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Research Paper

Southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids) responses to phosphorus deficiency

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ABSTRACT

Phosphorus (P) is a key element for plant growth and development. Phosphorus deficiency can affect plant survival and agronomic productivity. Southern highbush blueberry (SHB, *Vaccinium corymbosum* interspecific hybrids) responses to P deficiency are unknown. The objective of this research was to provide a detailed description of P deficiency responses of SHB. Two hydroponic experiments were conducted where 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' SHB plants were grown in a complete nutrient solution or a P free nutrient solution for eight weeks. We measured plant biomass accumulation, root architecture, P concentrations, gas exchange, root exudation, and leaf pigment concentration. We found that SHB plants respond to P deficiency by remobilizing P from older to younger organs, especially in older or larger plants. Therefore, older leaves were very informative about plant P status. Phosphorus deficient SHB plants also exhibited higher fine root mass and root length than control plants, and most varieties also increased acid phosphatase activity and root carbon exudation. Furthermore, P deficiency occasionally reduced carbon assimilation and increased root respiration and exudation rates, reducing plant daily carbon gain. A combination of P remobilization and P uptake-related traits led to the observed differences in P deficiency responses among SHB varieties.

1. Introduction

Phosphorus (P) is an essential macronutrient in plants because it is required for many physiological, biochemical, and cell signaling processes (Chan et al., 2021; Malhotra et al., 2018; Lambers, 2022; Liu, 2021) and a constituent of critical biomolecules such as ATP, NADPH, nucleic acids, phospholipids, and sugar-phosphates (Bechtaoui et al., 2021). Plants take up P as $H_2PO_4^-$ (often abbreviated Pi) (Vance et al., 2003). Pi is typically present at low concentrations in agricultural soils (Richardson et al., 2009; Balemi and Negisho, 2012; Menezes-Blackburn et al., 2018) due to its reactivity with soil cations depending on soil type and pH. Thus, Pi could be found as part of organic phosphates, iron and aluminum phosphates, or calcium phosphates (Schubert et al., 2020), leaving only a small amount of plant-available P (Gatiboni et al., 2021). Consequently, P deficiency is one of the main factors limiting crop production (Gerke, 2015).

Plants use several biochemical, physiological and morphological strategies to increase P bioavailability and uptake from the soil. Root architecture changes such as increasing root branching, elongation, density, and proliferation of fine roots and root hairs improve P uptake efficiency (PUpE) (Shen et al., 2018; Wen et al., 2017). Root exudation of protons, acid phosphatases, organic acids, amino acids, phenols, and sugars are also involved in higher PUpE (Tantriani et al., 2023; Vengavasi et al., 2021). Also, symbiotic associations with mycorrhizal fungi can increase PUpE (Scagel, 2005; Wang and Lambers, 2020). Something all these strategies have in common is that they are costly in terms of energy and carbon (C) for plants. Additionally, P solubilization and uptake strategies can affect agricultural plant productivity due to the allocation of photosynthates away from plant growth and development (typically called "stunted growth") (Brown et al., 2013; Malhotra et al., 2018; Tiziani et al., 2020). The decrease in growth when P is scarce is also related to the prominent role of P in enzyme activation, energy transfer (as a component of ATP and ADP), and cell division (as a component of DNA, RNA, and biological membranes) (Khan et al., 2023). Consequently, typical symptoms of P deficiency include increases in root:shoot dry mass ratio (Ramaekers et al., 2010), development of dark green, purple, red, or brown coloration in leaves (Malhotra et al., 2018) due to accumulation of anthocyanins for photoprotection (Ha and Tran, 2014), delays in flower bud initiation, reduced reproductive growth, and premature leaf senescence (Marschner, 2012).

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Fig. 1. Gas analysis system used to simultaneously measure blueberry branch net photosynthesis (A_{branch}) and root respiration (R_{root}).

