



## Article

# Calcium Carbonate Can Be Used to Manage Soilless Substrate pH for Blueberry Production

Michael J. Schreiber and Gerardo H. Nunez \*

Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA; schreiber@m@ufl.edu

\* Correspondence: g.nunez@ufl.edu

**Abstract:** Blueberry (*Vaccinium corymbosum* interspecific hybrids) production in soilless substrates is becoming increasingly popular. Soilless substrates have low pH buffering capacity. Blueberry plants preferentially take up ammonium, which acidifies the rhizosphere. Consequently, soilless substrates where blueberry plants are grown exhibit a tendency to get acidified over time. Agricultural lime ( $\text{CaCO}_3$ ) is commonly used to raise soil and substrate pH in other crops, but it is rarely used in blueberry cultivation. We hypothesized that substrate amendment with low rates of agricultural lime increases substrate pH buffering capacity and provides nutritional cations that can benefit blueberry plants. We tested this hypothesis in a greenhouse experiment with 'Emerald' southern highbush blueberry plants grown in rhizoboxes filled with a 3:1 mix of coconut coir and perlite. We found that substrate amendment with  $\text{CaCO}_3$  did not cause high pH stress. This amendment maintained substrate pH between 5.5 and 6.5 and provided Ca and Mg for plant uptake. When blueberry plants were grown in  $\text{CaCO}_3$ -amended substrate and fertigated with low pH nutrient solution (pH 4.5), they exhibited greater biomass accumulation than plants grown in unamended substrates. These results suggest that low rates of  $\text{CaCO}_3$  could be useful for blueberry cultivation in soilless substrates.

**Keywords:** *Vaccinium corymbosum*; container; ammonium uptake; southern highbush blueberry



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## 1. Introduction

Cultivation in containers filled with soilless substrates is rapidly becoming a popular growing system for blueberry (*Vaccinium corymbosum* interspecific hybrids) production. Soilless substrates based on sphagnum peat moss or coconut coir are generally acidic [1] and have high water holding capacity [2]. These substrate characteristics promote blueberry nutrient uptake and support vigorous growth [3,4]. As this growing system becomes widespread [5], there is a need for research focused on fertilization and management practices for substrate-grown blueberry.

Sphagnum peat moss and coconut coir have low pH buffering capacity [6,7]. Consequently, pH changes of up to 1 unit per month are not uncommon [3,4,8,9]. While blueberry roots exhibit limited ability to change the rhizosphere pH through  $\text{H}^+$  extrusion [10], ammonium uptake can lead to rapid rhizosphere acidification [11,12]. Considering blueberry growth and N content are enhanced by ammonium-based fertilization [11,13], substrate acidification appears inevitable in this production system.

Calcitic ( $\text{CaCO}_3$ ) and dolomitic [ $\text{CaMg}(\text{CO}_3)_2$ ] lime are commonly used to raise soilless substrate pH, but amendment rates and effects are crop-specific (reviewed in [14]). The carbonate moiety in lime acts as a buffer that maintains the rhizosphere approximately at pH 6.4 [14]. The cations in lime are nutritionally relevant Ca and Mg. Substrates used for cultivation of other acid-loving plants are routinely amended with lime to limit substrate pH change [15,16]. Nevertheless, the effects of lime amendments in substrate-grown blueberry remain understudied.

Lime is rarely used in soil-based blueberry cultivation because high liming rates can raise soil pH excessively and cause plant stress. When grown in high pH soils, blueberry

plants exhibit nutritional deficiencies, stunted growth, and lower yields [17–19]. Lime is only used in situations where soil pH is very low to deliver Ca and Mg [20]. Hence, lime amendments must be meticulously used to avoid stressing blueberry plants.

This research investigates the effect of substrate amendment with CaCO<sub>3</sub> on the substrate pH, growth, and nutrition of southern highbush blueberry. We hypothesized that substrate amendment with low rates of agricultural lime increases substrate pH buffering capacity and provides nutritional cations that can benefit blueberry plants. We tested this hypothesis in a greenhouse experiment with plants grown in rhizoboxes.

