysis. However, neither coarse perlite nor sand was a suitable substrate compared with the peat-based substrate as the pH increased higher than desired in the leachates of these treatments, and the root growth was lower. This is in agreement with the results of Taghavi et al. (2017), who also observed lower plant growth with perlite and sand compared with the peat-based blends. The DWC system also has the same advantages as sand or perlite, with the additional benefit of not having to separate the roots from any substrate. During this study, DWC also experienced pH drift, but in the opposite direction. Like plants grown in perlite or sand, aboveground plant growth in DWC was similar to that of plants grown in a peat-based substrate, and root growth was lower, in part because of the low pH. However, the ease of obtaining clean roots from the DWC system warranted additional investigation to determine whether the pH drift issue could be adequately managed.

One potential strategy to minimize pH flux in weakly or nonbuffered nutrient solutions is to balance the uptake of cations and anions. Dickson et al. (2016) hypothesized that the NO_3^-:NH_4^+ ratio could be adjusted to maintain a stable hydroponic nutrient solution pH based on differential uptake of cations and anions by different plant species. Lea-Cox et al. (1997) effectively demonstrated this with wheat (*Triticum aestivum*) using a species-specific NO_3^-:NH_4^+ ratio of 75:25. The NO_3^-:NH_4^+ ratio of the nutrient solution in our study (Expt. 1) was 80:20; although it was close to the ratio used by Lea-Cox et al. (1997), it did not adequately maintain pH within the desired range for strawberry. Therefore, we proceeded to evaluate different NO_3^-:NH_4^+ ratios as well as the addition of buffers to the nutrient solution for DWC production of strawberry to determine whether DWC could be a suitable growing system for nutrient-based research studies.

Expt. 2: Impact of NO_3^-:NH_4^+ on DWC nutrient solution pH. Among the six NO_3^-:NH_4^+ ratios evaluated, 0.8 mM $KHCO_3$ adequately buffered the 100% NO_3^- solution, and the average pH was 5.8 ± 0.04 throughout the trial (Fig. 1; Table 3). The slight increase in pH, relative to the initial nutrient solution pH, in the 100% NO_3^- treatment may have been attributable to the release of OH^- from roots to offset the NO_3^- uptake (Allen and Raven 1987; Kirkby and Knight 1977).

In contrast, the pH of solutions containing NH_4^+ decreased even with the addition of up to 2.6 mM $KHCO_3$ (Fig. 1A–E; Table 3). The mean pH across all NH_4^+-containing treatments decreased from 5.7 to 4.2 ± 0.1 during the 2 to 3 d after each pH re-adjustment. The decrease in pH of the NH_4^+-containing solutions is likely attributable to NH_4^+ uptake by the roots (Neumann and Römheld

Table 3. Impact of the nitrate-to-ammonium ratio (NO_3^-:NH_4^+) on the nutrient solution pH buffered by varied amounts of potassium bicarbonate ($KHCO_3$). Strawberry (*Fragaria ×ananassa* 'Fronteras') were grown in deep water culture for 15 weeks and nutrient solution pH was adjusted back to 5.7 every 2 to 3 d (n = 4). The pH decrease between adjustment timepoints was consistent throughout the study; therefore, the mean pH (±*SE*) before adjustment back to 5.7 is pooled across all time points, along with the magnitude of change (Δ pH). The pH was analyzed using a one-way analysis of variance (ANOVA), and means followed by a different letter are statistically different according to Tukey's honest significant difference (HSD) test at $\alpha = 0.05$.