Blueberry P deficiency responses are minimally understood. Previous research in northern highbush blueberry (*Vaccinium corymbosum* L.) described P deficiency symptoms as purple coloration of older leaves, darkened stems, and roots with grey discoloration (Ballinger, 1966; Korcak, 1989; Tamada, 1989). In rabbiteye blueberry (*Vaccinium ashei* Reade), P deficiency symptoms include darker and purple-red leaves, reduced leaf size, leaf senescence, and decreased plant growth (Minton et al., 1951; Spiers, 1983; Tamada, 1989). To date, there is no research of P deficiency symptoms in southern highbush blueberry (SHB, *Vaccinium corymbosum* interspecific hybrids). Also, other biochemical, physiological and morphological responses are unknown.

The objective of this study was to provide a detailed description of the P deficiency responses of SHB. SHB is a widely cultivated blueberry type in the tropics and subtropics (Fang et al., 2020). We pursued this objective in two separate hydroponic experiments.

2. Materials and methods

Plant material and growing conditions. In Expt. 1, one-year old rooted cuttings of 'Colossus' SHB (bushy, high vigor variety) were evaluated. In Expt. 2, one-year old rooted cuttings of 'Colossus' SHB, and three-month old rooted cuttings of 'Farthing' SHB (compact, high vigor variety), 'Keecrisp' SHB (upright, low vigor variety) and 'Sentinel' SHB (spreading, high vigor variety) were evaluated. Both experiments used the same hydroponic system. Each plant was transplanted to a 2-L reservoir after washing its roots clean of substrate using tap water. Reservoirs were filled with continuously aerated nutrient solution and wrapped with aluminum foil to avoid light infiltration. Plants were grown with a complete nutrient solution during a five-week acclimation period. The nutrient solution contained (mM): 0.25 $(NH_4)_2SO_4$, 0.5 K_2HPO_4, 1.0 $MgSO_4$, 0.5 $CaCl_2$, 0.045 H_3BO_3, 0.01 $MnSO_4$, and 0.01 $ZnSO_4$, with 0.3 μM $CuSO_4$ and 0.2 μM Na_2MoO_4, and 45 μM FeNa-EDTA. Nutrient solution P concentration was 0.12 mM (equivalent to 15 mg L^{-1}) during acclimation. Nutrient solutions were changed weekly to maintain nearly constant nutrient concentrations. The nutrient solution was buffered to pH 5.8 using 5.0 mM 2-(N-morpholino) ethane sulfonic acid. pH was measured and adjusted using 3.8 M KOH three times per week with a dosing system (pH Mini; Autogrow America, Eureka, CA).

After the acclimation period, the experiment continued for eight more weeks (treatment period). Half of the plants (+P) were grown in the previously described complete nutrient solution, while the other half were grown in a P free nutrient solution (-P). Phosphorus free nutrient solution was prepared by withholding K_2HPO_4 (0 mg L^{-1} P) and providing additional 0.1 mM KOH to maintain K concentrations identical between both treatments.

Both experiments were conducted in temperature-controlled polycarbonate greenhouses located in Gainesville, FL (29.6516° N, 82.3248° W). In Expt. 1 plants were exposed to day-length extension delivered by metal halide lamps from 06:00 to 20:00 HR, whereas in Expt. 2 plants received natural daylength (approximately 14 h). Environmental conditions in the greenhouse were measured with a data logger (WatchDog Weather Tracker 305; Spectrum Technologies, Inc., Plainfield, IL). Plants in Expt. 1 were grown under day and night air temperature, RH, and daily light integral (DLI) of 26.3 ± 2.9 °C, 22.7 ± 2.1 °C, 89.8 ± 10.5 %, and 10.3 ± 2.5 mol·m^{-2}·d^{-1}, respectively. Plants in Expt. 2 were grown under a day and night air temperature, RH, and DLI of 29.7 ± 4.1 °C, 25.2 ± 3.5 °C, 91.2 ± 8.4 %, and 14.2 ± 5.1 mol·m^{-2}·d^{-1}.