## 2. Materials and Methods

Rooted cuttings of 'Emerald' southern highbush blueberry (SHB; rooting volume = 3 cm<sup>3</sup>, average dry weight = 1.15 g, average height = 12 cm) were acquired from a commercial micropropagation nursery (Agristarts LLC, Apopka, FL, USA) and transplanted to benchtop rhizoboxes as per [21]. Rhizoboxes were built using two 35.56 cm × 35.56 cm plexiglass panels spaced 1.9 cm apart using wood inserts. Each rhizobox contained approximately 1.7 L of substrate and was irrigated or fertigated by two 1.89 l·h<sup>-1</sup> pressure-regulating emitters, spaced approximately 15.25 cm apart. Custom-made rhizobox stands kept roots in the dark at 33° inclination. There was one plant per rhizobox. Rhizoboxes were used as a tool to study root growth patterns in response to substrate amendment and fertigation pH treatments.

Rhizoboxes were filled with a 3:1 mixture of coconut coir (SpongEase™, Enroot Products LLC, Cromwell, CT, USA) and horticultural grade perlite (American Garden Perlite, LLC, Lake Wales, FL, USA) pre-treated to deliver two substrate amendment treatments. In one treatment, substrate was amended with CaCO<sub>3</sub> (Garden Lime, Austinville Limestone, Austinville, VA, USA) at a rate of 6.18 Kg·m<sup>-3</sup>. This rate corresponds to half of the rate used in [22] where lime amendments were used to stress azalea (*Rhododendron* spp.). In the other treatment, substrate was amended with Ca-containing fertilizer produced from neutralized CaCO<sub>3</sub> (Calexin®, Miller Chemical & Fertilizer Corporation, Hanover, PA, USA) at a rate of 100.3 L·m<sup>-3</sup>. Guaranteed analysis and product density information were used to calculate a Calexin application rate that delivered the same amount of Ca as the CaCO<sub>3</sub> amendment. Both amendments were incorporated into moist substrate 7 days before transplant.

Fertigation solution pH was a second variable in the experiment. Plants were fertigated with a solution containing 0.5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5 mM K<sub>2</sub>PO<sub>4</sub>, 1.0 mM MgSO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub>, 0.045 mM H<sub>3</sub>BO<sub>3</sub>, 0.01 mM MnSO<sub>4</sub>, 0.01 mM ZnSO<sub>4</sub> with 0.3 mM CuSO<sub>4</sub>, 0.2 mM Na<sub>2</sub>MoO<sub>4</sub>, and 45 mM Fe provided as Sequestrene 330 (10% iron(III)-diethylenetriamine pentaacetic acid) (Becker Underwood, Inc., Ames, IA, USA). Ammonium was the only form of N provided, in agreement with industry practices [20]. The low N rate was selected because blueberry microcuttings exhibited ammonium toxicity when fertigated with higher N rates in a preliminary experiment. Fertigation solution was buffered using 5.0 mM 2-(4-morpholino)-ethane sulfonic acid to pH 4.5 or pH 6.5 using HCl or KOH. These fertigation pH treatments are referred to as low pH and high pH respectively in relation to fertigation pH used in previous studies [3]. There were 21 fertigation/irrigation events per week. Each plant received 1.75 L of fertigation solution (delivered through 7 events) and 3.96 L of irrigation water per week (delivered through 14 events). Fertigation events preceded irrigation events. Fertigation and irrigation volumes were measured with graduated cylinders connected directly to emitters.

Substrate samples were collected at the start (day 0) and end (days 75–77) of the experiment and submitted for analysis at a commercial laboratory (Waters Agricultural Laboratory, Camilla, GA, USA). Ca, Mg, and K concentrations in the substrate were determined using inductively coupled plasma mass spectrometry [23]. Cation exchange capacity (CEC) was calculated from K, Ca, Mg, and H concentrations as per [24]. Substrate pH was measured in a 1:1 substrate:deionized water slurry [25].

