Preplant Fertilization Increases Substrate Microbial Respiration But Does Not Affect Southern Highbush Blueberry Establishment in a Coconut Coir-based Substrate

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Abstract. Coconut coir is widely used as a substrate component for southern highbush blueberry [(SHB) *Vaccinium corymbosum* L. interspecific hybrids] cultivation in containers. Coconut coir-based substrates can exhibit high potassium (K), sodium (Na), and chlorine (Cl) concentrations. Sodium in the substrate is particularly problematic because it can cause salinity stress and nutritional imbalances in young blueberry plants. Thus, Na removal is important to ensure transplant success. We hypothesized that preplant fertilization with large volumes of nutrient solution can reduce substrate salinity, replace Na with nutritional cations, and enhance blueberry establishment. We tested this hypothesis in a greenhouse experiment with ‘Snowchaser’ SHB grown in rhizoboxes filled with a 7:3 mix of coconut coir and perlite. Four different treatments were delivered every 24 hours starting 72 hours before transplant. Treatments included 1.75 g·L⁻¹ calcium nitrate (CN), 2.38 g·L⁻¹ monoammonium phosphate (MAP), deionized water, and well water. One rooted cutting was transplanted to each rhizobox. Rhizoboxes were fertigated during the 7-week cultivation period. We found that preplant fertilization increased nitrogen (N), phosphorus (P), and calcium (Ca) concentrations in the substrate without replacing Na. Thus, preplant fertilization increased substrate salinity. Preplant fertilization also promoted microbial respiration in the substrate at the start of the experiment. Treatments did not affect SHB root architecture, leaf area index, leaf greenness, or biomass accumulation, likely because nutrients delivered by the fertigation solution provided the plants with homogeneous optimal conditions. These findings suggest that preplant fertilization with large volumes of nutrient solution does not enhance blueberry establishment in coconut coir-based substrates.

Blueberry (*Vaccinium corymbosum* L. interspecific hybrids) production in containers filled with soilless substrates is rapidly expanding globally (Cronquist and Cook, 2020). In soilless substrates, blueberry exhibits high productivity from the first year of cultivation (Fang et al., 2020). One of the reasons for this vigorous growth pattern is that soilless substrates allow growers to optimize the crop rhizosphere by managing moisture content and nutrient concentrations, and by limiting edaphic stress.

Coconut coir is a common component of soilless substrates used in blueberry production (Kingston et al., 2017) because of its favorable physical and chemical characteristics (Abad et al., 2002; Evans et al., 1996). However,

coconut coir can have high Na, K, and Cl concentrations (Abad et al., 2002). Thus, some manufacturers rinse coconut coir before commercialization (Poulter, 2014). In hydrated coconut coir, cations (such as Na⁺ and K⁺) are found in dynamic equilibrium between the substrate adsorption sites and the substrate solution, whereas anions (such as Cl⁻) are found almost exclusively in the substrate solution. Thus, a combination of cation replacement and solution displacement can prepare coconut coir for optimal transplant.

Sodium is not an essential nutrient for plants (Maathuis, 2014). In addition, as a monovalent ion (Na⁺) with small atomic radius, Na has a high affinity for substrate adsorption sites and is a major contributor to substrate solution salinity (Havlin et al., 2013). Blueberry plants are particularly sensitive to salinity stress (Retamales and Hancock, 2018). In addition, high Na in the growing medium can affect blueberry uptake of other cations (Spiers, 1993). Thus, reducing Na content in the substrate is an important step for blueberry transplant success.

The objective of our study was to investigate the effects of preplant fertilizer application

on coconut coir-based substrate characteristics and blueberry establishment. We hypothesized that preplant fertilization with large volumes of nutrient solution can 1) reduce substrate salinity, 2) replace Na with nutritional cations, and 3) enhance blueberry establishment. Our rationale was that the fertilizer solution would displace the dissolved ion pool, whereas the cations in the fertilizer solution would replace adsorbed Na in the substrate.