NO_3^-:NH_4^+	$KHCO_3$ added to nutrient solution (mM)	pH	Δ pH
0:100	2.6	4.4 ± 0.08 b	−1.3
20:80	2.3	4.2 ± 0.06 bc	−1.5
50:50	2.0	4.1 ± 0.07 c	−1.6
60:40	1.8	4.0 ± 0.05 c	−1.7
80:20	1.6	4.2 ± 0.06 bc	−1.5
100:0	0.8	5.8 ± 0.04 a	0.1
ANOVA		<0.0001	
HSD		0.2	

2012). To maintain the charge balance, roots release cations into the rhizosphere to offset the uptake of nutrient cations, like NH_4^+. Root-excreted cations are predominantly H^+, which decrease pH, although they can include potassium (K^+), calcium (Ca^{2+}), and sodium (Na^+) (White 2012). As a result, the rhizosphere pH tends to decrease when plant uptake of NH_4^+ exceeds the uptake of NO_3^-. For example, the nutrient solution pH decreased from 6.5 to 4.4 when NH_4^+ was the sole N source provided to castor bean (*Ricinus communis*), whereas the pH increased to 6.9 when only NO_3^- was supplied (Allen and Smith 1986). Similarly, bean grown in a sandy loam soil experienced a decrease in pH from 6.5 to 4.5 in the rhizosphere when provided NH_4^+ plus a nitrification inhibitor (N-Serve) (Thomson et al. 1993).

Across the range of 20% to 100% NH_4^+ in our nutrient solutions, we observed a similar decrease in pH in these five treatments between adjustment timepoints. However, the decrease in pH did not increase in magnitude with increasing NH_4^+ fraction (Fig. 1A–E). Nutrient uptake is primarily driven by passive diffusion and bulk flow via transpiration and, therefore, often scales with plant biomass (van Beusichem et al. 1988). As a macronutrient, N has a large influence on the pH adjustment of the nutrient solution because of the need to maintain charge balance homeostasis. Similarly, in castor bean, the observed pH of nutrient solution averaged 4.4 regardless of an increase in the NH_4^+ concentration from 0.8 to 4 mol·m^{-3} (Allen and Smith 1986). Because NH_4^+ uptake by roots is passive and NO_3^- uptake is energy-requiring, there is often a preference for NH_4^+ uptake when both are supplied (Neumann and Römheld 2012; van Beusichem et al. 1988). As such, a nutrient solution for strawberries may need to be predominantly NO_3^--based to maintain pH equilibrium and/or well-buffered to offset the preferential NH_4^+ uptake.

Different species have different capacities to influence substrate or nutrient solution pH (Chapin et al. 1993; Marschner et al. 1991) based on net cation or anion uptake. For example, Dickson et al. (2016) supplied the same nutrient solution comprising a 50:50 ratio of NO_3^-:NH_4^+ to hydroponically grown plants and observed that geranium (*Pelargonium ×hortorum*) decreased the solution pH the most, impatiens (*Impatiens wallerana*) decreased the pH but to a lesser extent, and petunia (*Petunia ×hybrida*) decreased the pH the least. However, the nutrient solution pH did not fluctuate greatly for these three species when the N source provided was 100% NO_3^- (Dickson et al. 2016). The impact of species on nutrient solution pH may be attributable to genetic factors or soil conditions in which plants evolved.

Plants did not flower during Expt. 2. 'Fronteras' has a short-day photoperiod requirement (Larson and Shaw 2015); therefore, under the 16-h photoperiod, it did not initiate inflorescence development. With the treatments comprising the 0:20 to 100:0 ratios of NO_3^-:NH_4^+, runner number (10–15; $P = 0.52$), mother plant dry mass (5.1–8.7 g; $P = 0.63$), and root dry mass (0.9–1.5 g; $P = 0.24$) were similar (Supplemental Table 1). Data were not collected for the 100% NH_4^+ treatment because plants did not survive to the end of the study. Qualitatively, crown growth, runner number, and root growth of the 100% NH_4^+ treatment appeared to be similar to those of the other treatments early during the study. However, as the weeks progressed, crown and root growth became stunted, and all plants died by 90 d after the start of the treatment. In a related study, strawberry plants grown with 100% NH_4^+ solution declined and some died, especially as the root zone temperature increased from 25 to 32 °C (Neumann-Ganmore and Kafkafi 1983). Although plant death during our study could have been attributable to a combination of multiple factors, we suspect that NH_4^+ toxicity was a contributing factor. We observed crown and root growth suppression, as well as leaf curl, which are typical symptoms of NH_4^+ toxicity (Shilpha et al. 2023). Ammonium toxicity has been shown to be exacerbated at a low pH. Claussen and Lenz (1999) grew strawberry 'Senga Sengana' in quartz sand, with and without the addition of calcium carbonate ($CaCO_3$), and they observed more pronounced effects of NH_4^+ toxicity in plants grown without $CaCO_3$ (i.e., at a lower pH). Therefore, prolonged exposure to 100% NH_4^+, especially with a low substrate or solution pH, is not advised for strawberry production.