Substrate pH and electrical conductivity (EC) were monitored using the pour-through method [26]. Deionized water samples that eluted through the substrate (hereon, leachate) were collected on a weekly basis ( $n = 3$  per treatment). Rhizoboxes were removed from the stand and placed vertically on top of plastic trays (one rhizobox per tray) approximately 2 h after the last fertigation event. Then, 500 mL of deionized water were slowly poured on top of the substrate. Leachate was collected in the plastic tray for approximately 20 min. Then, leachate volume was measured with a graduate cylinder and 50 mL aliquots were transported to the laboratory for immediate measurement of leachate pH (Accumet AP110 Portable pH Meter, Thermo Fisher Scientific, Hampton, NH, USA) and EC (Accumet Excel Conductivity Meter, XL30, Thermo Fisher Scientific, Hampton, NH, USA) using standardized electrodes. In this manuscript and elsewhere [26], it is assumed that leachate pH and EC represent rhizosphere conditions. Leaf greenness was measured on the youngest fully expanded leaf of each plant using a SPAD-502 meter (Konica Minolta, Inc., Ramsey, NJ, USA).

Rhizoboxes were scanned using a flatbed scanner (LX1100, Seiko Epson Corp., Tokyo, Japan) at a resolution of 1000 dots per inch (dpi). The scanner was held at an inclination of  $30^\circ$  during scanning to avoid substrate loss. Rhizoboxes were scanned on a weekly basis starting on week 2 of the experiment. Rhizobox images were used to measure root system convex hull area using ImageJ version 1.51 [27]. Convex hull area is the area of the polygon formed by lines connecting the most distal root tips in a plant. Root system spread was computed as the ratio of the convex hull area to root dry weight.

Rhizoboxes were disassembled 75 to 77 days after the start of the experiment. Roots were washed clean of substrate using tap water. A subset of the root systems ( $n = 4$  per treatment except for  $\text{CaCO}_3 + \text{pH } 6.5$  where one root image was lost due to human error) were scanned floating in water using the transparency unit of the flatbed scanner at 1000 dpi. Images were divided into 5 tiles using ImageJ. Then, total root length was determined using WinRhizo Pro 2013b (Regent Instruments, Quebec, QC, Canada). Organ and whole plant fresh weight were measured. Leaves were laid flat and photographed at a distance of 48.25 cm from the lens using mobile phone cameras (iPhone 7 and iPhone X, Apple Inc., Cupertino, CA, USA) on a white background with a scale bar of known size. Total leaf area was measured using ImageJ. Plant tissues were weighted after drying at  $72^\circ\text{C}$  for a week. Dry tissue was ground until it passed through a size-20 mesh (sieve opening = 0.841 mm). Then, tissue was submitted for elemental analysis at a commercial laboratory (Waters Agricultural Laboratory, Camilla, GA, USA).

The experiment was conducted in a greenhouse where average temperature and relative humidity were  $22.53^\circ\text{C}$  and 70.19%, respectively. The experiment followed a completely randomized design with treatments in a  $2 \times 2$  factorial arrangement. There were 10 single-plant replications per amendment  $\times$  pH combination. Unless otherwise stated,  $n = 10$  per treatment. Treatment effects on biomass accumulation, leaf area, substrate characteristics, elemental content, and root traits were assessed using two-way analysis of variance (R package agricolae, [28]). Where significant effects were identified, pairwise comparisons were made using the least significant difference method. Leachate pH and EC data were analyzed through linear mixed-effect analysis (R package lme4, [29]). Fertigation solution pH, substrate amendment, and their interaction were considered fixed effects. Repeated measures per plant and week were considered random sources of error. Leachate pH and EC were response variables analyzed in separate models. Statistical significance was determined by likelihood ratio tests comparing the full model against a model without the effect being investigated. All statistical analyses were conducted in R version 3.6.2 [30]. Data were illustrated using ggplot2 [31].

### 3. Results

At the start of the experiment, substrates amended with  $\text{CaCO}_3$  exhibited higher pH, percentage base saturation, Mg content, and K content than substrates amended with Calixin (Table 1). Substrate CEC and Ca content were not different between the amend-

ment treatments. At the end of the experiment, substrate pH was not different among treatments (Table 2). Substrates amended with CaCO<sub>3</sub> exhibited higher CEC, percentage base saturation, Ca concentration, and Mg concentration than substrates amended with Calixin. Substrates that were fertigated at pH 4.5 exhibited lower K concentration than substrates that were fertigated at pH 6.5. The interaction of substrate amendment and fertigation pH did not affect substrate characteristics ( $p \geq 0.158$ ).