Materials and methods

Plant cultivation and measurements. This experiment was carried out in a previously described rhizobox system (Schreiber and Nunez, 2021). Rhizoboxes contained ≈1.7 L of a substrate composed of 70% hydrated coconut coir (SpongeEase™, Enroot 65 Products LLC, Cromwell, CT) and 30% horticultural-grade perlite (American Garden Perlite, 66 LLC, Lake Wales, FL) (Fig. 1). Coconut coir bricks (70.8 L) were hydrated overnight with greenhouse water before mixing with perlite. Substrate in each rhizobox was treated with ≈350 mL of treatment solution 72 h, 48 h, and 24 h before transplant. Treatment solutions included 1.75 g·L⁻¹ CN (Southern Ag, Rubonia, FL), 2.38 g·L⁻¹ MAP (Greenway Biotech, Santa Fe Springs, CA), deionized water [pH, 5.4; electrical conductivity (EC), 0.03 dS·m⁻¹], and well water from the greenhouse irrigation system (pH, 7.9; EC, 0.40 dS·m⁻¹). Well water analysis results are presented in Supplemental Table 1. Fertilizer solutions were prepared with deionized water to yield a final EC of 2.0 dS·m⁻¹ according to the method outlined in Fisher et al. (2014).

Rooted cuttings of ‘Snowchaser’ SHB (*Vaccinium corymbosum* interspecific hybrids) were obtained from a commercial nursery (AgriStarts LLC, Apopka, FL) and transplanted into the rhizoboxes. There was one plant in each rhizobox and 10 rhizoboxes per treatment. Ten additional plants were harvested destructively before transplant and used to determine the starting dry weight of the transplants. Each rhizobox was fertigated with two 2-L·h⁻¹ pressure-compensating emitters (Netafim Irrigation, Fresno, CA). The stock fertigation solution was prepared with 23.8 g·L⁻¹ soluble fertilizer (21N–3.0P–5.9K, Peters Professional Acid Special; ICL Specialty Fertilizers, Summerville, SC), with 11.47% and 9.53% of the N provided as ammonium and urea, respectively. Sulfuric acid (35%) was used to bring the fertigation solution to pH 6.0. A hydraulic injector (Dosa-tron, Clearwater, FL) calibrated for an injection rate of 1:50 was used. Plants were fertigated four times a day for 8 min with a nutrient solution that contained 100 mg·L⁻¹ N, 16.7 mg·L⁻¹ P, 27.6 mg·L⁻¹ K, 2.86 mg·L⁻¹ magnesium, 61.9 mg·L⁻¹ sulfur, 0.71 mg·L⁻¹ iron, 0.23 mg·L⁻¹ manganese, 0.14 mg·L⁻¹ boron, and 0.04 mg·L⁻¹ molybdenum. By week 4 of the experiment, plants in all treatments exhibited irregular discoloration in older leaves—a symptom associated with salinity stress or nutritional imbalances in blueberry. Thus, the injection rate was adjusted to 1:100 to reduce the fertigation

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Fig. 1. Rhizobox system used to investigate southern highbush blueberry responses to preplant fertilizer applications. (A) Rhizoboxes contained 1.7 L of a substrate composed of 70% hydrated coconut coir and 30% horticultural grade perlite. (B) Rhizoboxes were fertigated with two pressure-compensating emitters and were kept in a custom-built stand.

solution concentrations by half. These symptoms were not observed in leaves developed after week 4.

Canopy and root system data collection took place on weeks 1, 3, 5, and 7 of the experiment. A ruler was used to measure plant height above the substrate line. In addition, plants and a 5-cm scale bar were photographed using a digital camera (Samsung SH100, 14.2 MP; Samsung Electronics Co. Ltd., Seoul, South Korea) placed 55 cm above the rhizoboxes. Photos were used to measure the leaf area index (LAI) using ImageJ software version 1.51w (National Institutes of Health, Bethesda, MD). Leaf greenness [soil plant analysis development (SPAD)] was measured on the youngest fully expanded leaf of each plant with a leaf spectrometer (SpectraVue CI-710; CIDBio Science, Inc., Camas, WA). Each rhizobox was scanned using a flatbed scanner (Epson LX1100; Epson, Tokyo, Japan) at a resolution of 1000 dpi. Scans were used to measure root system convex hull area, width, and maximum depth. First, the polygon that connects the outermost points in a root scan was drawn. Then, the area of the polygon, the maximum width, and the maximum height were measured using the Analyze tool in ImageJ.