Gas exchange measurements. In Expt. 1, leaf net photosynthesis (A_{leaf}) was measured individually on two young fully expanded leaves per plant using an infrared gas analyzer (IRGA) (CIRAS-4, PP Systems, Amesbury, MA, USA). The reference leaf temperature, photosynthetic photon flux density (PPFD) provided by an LED light (38 % red 37 % green 25 % blue), and CO_2 concentration inside the cuvette were set at 25 °C, 2000 μmol·m^{-2}·s^{-1}, and 400 μmol·mol^{-1}, respectively.

Root tip respiration (R_{tip}) was measured in root samples (~1 cm long) collected from each plant. Root samples were collected in vials filled with nutrient solution and transferred on ice to the laboratory. Then, they were lightly blotted with a paper towel and placed into 50-cm^3 cylindrical chambers connected to an IRGA (CIRAS-3, PP Systems, Amesbury, MA, USA). Reference CO_2 concentration was set to 400 μmol·mol^{-1}. CO_2 differentials between the reference and the head space in the chamber were measured automatically every minute for a period of 15 minutes. The data used was the highest value measured once they reached a plateau and the variation among them did not exceed 2 %. R_{tip} was calculated by dividing the CO_2 differential (output CO_2 - reference CO_2) by the fresh biomass of the root tip sample.

In Expt. 2, photosynthetic and respiration rates were measured simultaneously in a branch (A_{branch}) and the whole root system (R_{root}). A_{branch} was measured using a custom-built cylindrical chamber connected to an IRGA (model CIRAS-4, PP Systems, Amesbury, MA, USA). The chamber had a volume of 2.6 L and it was equipped with an LED grow light (SMD-5050; Birddog Lighting, Belgrade, MT) that delivered an average of 256 μmol·m^{-2}·s^{-1} PPFD (80 % red 20 % blue). CO_2 concentration inside the chamber was set at 400 μmol·mol^{-1}. A_{branch} was measured by placing a representative branch inside the chamber and collecting CO_2 differential data continuously for 15 minutes until stabilization as described above. Photos of each branch were taken immediately after gas exchange measurements and used to determine the leaf area index using ImageJ software version 1.51w (Schneider et al., 2012). SHB exhibits green leaves and green stems at this growth stage. Therefore, leaf area index represents the vertically projected area of all the green organs. A_{branch} was normalized by division with leaf area index. For small plants ('Farthing', 'Keecrisp', and 'Sentinel' SHB), the entire plant shoot fit inside the chamber. Therefore, A_{branch} represents whole canopy photosynthetic rates.

R_{root} was measured by placing the entire root system into an opaque 2-L plastic chamber connected to an IRGA (model CIRAS-3, PP Systems, Amesbury, MA, USA). Settings and data collection proceeded as in R_{tip} (Fig. 1). Respiration rate was calculated by dividing the CO_2 differential (output CO_2 - reference CO_2) by the fresh biomass of the entire root system. In both experiments, gas exchange measurements were performed between 11:00 and 14:00 HR on weeks four (half way) and eight (end) of the treatment period.

Acid phosphatase activity (APA). APA was measured on week four and eight, as per Tabatabai and Bremner (1969). Root tip samples (approximately 1 cm long) were collected in 20-mL glass vials containing 50 mM sodium acetate at pH 5.5 on ice. Later, samples were lightly blotted with a paper towel and transferred (in triplicate) to an assay solution containing 50 mM sodium acetate (pH 5.5) and 1.5 mM p-nitrophenol. Root samples were incubated for one hour at 30 °C under continuous shaking (500 rpm) in the dark as per Zhang et al. (2016). After incubation, APA was inhibited by adding 0.50 M NaOH and vortexing. Finally, absorbance (405 nm) of inhibited solution was measured using a microplate spectrophotometer (Synergy 2 Multi-Mode; BioTek Instruments Inc., Winooski, VT, USA). *p*-nitrophenol concentration was determined using a molar extinction coefficient of 1.83×10^4 M^{-1} cm^{-1}. Root samples were weighed immediately after the assay, and APA was normalized by division with root fresh mass (μmoles·g^{-1} of fine roots FM).