**Table 1.** Substrate characteristics before transplant. A substrate composed of a 3:1 mixture of coconut coir and perlite was amended with CaCO<sub>3</sub> or a Ca-containing fertilizer (Calixin) 7 days before transplanting ‘Emerald’ southern highbush blueberry.

Amendment	pH	Cation Exchange Capacity (meq·100 g <sup>-1</sup> Substrate)	Base Saturation (%)	Ca (mg·Kg <sup>-1</sup> )	Mg (mg·Kg <sup>-1</sup> )	K (mg·Kg <sup>-1</sup> )
CaCO <sub>3</sub>	6.4	9.50	71.77	932.17	158.67	325.67
Calixin	4.4	10.97	57.27	1089.00	43.84	194.00
<i>p</i> value <sup>z</sup>	<0.001	0.084	0.021	0.283	<0.001	0.009

<sup>z</sup> Treatments were compared using ANOVA.

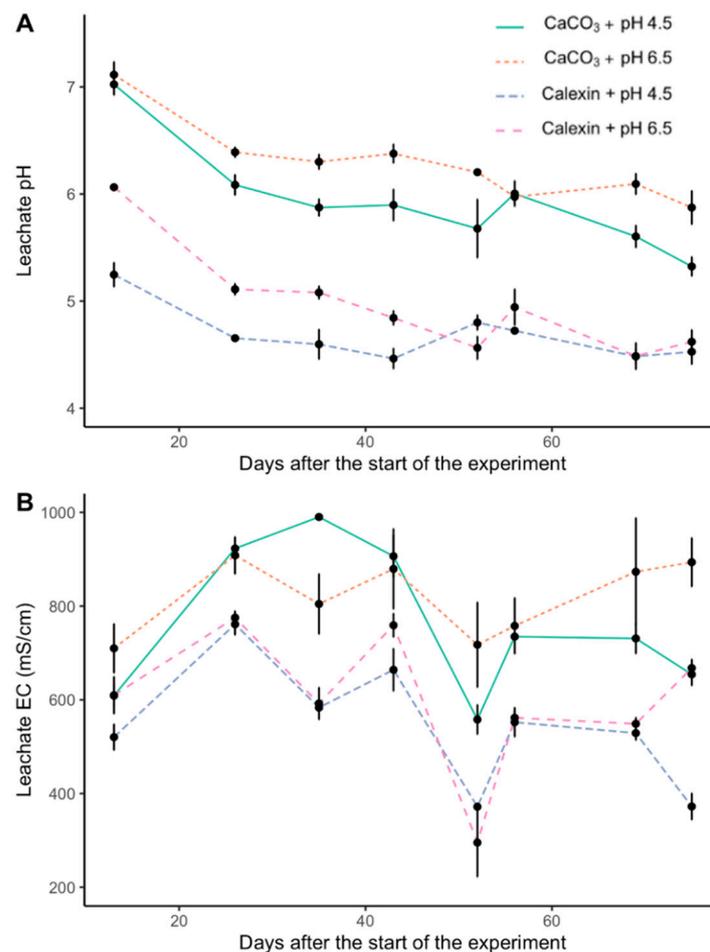
**Table 2.** Substrate characteristics after 75–77 days of growing ‘Emerald’ southern highbush blueberry with contrasting substrate amendments and fertigation pH.

Treatment	Substrate pH	Cation Exchange Capacity (meq·100 g <sup>-1</sup> Substrate)	Base Saturation (%)	Ca (mg·Kg <sup>-1</sup> )	Mg (mg·Kg <sup>-1</sup> )	K (mg·Kg <sup>-1</sup> )
CaCO <sub>3</sub>	4.9	8.05	57.38	1298.33	307.50	99.17
Calixin	4.7	6.73	34.60	601.83	169.67	95.83
<i>p</i> value <sup>z</sup>	0.094	0.009	<0.001	<0.001	0.003	0.596
pH 6.5	4.8	7.30	48.95	958.33	248.67	164.67
pH 4.5	4.8	7.48	43.03	941.83	228.50	30.33
<i>p</i> value	0.999	0.643	0.144	0.901	0.563	<0.001

<sup>z</sup> Data were analyzed by two-way ANOVA. The interaction of fertigation pH and substrate amendment did not affect substrate characteristics ( $p \geq 0.158$ ).