Plants were harvested destructively 7 weeks after transplant. Rhizoboxes were opened and root systems were rinsed with tap water to remove the substrate. Plants were separated into leaves, canes, and roots. Discolored (older) and healthy (younger) leaves were kept

separate. Roots were scanned floating in water using the transparency unit of the flatbed scanner. Total root length was determined using WinRHIZO Pro 2013b (Regent Instruments Inc., Quebec, Canada). Leaves, canes, and roots were dried at 65 °C to a constant weight, and organ dry weight was determined. Subsequently, healthy leaf tissue was ground until it passed a sieve with 0.40-mm pores, and was submitted to an institutional laboratory (University of Florida, Institute of Food and Agricultural Sciences Analytical Research Laboratories, Gainesville, FL) for elemental analysis using automated colorimetry (U.S. Environmental Protection Agency, 1993).

The experiment was conducted in a greenhouse located in Gainesville, FL, under natural photoperiod conditions between Aug. and Sept. 2020. Air temperature and humidity were measured and recorded with a data logger (HOB0 Model MX301; Onset, Cape Code, MA) every 10 min. The average temperature was 25.4 °C and the average relative humidity was 95.5% during the experiment.

Substrate analysis. Substrate pH and EC were monitored using the pour-through method (Cavins et al., 2000) on weeks 1, 3, 5, and 7 of the experiment. Leachate was collected by placing each rhizobox partially inside a 40.6 × 45.7-cm plastic bag and then pouring 500 mL deionized water. About 400 mL leachate was collected from each rhizobox. Leachate EC (Hanna Combo HI98129; Hanna Instruments, Woonsocket, RI) and pH

(S220 SevenCompact; Mettler Toledo, OH) were measured within 2 hours of collection using previously calibrated sensors.

Substrate samples were collected before transplant (week 0) and after destructive harvest (week 7), and submitted to a commercial laboratory for analysis (Waters Agricultural Laboratory, Camila, GA). Additional substrate samples were collected on weeks 1 and 7, air-dried at room temperature, and used for a substrate respiration assay. Substrate respiration is a measure of microbial activity in the substrate (Alsanius and Wohanka, 2019). First, substrate samples were soaked with a 1% w/v sucrose solution, and gravitational water was allowed to drain for ≈5 min. Then, 5 g of moist substrate was placed in an airtight chamber (500 mL). Headspace carbon dioxide (CO₂) concentrations were measured with an infrared gas analyzer (MAP Gas Analyzer; Bridge Analyzers Inc. Bedford Heights, OH) immediately after closing the jars. A second measurement was made after incubating for 24 h at 23 °C. Last, substrates were dried at 65 °C to a constant weight. Substrate respiration rates were calculated per Setia et al. (2011). Each substrate sample was tested in quadruplicate. Autoclaved substrate soaked with sucrose solution, substrate soaked with deionized water, and empty chambers were used as assay controls. Assay controls were tested in triplicate.

Statistical analysis. The experiment followed a completely randomized design with

four treatments (CN, MAP, deionized water, and well water) and 10 single-plant replications per treatment. Data were analyzed using one-way analysis of variance using agricolae (Mendiburu, 2021) in R (v. 4.1.1; R Foundation for Statistical Computing, Vienna, Austria). When significant effects were identified, mean separation was performed using the least significant difference test. In the substrate respiration assay, comparisons among treatments and assay controls were performed using two-tailed independent sample *t* tests. Results were illustrated using ggplot2 (Wickham, 2011) in R.

Results

Preplant fertilization treatments changed substrate nutrient concentrations at the start of the experiment (Table 1). Substrates treated with CN exhibited greater Ca concentrations than substrates treated with deionized water or well water, and greater nitrate (NO_3^-) concentrations than all other treatments. Substrates treated with MAP exhibited more ammonium (NH_4^+) and P than substrates in all other treatments. Substrates treated with preplant fertilizers exhibited similar or greater Na and K concentrations than substrates treated with deionized water or well water. Substrates treated with well water exhibited greater Cl concentrations than all other treatments. Substrates treated with preplant fertilizers exhibited higher EC values and lower pH values than substrates treated with deionized water or well water. At the end of the experiment, NH_4^+ , NO_3^- , P, and Cl concentrations were not different among treatments. Substrates treated with MAP exhibited lower Ca concentrations than substrates treated with deionized water or well water. These substrates also exhibited lower K and Na concentrations, and lower EC values than substrates in all other treatments. Substrate pH was similar among all treatments at the end of the experiment.