During Expt. 1, the DWC system produced a similar number of 'Albion' runners as the peat-based substrate (five and four runners, respectively, after 6 weeks). Although runner capacity is cultivar-specific and varies depending on plant size, environmental conditions, and duration between harvests, the number of runners produced in our study in DWC was comparable to that of other growth chamber-based studies. 'Albion' produced three to four runners when grown in a peat-based substrate for 12 weeks (Xu and Hernández 2020). During Expt. 2, the runner number of 'Fronteras' was 10 to 15, depending on the NO_3^-:NH_4^+ treatment provided. This greater number was likely attributable to a combination of cultivar and longer experimental period (15 weeks of treatment).

The use of clay pebbles during Expt. 2 to support strawberry crowns in a net pot was easy to use, inexpensive, and provided adequate stability. However, the ability of the clay pebbles to move water through capillary action and retain water in their pores can be detrimental to strawberry growth if the pebbles remain in contact with the nutrient solution and are densely packed around the crown. This can keep the crown too wet and lead to crown rot. When nutrient solution is added, care should be taken to fill the reservoir to just below the net pot to minimize direct contact of the nutrient solution with the clay pebbles but allow for good root contact with the nutrient solution. To minimize the potential for excessive moisture around the strawberry crown, phenolic foam was selected as an alternative substrate for Expt. 3.

To grow strawberry plants in DWC, in the presence of NH_4^+, we determined that a greater buffering capacity of the nutrient solution was needed to maintain pH and provide a suitable environment for healthy root growth. We did not evaluate $KHCO_3$ concentrations above 2.6 mM in the nutrient solution because we did not want to increase nutrient solution EC too much and unintentionally induce osmotic stress. Therefore, we considered MES as a potential buffer because of its effectiveness in hydroponic systems when supplied at concentrations of 1 to 10 mM (Bugbee and Salisbury 1985; Frick and Mitchell 1993; Miyasaka et al. 1988).

Expt. 3: Comparison of the effect of MES or $KHCO_3$ on the buffering capacity of strawberry grown in DWC. The MES provided greater buffering capacity than $KHCO_3$ for hydroponic strawberry 'Monterey' production. Consistent with Expt. 2, the addition of 2 mM $KHCO_3$ to a nutrient solution with a 60:40 ratio of NO_3^-:NH_4^+ was ineffective for buffering pH, and pH adjustment back to 5.8 with either 1 M $KHCO_3$ or 1 M KOH did not maintain a stable pH (Fig. 2A and B; Table 4). With the MES treatments, the buffering capacity of the nutrient solution increased