Substrate amendments and fertigation pH created contrasting leachate pH and EC during most of the experiment (Figure 1). Leachate pH gradually decreased in all treatments ( $\chi^2 = 11.74$ ,  $df = 1$ ,  $p \leq 0.001$ , estimate =  $-0.42$ ). Amendment with CaCO<sub>3</sub> ( $\chi^2 = 93.34$ ,  $df = 1$ ,  $p < 0.001$ ) and high pH fertigation ( $\chi^2 = 28.69$ ,  $df = 1$ ,  $p < 0.001$ ) led to high leachate pH. The interaction of substrate amendment and fertigation pH did not affect leachate pH ( $\chi^2 = 2.57$ ,  $df = 3$ ,  $p = 0.11$ ). Amendment with CaCO<sub>3</sub> led to higher leachate EC ( $\chi^2 = 5.26$ ,  $df = 1$ ,  $p = 0.02$ ). Fertigation pH ( $\chi^2 = 1.13$ ,  $df = 1$ ,  $p = 0.29$ ), time ( $\chi^2 = 0.22$ ,  $df = 1$ ,  $p = 0.63$ ), and the interaction of substrate amendment and fertigation pH ( $\chi^2 = 7.33$ ,  $df = 3$ ,  $p = 0.06$ ) did not affect leachate EC.

Substrate amendments and fertigation pH affected plant biomass accumulation (Table 3). Plants grown with a combination of Calixin amendment and low pH fertigation solution exhibited lower cane, leaf, and total dry weight than plants grown with CaCO<sub>3</sub> amendments. Within a substrate amendment, fertigation pH did not affect biomass accumulation. Plants grown in substrates amended with CaCO<sub>3</sub> exhibited larger root systems than plants grown in substrates amended with Calixin. Leaf area followed the same trends as leaf dry weight (data not shown). Leaf greenness was not affected by the treatments (average = 24.68,  $p = 0.23$ ).



**Figure 1.** Leachate pH (A) and electrical conductivity (EC) (B) collected from rhizoboxes where ‘Emerald’ southern highbush blueberry grew with contrasting substrate amendments (CaCO<sub>3</sub> or Ca-containing fertilizer Caalexin) and fertigation pH (pH 4.5 and pH 6.5).

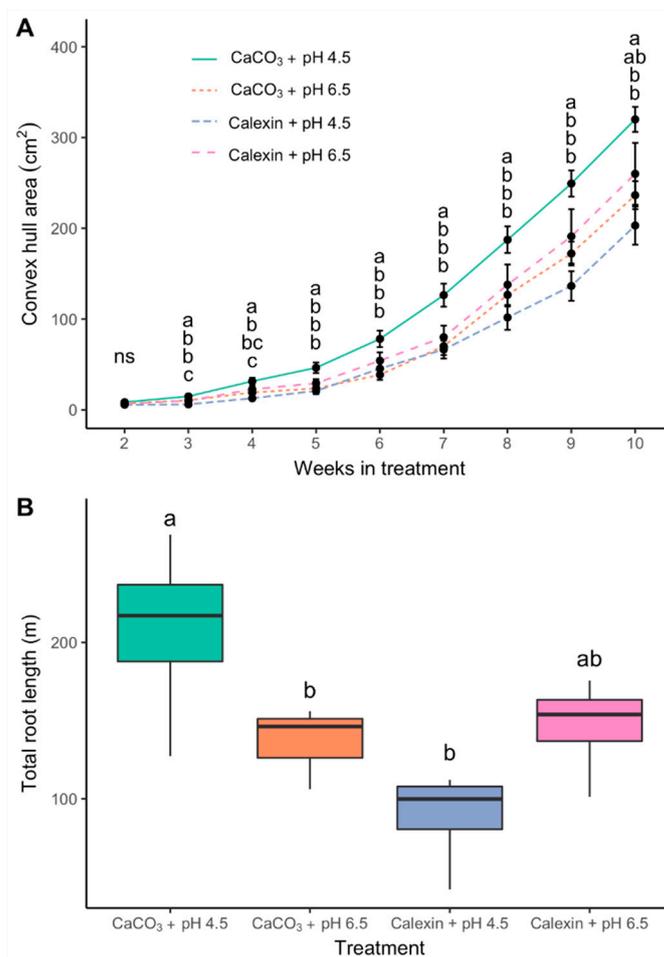
**Table 3.** Biomass accumulation of ‘Emerald’ southern highbush blueberry plants grown in rhizoboxes with contrasting substrate amendments and fertigation pH.