Root carbon exudation. Root carbon exudation was determined indirectly by measuring hexose concentration in nutrient solutions and then calculating the carbon molar fraction. Nutrient solution samples (20 mL) were collected 24 hours after changing the nutrient solution on week eight of the treatment period. Samples were immediately acidified with one drop of 12 M HCl. Then, 800 μL of acidified nutrient solution were centrifuged for 30 minutes at 15,000 rpm to precipitate suspended materials. The supernatant was discarded, and 250 μL of 90 % v/v ethanol was added to each tube before a second centrifugation step. Ethanol was allowed to evaporate overnight in a continuous flow hood at room temperature to form pellets. Pellets were resuspended in 200 μL of ultrapure water. Quantitation of hexose-equivalents was performed using the anthrone method as per Landhäusser et al. (2018) and Sperling et al. (2015). Briefly, 200 μL of the resuspended solution were mixed with 600 μL of anthrone stock solution (10 g·L^{-1} of conc. H_2SO_4) and incubated at 100 °C with continuous shaking for 10 minutes. Absorbance (620 nm) was measured using a microplate spectrophotometer (Synergy 2 Multi-Mode; Agilent BioTek Instruments Inc., Winooski, VT, USA). Hexose concentration was measured using a glucose standard curve. Glucose standard solution was prepared at 1 g·L^{-1} of ultrapure water and then a standard curve was made based on eight points from 0 to 0.150 g·L^{-1}. Exudate C content was calculated as 40 % of the hexose concentration.

Leaf spectrometry. In Expt. 2, SPAD index and anthocyanin reflectance index (ARI1) were measured using a portable narrow-bandwidth leaf spectrometer (CI-710; CID Bio-Science, Camas, WA). Measurements took place at the end of the acclimation period and on weeks four and eight of the treatment period. Two mature leaves and two young leaves per plant were measured. Mature leaves were randomly selected between 10 to 15 cm from the plant crown, and recently fully expanded young leaves were selected 5 to 10 cm from the tip of the canes. Data were averaged and treated as a single data point per replication.

Destructive harvest. A subset of plants in each experiment was destructively harvested at the end of the acclimation period and at the end of the treatment period. Plants were divided into new leaves, mature leaves, canes, and roots to determine their fresh mass. Root systems were further divided into fine roots and woody roots. Then, each organ was oven-dried to a constant weight at 65 °C, ground until it passed through a mesh with 1 mm holes and submitted for P concentration analysis at a commercial laboratory (Waters Laboratory, Camila, GA, USA). Phosphorus content of each organ was computed by multiplying dry mass and P concentration.

In Expt. 2, the following indicators were calculated as per Kreutz et al. (2023). The amount of P applied per week was calculated as the product of the nutrient solution volume multiplied by the nutrient solution P concentration. The total amount of P applied was the sum of the weekly amounts. Phosphorus use efficiency (PUE) was calculated as the ratio of total plant dry mass divided by the total amount of P applied (g TDM g^{-1} P applied). Phosphorus uptake efficiency (PUpE) was calculated as total plant P content divided by the total amount of P applied (mg P g^{-1} P applied). Phosphorus utilization efficiency (PUtE) represents the total plant dry mass produced per unit of P content (g TDM mg^{-1} P).

Root architecture measurements. Roots were scanned (600 dots per inch resolution) floating in water on a flatbed scanner (Expression 11000XL, Seiko Epson Corp., Tokyo, Japan) with a transparent acrylic tray. Then, total root length (TRL) was measured using RhizoVision Explorer (Seethepalli and York, 2020) and specific root length (SRL) was computed as the total root length to dry mass ratio.

Table 1
Average final dry mass of different organs of 'Colossus' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks.

Treatment	Dry mass (g)					
	Roots	Canes	Mature leaves	Young leaves	Total	Root: shoot ratio
+P	16.61	27.89**	7.32*	2.76*	54.51	0.44*
-P	18.70	23.48	10.78	4.48	57.43	0.49

***, **, *, indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level, respectively, based on one-way ANOVA ($n = 8$). Data from each organ was analyzed separately.