Substrate salinity was also detected in the leachate EC measurements (Fig. 2). In weeks 1 and 3, substrates treated with MAP exhibited higher leachate EC values than substrates

treated with deionized water and well water. This effect was not observed later in the experiment. EC was not different among other treatments. Leachate EC decreased between weeks 3 and 5, when the fertilizer injection rate was adjusted ($P < 0.001$). Treatments did not affect leachate pH for the duration of the experiment.

Preplant fertilization affected substrate microbial respiration during the first week of the experiment (Fig. 3). At the start of the experiment, substrates treated with preplant fertilizers exhibited greater respiration rates than substrates treated with deionized water and well water. In addition, substrates treated with CN exhibited greater respiration rates than substrates treated with MAP. At the end of the experiment, all treatments exhibited similar substrate respiration rates. Respiration rates decreased between the start and end of the experiment in substrates treated with preplant fertilizers ($P < 0.001$ in all cases), but they increased in substrates treated with well water or deionized water ($P < 0.002$ in all cases). Treatment substrates exhibited greater respiration rates than water-soaked substrates (average, $0.02 \text{ mg CO}_2 \cdot \text{g}^{-1} \text{ substrate} \cdot \text{d}^{-1}$; $P < 0.001$) and autoclaved substrates (average, $0.00 \text{ mg CO}_2 \cdot \text{g}^{-1} \text{ substrate} \cdot \text{d}^{-1}$; $P < 0.001$).

Average plant dry weight was 0.49 g at the time of transplant. All plants grew during the experiment. Preplant fertilization did not affect cane (average, 0.95 g; $P = 0.43$), leaf (average, 2.01 g; $P = 0.27$), or root (average, 0.38 g; $P = 0.13$) biomass accumulation. In addition, preplant fertilization did not affect leaf area index (average, 323.80 cm^2 ; $P = 0.49$), plant height (average, 22.83 cm; $P = 0.87$), or leaf greenness (average, 17.85 SPAD units; $P = 0.18$). Nevertheless, leaf N concentrations at the end of the experiment were different among treatments. Young healthy leaves of plants grown in substrates treated with CN exhibited greater N concentrations than leaves of plants grown in substrates treated with MAP or deionized water, but not well water ($P = 0.02$). Leaf N concentrations ranged between 1.81% and 2.04% in all treatments.

Preplant fertilization treatments had minimal effects on root architecture traits (Supplemental Fig. 1). Plants in all treatments exhibited similar convex hull areas for the duration of the experiment ($P > 0.52$ in all cases). In addition, plants in all treatments exhibited similar total root length at the end of the experiment (average, 66.2 m; $P = 0.29$). Treatment effects on root system width and depth were statistically significant at times, but small and inconsistent.

Discussion

Substrates that contain coconut coir can provide ideal rhizosphere conditions for container blueberry growth if salinity at transplant can be managed. Previous research has shown blueberry root and leaf growth peak between 0.10 and $0.50 \text{ dS} \cdot \text{m}^{-1}$ (Machado et al., 2014). In our study, the starting salinity in coconut coir that was treated with well water (our best approximation to grower conditions) was $0.34 \text{ dS} \cdot \text{m}^{-1}$. These substrate chemical characteristics can be considered average (Abad et al., 2002), and they are likely caused by high K, Na, and Cl concentrations. Because Na and Cl are not nutritionally relevant, and high Na concentrations can lead to plant stress (Spiers, 1993), we aimed to replace these ions by treating the substrate with large volumes of fertilizer solutions applied before planting. We found that neither of the tested preplant fertilizers replaced these ions. In addition, preplant fertilization increased substrate salinity at transplant and for the first 3 weeks of the experiment. Thus, our first and second hypotheses were not supported.