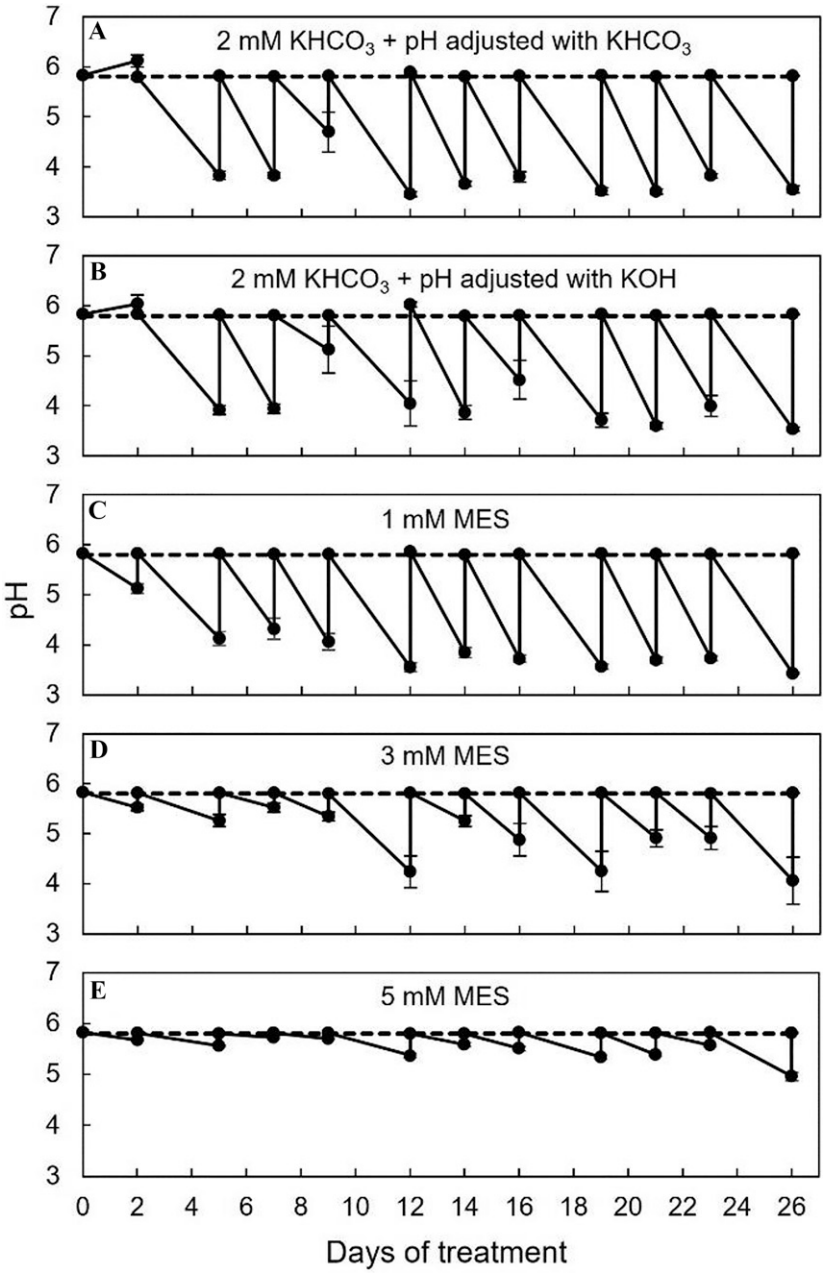

Fig. 2. The effect of 2(N-Morpholino)ethanesulfonic acid (MES) or potassium bicarbonate ($KHCO_3$) on the buffering capacity of a 60:40 nitrate (NO_3^-) to ammonium (NH_4^+)-based nutrient solution on strawberry (*Fragaria ×ananassa* 'Monterey') grown in deep water culture (n = 4). Data points are means ± *SE*. Buffering treatments were 2 mM $KHCO_3$ in the nutrient solution with pH adjustment using 1 M $KHCO_3$ (**A**), 2 mM $KHCO_3$ in the nutrient solution with pH adjustment using 1 M potassium hydroxide (KOH) (**B**), and 1 mM MES (**C**), 3 mM MES (**D**), and 5 mM MES (**E**) in the nutrient solution with pH adjustment using 1 M KOH. The initial nutrient solution pH was 5.8 (horizontal dotted line), and it was readjusted back to 5.8 every 2 to 3 d.

as the MES concentration increased. The lowest concentration evaluated (1 mM) was not sufficient to maintain pH in the desired range. The 3 mM MES provided some buffering, especially initially, when plants were small. The highest concentration of MES evaluated (5 mM) provided sufficient buffering capacity over the 4-week experiment (Fig. 2C–E; Table 4). The addition of 5 mM MES to the nutrient solution stabilized the pH (5.6 ± 0.2, Δ pH = −0.2 every 2 to 3 d before adjustment back to 5.8) and allowed it to remain within the recommended range previously reported for optimal nutrient solubility (Argo and Biernbaum 1996; Handreck and Black 1994). The response observed with 5 mM MES is generally consistent with that reported by other studies. Bugbee and Salisbury (1985) observed adequate buffering with 1 to 3 mM MES when growing bean (*Phaseolus vulgaris* 'Bush Blue Lake'), corn (*Zea mays* 'JX L.1'), lettuce (*Lactuca sativa* 'Grand Rapids'), tomato (*Lycopersicon esculentum* 'Heinz 1320'), and wheat 'Fremont' for 3 to 4 weeks. The efficacy observed at a slightly lower MES concentration may have been attributable to the species selection or the use of a nutrient solution with a higher NO_3^-:NH_4^+ (8:1.5) than what we used in our study. The addition of 5 mM MES was sufficient for hydroponic production of rapeseed (*Brassica napus*) (Frick and Mitchell 1993) and winter wheat 'Centurk' (Miyasaka et al. 1988).