Statistical analysis. Expt. 1 followed a completely randomized design with eight single-plant replications per P treatment. Expt. 2 followed a completely randomized design with factorial arrangement of treatments and six single-plant replications per P treatment × Variety combination. Plants were physically arranged in two greenhouse benches, but environmental variability between the benches was minimal. Therefore, blocking was not used for analysis.

Two-sample t-tests were conducted to determine growth among the plants at the end of the acclimation period and those at the end of the treatment period. In Expt. 1, data were analyzed through one-way ANOVA (P treatment as a factor). In Expt. 2, data were analyzed through two-way ANOVA where variety (V), P treatment (P), and their interaction were included. Leaf spectrometry data were analyzed through two-way ANOVA, including leaf age, P treatment, and their interaction. Tukey's HSD test was used for group separation when statistical differences were present ($P \leq 0.05$). All ANOVAs were conducted using 'agricolae' package (de Mendiburu, 2021) in R program version 4.1.2 (R Development Core Team, 2021).

3. Results

3.1. Experiment 1

All 'Colossus' SHB plants grew during the experiment and after 8 weeks growing under treatments, they had 106.8 % more total dry mass (DM) with respect to the plants after acclimation ($P = 1.0 \times 10^{-09}$, data not shown). Treatments affected DM, as +P plants had 18.8 % more cane DM, but 32.1 % and 38.4 % less mature leaf DM and young leaf DM, respectively, than -P plants (Table 1). No differences in root dry DM and total DM were observed between treatments. Root:shoot ratio was higher in -P plants than +P plants.

At the end of the acclimation period, mature and young leaves from all plants exhibited 0.12 % P or higher (data not shown) which is the standard threshold for P deficiency in blueberries (Krewer and NeSmith, 1999). By the end of the treatment period, -P plants were P deficient (mature leaves average = 0.09 % P), while +P were not (mature leaves average = 0.14 % P) (Fig. 2A). Phosphorus deficient plants exhibited lower root and cane P concentration compared to +P plants, but there were no differences in P concentration in young leaves between treatments. There were differences in P content between treatments across plant organs except for mature leaves (Fig. 2B). +P plants exhibited

Table 2
Leaf photosynthesis (A_{leaf}) and root tip respiration (R_{tip}) of 'Colossus' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) at 4 and 8 weeks.

Treatment	4 weeks		8 weeks	
	A_{leaf} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	R_{tip} (μg $CO_2 \cdot g$ of $roots^{-1} \cdot min^{-1}$)	A_{leaf} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	R_{tip} (μg $CO_2 \cdot g$ of $roots^{-1} \cdot min^{-1}$)
+P	1.72	0.71*	2.64	0.63***
-P	1.45	0.97	2.87	1.49

***, **, *, indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level, respectively, based on one-way ANOVA ($n = 8$).