Amendment	Fertigation pH	Root Dry Weight (g)	Cane Dry Weight (g)	Leaf Dry Weight (g)	Total Dry Weight (g)
CaCO <sub>3</sub>	6.5	2.38 a	3.40 ab	5.06 ab	10.84 ab
	4.5	2.38 a	4.91 a	6.99 a	14.32 a
Caalexin	6.5	1.16 b	2.33 bc	4.01 bc	7.50 bc
	4.5	0.58 b	1.33 c	2.59 c	4.2 c
Effect <sup>z</sup>					
Amendment		<0.001	<0.001	<0.001	<0.001
Fertigation pH		0.272	0.574	0.672	0.947
Amendment x pH		0.208	0.009	0.008	0.017

<sup>z</sup> Data were analyzed by two-way ANOVA. Means followed by the same letter were not significantly different according to Tukey LSD at  $\alpha = 0.05$ .

Substrate amendment and fertigation pH also affected root system characteristics. Root systems of plants grown with low pH fertigation and CaCO<sub>3</sub> amendments exhibited larger convex hull areas than all other treatment combinations between weeks 3 and 9 (Figure 2A). Root systems of plants grown with low pH fertigation and Caalexin amendments had smaller convex hull area than all other treatments initially (weeks 3 and 4). Plants grown with low pH fertigation and CaCO<sub>3</sub> amendment exhibited higher total root length than plants grown with high pH fertigation and CaCO<sub>3</sub> amendments and plants grown with low pH fertigation and Caalexin (Figure 2B). High pH fertigation so-

lution ( $274.53 \text{ cm}^2 \cdot \text{g}^{-1}$  vs.  $191.66 \text{ cm}^2 \cdot \text{g}^{-1}$ ) and  $\text{CaCO}_3$  amendments ( $338.97 \text{ cm}^2 \cdot \text{g}^{-1}$  vs.  $127.22 \text{ cm}^2 \cdot \text{g}^{-1}$ ) reduced root system spread ( $p < 0.008$  in all cases). Root system spread was not affected by the interaction of substrate amendment and fertigation solution pH ( $p = 0.29$ ).



**Figure 2.** Root system characteristics of ‘Emerald’ southern highbush blueberry grown with contrasting substrate amendments and fertigation pH. (A) Convex hull area during the treatment period. (B) Total root length after 77 days of cultivation. Means followed by the same letter were not significantly different according to Tukey LSD at  $\alpha = 0.05$ .

Substrate amendment and fertigation pH affected root and leaf nutrient concentrations (Table S1). High pH fertigation decreased N, Zn, and Cu concentrations and increased Ca concentration in roots. Substrate amendment with  $\text{CaCO}_3$  decreased K, S, B, and Cu concentrations and increased Fe concentrations in roots. Other elements were not affected. The interaction of fertigation pH and substrate amendment did not affect root nutrient concentrations ( $p \geq 0.078$ ). Plants grown in substrates amended with Calixin and fertigated with low pH solution exhibited the highest leaf N, P, Ca, Mg, S, Fe, Zn, and Cu concentrations (Table S2). Plants grown in substrates amended with  $\text{CaCO}_3$  generally exhibited the lowest leaf concentrations of these elements. Plants grown with low pH fertigation exhibited higher leaf Mn concentrations than plants grown with high pH fertigation. Plants grown in substrates amended with  $\text{CaCO}_3$  exhibited lower leaf K, Mn, and B. With the exception of K, treatment effects on nutrient concentration did not exhibit the same trends in roots and leaves.

#### 4. Discussion

Soilless substrates have limited pH buffering capacity [6], which allows large pH changes over the cultivation period [3,4,8,9]. In this experiment, 'Emerald' SHB plants were fertigated with a nutrient solution where ammonium was the only form of N. Ammonium uptake leads to rhizosphere acidification [11,12]. As expected, leachate pH gradually decreased in all treatments. Similar leachate acidification has been previously observed in experiments with substrate-grown blueberry [4,12] and azalea [32].