Adding MES to the nutrient solution did not affect plant growth, as evaluated by runner production (7–9; $P = 0.58$), plant morphology (height and width: $P = 0.11$ and 0.48, respectively), shoot biomass (12.9–16.8 g; $P = 0.22$), and root biomass (2.1–2.9 g; $P = 0.47$), which were similar across the treatments (Supplemental Table 2). The concentration of MES required to maintain the solution pH will likely depend on plant species, plant size, water uptake, DWC reservoir capacity, study duration, N concentration, and N ratio. Early during production, when the plant size is small relative to the reservoir size, a lower concentration of MES will be needed to buffer nutrient solution pH. Later in production, as the plant size and water and nutrient uptake increases, a higher concentration of MES may be needed to maintain nutrient solution pH. For example, in Expt. 3, the solution pH during weeks 1 to 3 were similar for the 3-mM and 5-mM MES treatments. By week 4, however, the 3-mM MES treatment was unable to maintain a pH similar to that of the 5-mM treatment, and greater pH drift began to occur between adjustment timepoints. This likely would have increased in amplitude if the study had been extended for a longer duration. Likewise, increasing the volume of buffered nutrient solution can aid in pH maintenance. A transition to a larger reservoir may have extended the effectiveness of the 3-mM MES treatment. When the reservoir size was increased from 3.3 to 6.0 L, the effectiveness of a 3-mM MES treatment was extended by an additional 2 weeks for strawberry (data not shown).

Although different strawberry cultivars were used across the three experiments, their impact on nutrient solution pH was consistent. In a pilot study, 'Albion', 'Fronteras', and 'Monterey' were grown in DWC with an 80:20 ratio of NO_3^-:NH_4^+ nutrient solution (same as Expts. 1 and 2) and 1 mM $KHCO_3$. Over a 4-week treatment duration, the nutrient solution pH decreased consistently to 4.0 to 4.5 in all three cultivars between pH adjustment back to 5.8 (Fig. 3). At all timepoints, a one-way ANOVA for cultivar was nonsignificant ($P > 0.05$). Therefore, we anticipate that MES can effectively buffer the nutrient solution of strawberry. However, evaluating the MES concentration required to maintain the desired target pH range for other cultivars not used in these studies is recommended in case of a cultivar-specific response.

When selecting a buffer, material costs, labor costs for checking and adjusting pH (if performed manually), reservoir volume, reservoir-to-reservoir variability, and monitoring frequency are important factors to consider in addition to buffer efficacy. Although MES was more effective than $KHCO_3$ at maintaining DWC nutrient solution pH when

Table 4. Effect of 2(N-Morpholino)ethanesulfonic acid (MES) or potassium bicarbonate ($KHCO_3$) on the nutrient solution buffering capacity. Strawberry (*Fragaria ×ananassa* 'Monterey') were grown in deep water culture (DWC) for 4 weeks (n = 4), and the initial buffering treatments were 2 mM $KHCO_3$ or 1, 3, or 5 mM MES. Nutrient solution pH was adjusted back to 5.8 every 2 to 3 d. The MES-buffered treatments were adjusted with 1 M KOH. In the $KHCO_3$-buffered treatments, one was adjusted with 1 M KOH, and the other was adjusted with 2 mM $KHCO_3$. Solution pH, measured 2 d after solution replacement, was analyzed using a one-way analysis of variance (ANOVA), and means (±*SE*) within a column followed by a different letter are statistically different according to Tukey's honest significant difference (HSD) test at $\alpha = 0.05$. A change in pH (Δ pH) represents the difference between the starting pH of 5.8 and measured pH 2 d after nutrient solution replacement.