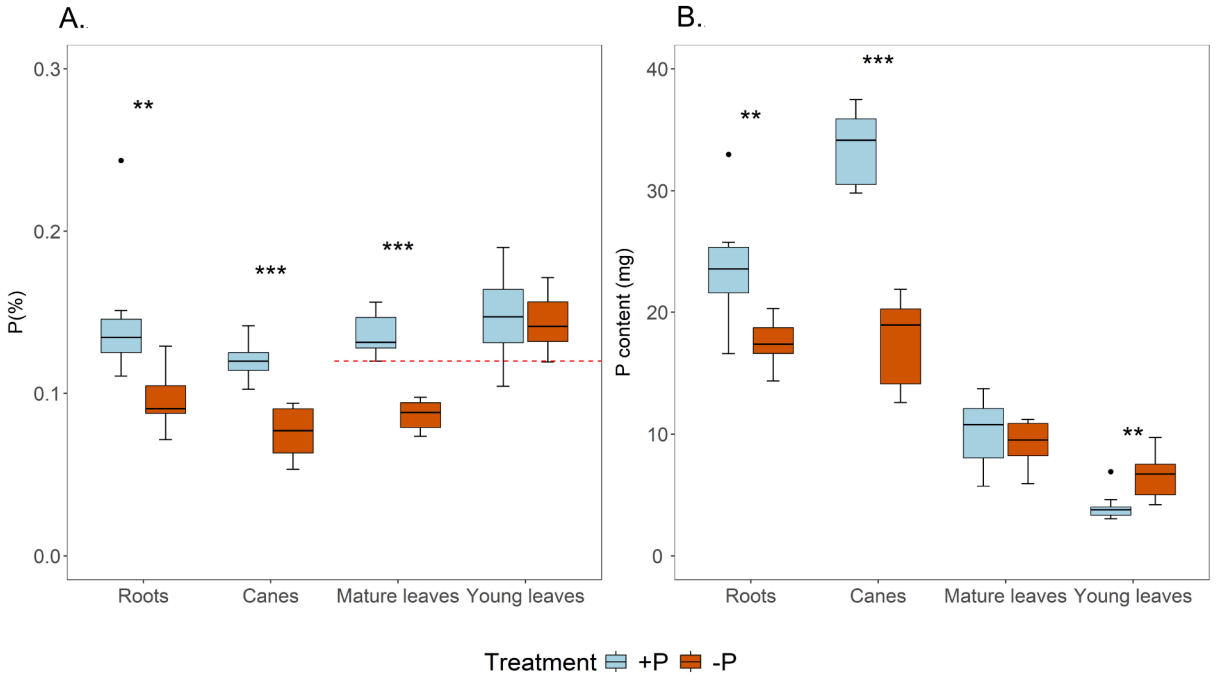

Fig. 2. Phosphorus concentration (P%) (A) and content (B) of different organs of 'Colossus' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} of phosphorus (+P) or 0 mg L^{-1} of P (-P) for 8 weeks in experiment 1. Data from each organ was analyzed separately. Red dotted line indicates baseline for P deficiency in leaves. ***, **, *, indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level, respectively, based on one-way ANOVA ($n = 8$). Dots represent outliers.