On the other hand, lower substrate salinity at the time of transplant was observed in substrates that were treated with deionized water. Sodium, K, and Cl concentrations were lower in these substrates. This suggests that using large volumes of high-quality water (low pH, low EC) is an alternative to manage salinity in coconut coir-based substrates. This is consistent with the “substrate washes” with clean water that growers use to manage salinity

Table 1. Initial (week 0) and final (week 7) chemical composition of the substrate treated with different preplant fertilizers used to grow ‘Snowchaser’ blueberry in a rhizobox experiment.

Treatments	Nutrient concn (mg·kg ⁻¹)							EC (dS·m ⁻¹)	pH
	NH ₄ ⁺	NO ₃ ⁻	P	Ca	K	Na	Cl		
Week 0									
Deionized water	10.97 b ^z	6.65 b	0.68 b	2.84 b	13.48 c	5.38 b	17 b	0.08 b	6.90 a
Well water	9.45 b	7.47 b	3.42 b	3.16 b	65.06 b	24.23 a	50 a	0.34 c	6.48 b
Calcium nitrate	12.72 b	58.57 a	1.06 b	6.34 a	119.3 a	31.65 a	20 b	0.61 a	5.99 c
Monoammonium phosphate	34.07 a	7.7 b	124.10 a	5.27 ab	94.61 ab	30.44 a	32 b	0.58 a	5.74 d
<i>P</i> value	<0.001	<0.001	<0.001	0.038	<0.001	<0.001	0.005	<0.001	<0.05
Week 7									
Deionized water	26.13	28.23	4.80	34.37 a	10.49 a	8.39 a	26	0.46 a	6.57
Well water	23.33	29.17	4.81	34.33 a	11.15 a	8.75 a	26	0.48 a	6.47
Calcium nitrate	31.27	42.00	5.26	29.01 ab	11.53 a	8.77 a	26	0.46 a	6.06
Monoammonium phosphate	28.47	21.93	4.06	21.39 b	8.79 b	7.18 b	26	0.36 b	6.27
<i>P</i> value	0.135	0.195	0.222	0.0327	0.020	0.0322	0.441	0.015	0.347

^zDifferent letters indicate significant differences among substrate conditioning treatments within a week, according to least significant difference tests ($P \leq 0.05$).

Ca = calcium; Cl = chlorine; EC = electrical conductivity; K = potassium; Na = sodium; NH_4^+ = ammonium; NO_3^- = nitrate; P = phosphorus

Fig. 2. Leachate (A) electrical conductivity and (B) pH in substrates that received calcium nitrate (CN), monoammonium phosphate (MAP), deionized water, or well water 72 h before transplanting 'Snowchaser' southern highbush blueberry. ns, *, **Not significant at $P \geq 0.05$, or significant at $P < 0.05$ and $P < 0.01$, respectively.

buildup during the production cycle (Y. Fang, personal communication). These results underscore the importance of water quality for substrate-based blueberry production.

Preplant fertilizer application did not enhance 'Snowchaser' establishment. Thus, our third hypothesis was not supported. It is possible that other blueberry varieties might respond differently. Blueberry plants can take up N as either NH_4^+ or NO_3^- , but the best performance is usually observed in NH_4^+ -abundant conditions (Imler et al., 2019; Tamir et al., 2021). In our study, plants responded similarly to preplant fertilization with either N form. It is possible that NH_4^+ from the fertigation solution or nitrification in the substrate obliterated the effect of N form in the preplant application. Similar NO_3^- and NH_4^+ concentrations in the

substrate at the end of the experiment suggest that the latter phenomenon might explain the plant responses. Although there were differences in leaf N concentration, plants in all treatments exhibited leaf N concentrations in the optimum range for commercial production (Strik and Vance, 2015).