CaCO<sub>3</sub> is routinely used to raise soil or substrate pH in other crops [14–16], but not in blueberry. When blueberry and other acid-loving plants are grown in soils or substrates amended with high CaCO<sub>3</sub> rates, they exhibit high pH stress symptoms such as interveinal chlorosis and stunted growth [17–19,33]. In this experiment, CaCO<sub>3</sub> in the substrate did not cause high pH stress in 'Emerald' SHB, probably due to the low rate used. Plants grown in substrates amended with CaCO<sub>3</sub> did not exhibit Fe deficiency symptoms either, but leaf Fe concentrations were lower than published recommendations [20]. These results suggest that even though CaCO<sub>3</sub> raised substrate pH, the effect was mild enough to avoid causing high pH stress in 'Emerald' SHB. Further research will be necessary to determine if the CaCO<sub>3</sub> rate used here is appropriate for other blueberry varieties.

In this experiment, CaCO<sub>3</sub> in the substrate acted as a pH buffer that partially neutralized H<sup>+</sup> from ammonium uptake and the fertigation solution, maintaining leachate pH between pH 5.5 and pH 6.5 for most of the experiment. Additionally, CaCO<sub>3</sub> amendment replaced cations from the substrate adhesion sites with Ca and Mg. The combination of acidic substrate and nutritional cation availability supported vigorous growth above- and below-ground in 'Emerald' SHB, especially when CaCO<sub>3</sub> amendment was matched with low pH fertigation solution.

When the substrate did not contain carbonates, leachate pH ranged between pH 4.5 and pH 5.0 and almost half of the adhesion sites were occupied by non-nutritional cations. The lack of nutritional cations was likely caused by the abundance of H<sup>+</sup> and/or Ca<sup>2+</sup> leaching out of the rhizoboxes. These substrate conditions affected shoot and root growth, particularly when the fertigation solution pH was low. Low pH increases Al solubility [34], which can cause Al toxicity in blueberry [35]. Perlite contains 10–15% Al<sub>2</sub>O<sub>3</sub> [36]. Thus, it is possible that Al toxicity might have affected 'Emerald' SHB growth when substrate pH was extremely low. Al concentrations in the rhizosphere were not measured in this experiment. Further research will be necessary to establish if Al toxicity impacted plant responses.

Substrate characteristics affected blueberry root abundance and distribution. CaCO<sub>3</sub> amendments increased 'Emerald' SHB root dry weight and, in combination with low pH fertigation, they led to large root systems that reached most of the substrate in the rhizoboxes. Nevertheless, large root systems were not always better at taking up nutrients. Previous research has shown that CaCO<sub>3</sub> can affect nutrient uptake through pH-dependent and pH-independent effects [37]. Thus, fertilization practices might need to be adapted to maintain optimum plant nutrition in substrates amended with CaCO<sub>3</sub>.

Irrigation water can contain carbonates and bicarbonates (collectively called alkalinity). Alkaline water sources are not uncommon in blueberry production areas [38], but water acidification through sulfuric acid injection or sulfur dioxide generators is routinely used to neutralize alkalinity [39]. Our results suggest that increasing substrate pH buffering capacity can be beneficial for blueberry. Thus, it is important to recognize the tradeoff between irrigation water pH and pH buffering capacity when irrigation water is acidified. Considering the substrate acidification tendency observed here and elsewhere [4,12], it is tempting to speculate on the utility of alkaline water for substrate pH management. Future research should explore this potential management strategy.

Altogether, our results indicate that substrate amendment with low rates of CaCO<sub>3</sub> is a viable tool to increase pH buffering capacity in coconut coir-based substrates used for blueberry cultivation. CaCO<sub>3</sub> neutralized H<sup>+</sup> and contributed Ca and Mg for plant uptake. Access to a weakly acidic substrate with abundant nutritional cations supported vigorous

growth in ‘Emerald’ SHB. Further research should evaluate other CaCO<sub>3</sub> amendment rates and other blueberry varieties to facilitate decision making when using CaCO<sub>3</sub> in substrate-based blueberry cultivation.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2311-7524/7/4/74/s1>, Table S1: Nutrient concentrations in roots of ‘Emerald’ southern highbush blueberry, Table S2: Nutrient concentrations in leaves of ‘Emerald’ southern highbush blueberry.

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