	Week 1		Week 2		Week 3		Week 4	
Buffer	pH	Δ pH	pH	Δ pH	pH	Δ pH	pH	Δ pH
2 mM $KHCO_3$ + pH adjusted with $KHCO_3$	6.1 ± 0.1 a	−0.3	4.7 ± 0.4 ab	−1.1	3.8 ± 0.1 b	−2.0	3.8 ± 0.1 c	−2.0
2 mM $KHCO_3$ + pH adjusted with KOH	6.0 ± 0.2 a	−0.2	5.1 ± 0.5 ab	−0.7	4.5 ± 0.4 ab	−1.3	4.0 ± 0.2 c	−1.8
1 mM MES	5.1 ± 0.1 c	−0.7	4.1 ± 0.2 b	−1.8	3.7 ± 0.1 b	−2.1	3.7 ± 0.1 c	−2.1
3 mM MES	5.5 ± 0.1 bc	−0.3	5.4 ± 0.1 a	−0.5	4.9 ± 0.3 a	−0.9	4.9 ± 0.2 b	−0.9
5 mM MES	5.7 ± 0.0 ab	−0.1	5.7 ± 0.0 a	−0.1	5.5 ± 0.0 a	−0.3	5.6 ± 0.0 a	−0.2
ANOVA	<0.0001		0.01		0.0003		<0.0001	
HSD	0.5		1.3		1.0		0.6	

NH_4^+ was included during this study, its cost is considerably higher. Based on the current prices for laboratory-grade reagents, the addition of 2 mM $KHCO_3$ to 3.5 L of nutrient solution costs $0.11, whereas the addition of 5 mM MES costs $5.60 (Table 5). Initially, the time dedicated to monitoring and adjusting pH will be similar for both buffers until enough historical data have been compiled for a site to determine a preferred monitoring interval. Over time, the frequency of manually checking pH can be less for MES if it is supplied at a sufficiently high concentration, depending on the amplitude of deviation from the initial nutrient solution pH that a researcher or grower deems acceptable. During Expt. 3, the 5-mM MES treatment was most consistent; it had the smallest variability between reservoirs during all 4 weeks (*SE* = 0) (Table 4). However, within-treatment variability was consistent for the lower MES concentrations and the $KHCO_3$-buffered solutions (*SE*s ranged between 0.1 to 0.5). Taken collectively, although 5 mM MES effectively buffered strawberry in DWC, it may not be a practical option for all situations. Its use may be limited to small-scale production for which very tight control of nutrient solution pH is desired (e.g., research applications).

In summary, our initial objective was to identify an effective growing system and protocol that would allow us to easily and effectively monitor both root and shoot growth of strawberry. The peat-based, perlite, and sand substrates and the DWC system all produced similar runner numbers and mother plant dry mass. The main difference was in the root growth. Plants grown in the peat-based substrate had a higher root mass, whereas root mass of plants grown in perlite, sand, and DWC were lower but similar. Because the ease of root collection was a key priority, DWC was considered preferable to sand or perlite. Therefore, the preferred system was narrowed down to the peat-based substrate and DWC, with the main tradeoff being root mass vs. ease of collection. Strawberry plants in the DWC system developed sufficient root mass for elemental analysis, but the low pH of the minimally buffered nutrient solution needed to be addressed. Adjusting the NO_3^-:NH_4^+ ratio (with additional buffering from $KHCO_3$) was insufficient to maintain pH within the desired range, but MES at 5 mM was effective. A nutrient solution with a high NO_3^-:NH_4^+ ratio (up to 80% NO_3^-) did not impact mother plant growth, but it was insufficient to mitigate pH drift when $KHCO_3$ was provided as a buffering agent. Additional evaluation is required to determine the minimum %NH_4^+ that can be provided in a $KHCO_3$-buffered nutrient solution and maintain pH. Adding MES at 5 mM was effective at maintaining the pH of an NO_3^-:NH_4^+ nutrient solution with a ratio of 60:40. Lower MES concentrations may be required if the nutrient solution has a higher NO_3^-:NH_4^+, but that was not evaluated in this set of experiments. One drawback to using DWC is that it does not exactly mimic soilless substrate systems; however, hydroponic liquid culture systems are frequently used for research applications, and the use of hydroponics systems for commercial strawberry production continues to increase.