Previous research has shown that fertilization with P-containing fertilizers can promote root growth in blueberry (Haby et al., 1991). Nevertheless, high P concentrations in substrates treated with MAP did not enhance root growth or affect root architecture in our experiment. By the end of the experiment, P concentrations in substrates treated with MAP decreased nearly 30-fold, making P concentrations in all substrates similar. Although it is likely that some P was taken up by the plants,

it is safe to assume that most P leached out of the rhizoboxes thanks to the high leaching fraction used in this experiment and the negative charges in phosphates. Considerable P leaching has been observed in previous experiments with blueberry (Bandaranayake et al., 2020) and crape myrtle (Shreckhise et al., 2020) grown in pine bark-based substrates, suggesting that large P discharges might be a common characteristic of substrate-based production systems.

Calcium concentrations were twice as high in substrates treated with CN than in substrates treated with well water, but this did not elicit plant responses. Calcium exhibited a tendency to accumulate in the substrate of all treatments. At the end of the experiment, Ca concentrations were 4- to 12-fold higher than before

Fig. 3. Substrate microbial respiration rates at the start (week 1) and end (week 7) of a rhizobox experiment in which 'Snowchaser' southern highbush blueberry plants were grown with preplant applications of calcium nitrate (CN), monoammonium phosphate (MAP), deionized water, or well water. The substrate was composed of 70% coconut coir and 30% perlite. Respiration rates were measured on substrate samples saturated with 1% sucrose. Different letters indicate significant differences among substrate conditioning treatments within a week. CO₂, carbon dioxide; ns, not significantly different.

transplant. Calcium concentrations increased while K and Na concentrations decreased, suggesting that replacement from substrate adsorption sites took place. Because replacements are isoelectric (one Ca^{2+} atom per two Na^+ or K^+ atoms), Ca accumulation can reduce substrate EC, at least temporally. However, it is possible that Ca accumulation can lead to salinity issues in the long term.

Soiless substrates exhibit limited microbial activity before transplant. It is assumed that the limiting factor is scarcity of labile carbon (C) sources (Alsanius and Wohanka, 2019). Respiration patterns of substrates treated with well water or deionized water in this experiment corroborate this notion. These substrates exhibited lower respiration rates before transplant than after 7 weeks of cultivation, suggesting that lack of labile C limited microbial activity initially, and blueberry root exudation promoted microbial activity during the experiment. This is consistent with the rapid, plant-driven microbial community assembly observed in other substrate-grown crops (De Tender et al., 2016; Rosberg et al., 2014). Nevertheless, our results suggest there may be other limitations to microbial activity in soiless substrates. By performing our substrate respiration measurements in C-abundant conditions, we identified mineral nutrient availability as a secondary constraint. Preplant fertilization enriched the substrate with N, P, and Ca at the start of the experiment, and this promoted microbial activity in the substrate. These results invite us to think about preplant fertilization as a tool to prime substrate microbial communities before transplant. Considering the impact substrate microbial communities can have on plant resilience (Montagne et al., 2017) and nutrient use efficiency (Nunez et al., 2016), this is an area that merits further study.

Altogether, our results show that preplant fertilization with 1.75 g-L^{-1} CN and 2.38 g-L^{-1} MAP can change substrate ion concentrations and promote microbial activity, but not enhance blueberry establishment in coconut coir-based substrates.

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Supplemental Table 1. Analysis results of a well water sample used in an experiment in which ‘Snowchaser’ southern highbush blueberry plants were grown with contrasting preplant fertilization treatments.

Element	Concn (mg·L ⁻¹)
Nitrogen	0.88
Phosphorus	0.01
Potassium	1.47
Magnesium	22.92
Calcium	36.21
Sodium	11.31
Boron	0.01
Iron	0.04
Manganese	0.01
Sulfate	110.04
Chloride	28
Carbonate	0
Bicarbonate	75.64

Supplemental Fig. 1. Root architecture of ‘Snowchaser’ southern highbush blueberry in response to different preplant fertilizer applications. Substrates were treated with calcium nitrate (CN), monoammonium phosphate (MAP), deionized water, or well water starting 72 hours before transplant. (A) Convex hull area, (B) root system width, and (C) maximum root depth were measured periodically in root images collected with a flatbed scanner (resolution, 1000 dpi). (D) Total root length was measured after washing root systems clean of substrate after 7 weeks of cultivation. Different letters indicate significant differences among treatments. ns, not significantly different. Mean separation letters are presented vertically in the same order the treatments are presented in the legend.