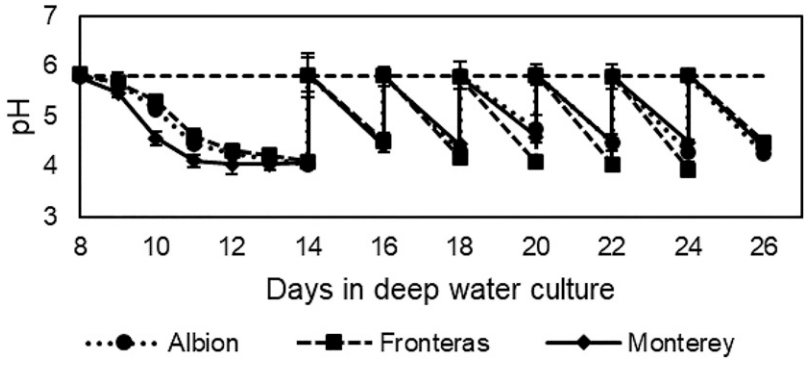

Fig. 3. A comparison of the nutrient solution pH of three strawberry (*Fragaria ×ananassa*) cultivars ('Albion', 'Fronteras', and 'Monterey') grown in a deep water culture (DWC) system. The nutrient solution supplied 100 mg·L^{-1} N at a nitrate (NO_3^-)-to-ammonium (NH_4^+) ratio of 80:20, and it was buffered with 1 M potassium bicarbonate ($KHCO_3$). The initial nutrient solution pH was 5.7 (horizontal dotted line), and it was readjusted back to 5.7 on days 7 and 14, and then every 2 d using potassium hydroxide. Data points are means ± *SE* (n = 4). At each timepoint, the nutrient solution pH before pH adjustment was analyzed using a one-way analysis of variance (ANOVA) and cultivar was nonsignificant ($P > 0.05$) on all days.

Conclusion

A DWC system for strawberry production can be suitable if the nutrient solution is adequately buffered to maintain a suitable pH. A 100% NO_3^- nutrient solution could maintain the pH range desired for optimal nutrient solubility with the addition of 0.8 mM $KHCO_3$. We provided a protocol that accounts for a decrease in pH that occurs when NH_4^+ is included in a nutrient solution. The nutrient solution pH decreased when as little as 20% of total N was supplied in the form of NH_4^+, even with the addition of up to 2.6 mM $KHCO_3$. Growing strawberry in DWC required the use of a different buffer in the nutrient solution to counter acidification by roots. The addition of 5 mM MES provided desired pH management. Given the cost of MES, this may be practical only for small-scale research applications, especially for nutrient studies in which access to clean roots for elemental analysis is desired.

Table 5. Comparison of 2(N-Morpholino)ethanesulfonic acid (MES) or potassium bicarbonate ($KHCO_3$) as buffers for hydroponic deep water culture (DWC) of strawberry (*Fragaria ×ananassa*).

Buffer	Concn (mM)	Cost ($)/3.5 L reservoir volume	Buffer efficacy[iii]	Labor[iv]
MES[i]	1	1.12	Low	Medium
MES	3	3.36	Medium	Medium
MES	5	5.60	High	Low
$KHCO_3$[ii]	2	0.11	Low	Medium

[i] More than 99.0% purity, molecular weight of 213.25, and cost of $750/500 g.

[ii] Purity of 99.7%, molecular weight of 100.12, and cost of $81.10/500 g.

[iii] Based on the magnitude of pH drift, where pH < 4 is low, pH of 4.1 to 5.3 is medium, and pH of 5.4 to 6.2 is high.

[iv] Based on the days per week required to measure and adjust the pH, where 1 to 2 is low, 3 to 4 is medium, and ≥5 is high.

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