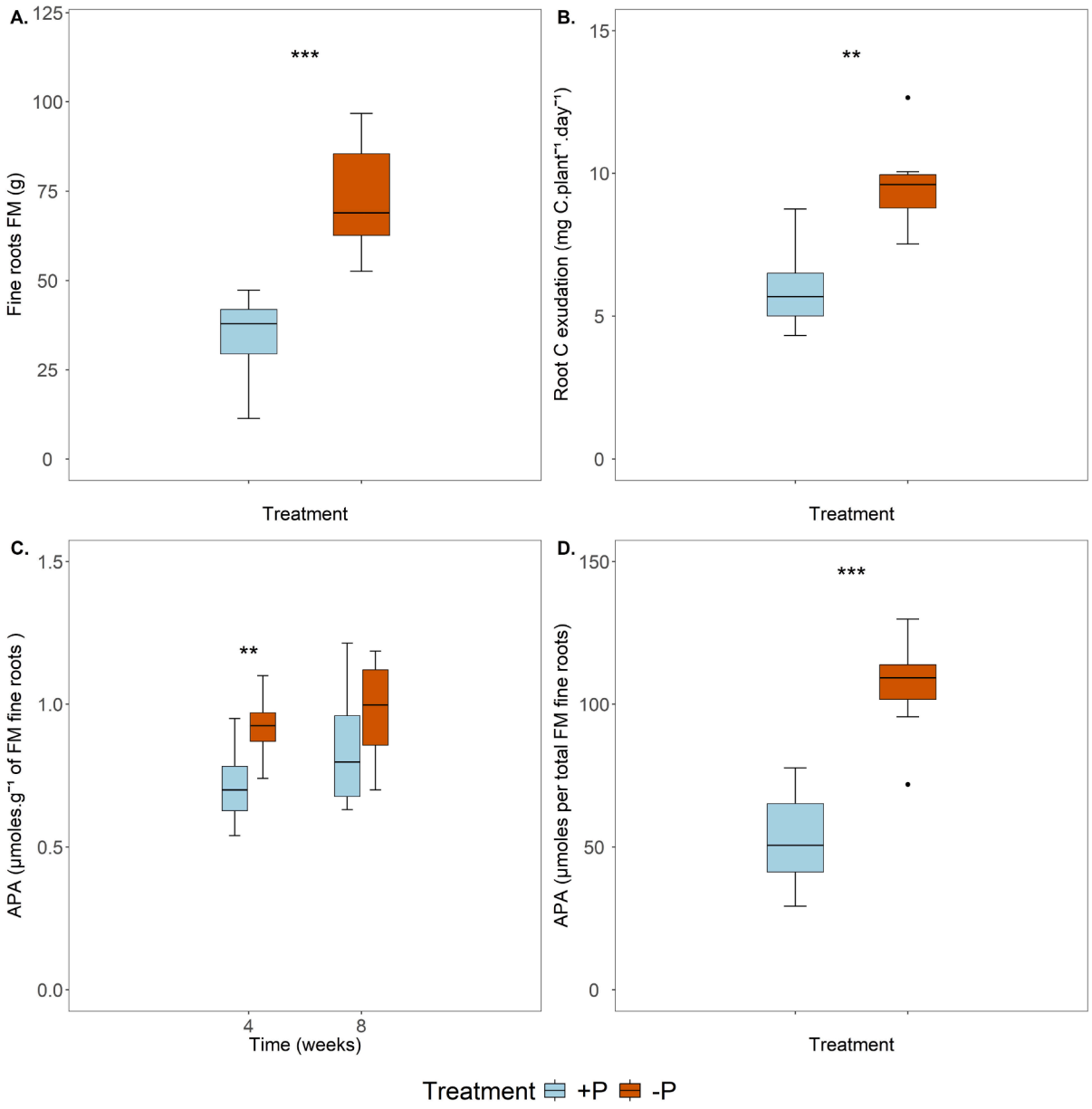

Fig. 3. Fine root fresh mass (FM) (A) and woody roots FM (B) of 'Colossus' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks. ***, **, *, indicate statistical significance at the 0.001, 0.01, and 0.05 $P \leq$ level, respectively, based on one-way ANOVA ($n = 8$). Dots represent outliers.

higher root and cane P content but lower young leaves P content than -P plants.

As plants grew, A_{leaf} exhibited a tendency to increase over time in all plants (Table 2). There were no differences in A_{leaf} between treatments. In contrast, -P plants had 36.6 % (at week 4) and 136.5 % (at week 8) higher R_{tip} than +P plants.

P deficiency also caused root morphological and biochemical responses (Fig. 3). -P plants exhibited higher biomass of fine roots and more APA per gram of fine roots than +P plants. When scaled to account for fine root biomass differences, -P plants exhibited approximately double total APA that +P plants Additionally, root C exudation was 61.0 % higher in -P plants than +P plants. No differences in woody root FM were observed between treatments (average = 41.8 g, data not shown).

3.2. Experiment 2

In this follow-up experiment, four blueberry varieties with different growth characteristics were evaluated (Fig. 4). All plants grew during the treatment period regardless of P treatment. 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' exhibited 136.5 % ($P = 8.8 \times 10^{-08}$), 236.8 % ($P = 5.2 \times 10^{-05}$), 110.3 % ($P = 4.3 \times 10^{-06}$), and 158.3 % ($P = 3.2 \times 10^{-06}$) more dry mass than plants harvested after acclimation, respectively (data not shown). Overall, 'Colossus' exhibited higher dry mass in all plant organs than the other varieties (Table 3). There was no V × P interaction in root or young leaves dry mass. Phosphorus deficiency affected other organs aboveground. In all varieties, -P plants exhibited less cane and mature leaves dry mass than +P plants. Root:shoot ratio was interactively affected by variety and P treatment. 'Farthing' plants responded to P deficiency by increasing their root:shoot biomass ratio, while other varieties did not.

At the end of the acclimation period, leaf P concentrations in plants of all varieties were above the reference deficiency threshold (data not shown). At the end of the treatment period, mature and young leaves of -P plants of all varieties exhibited P concentration below 0.12 %,

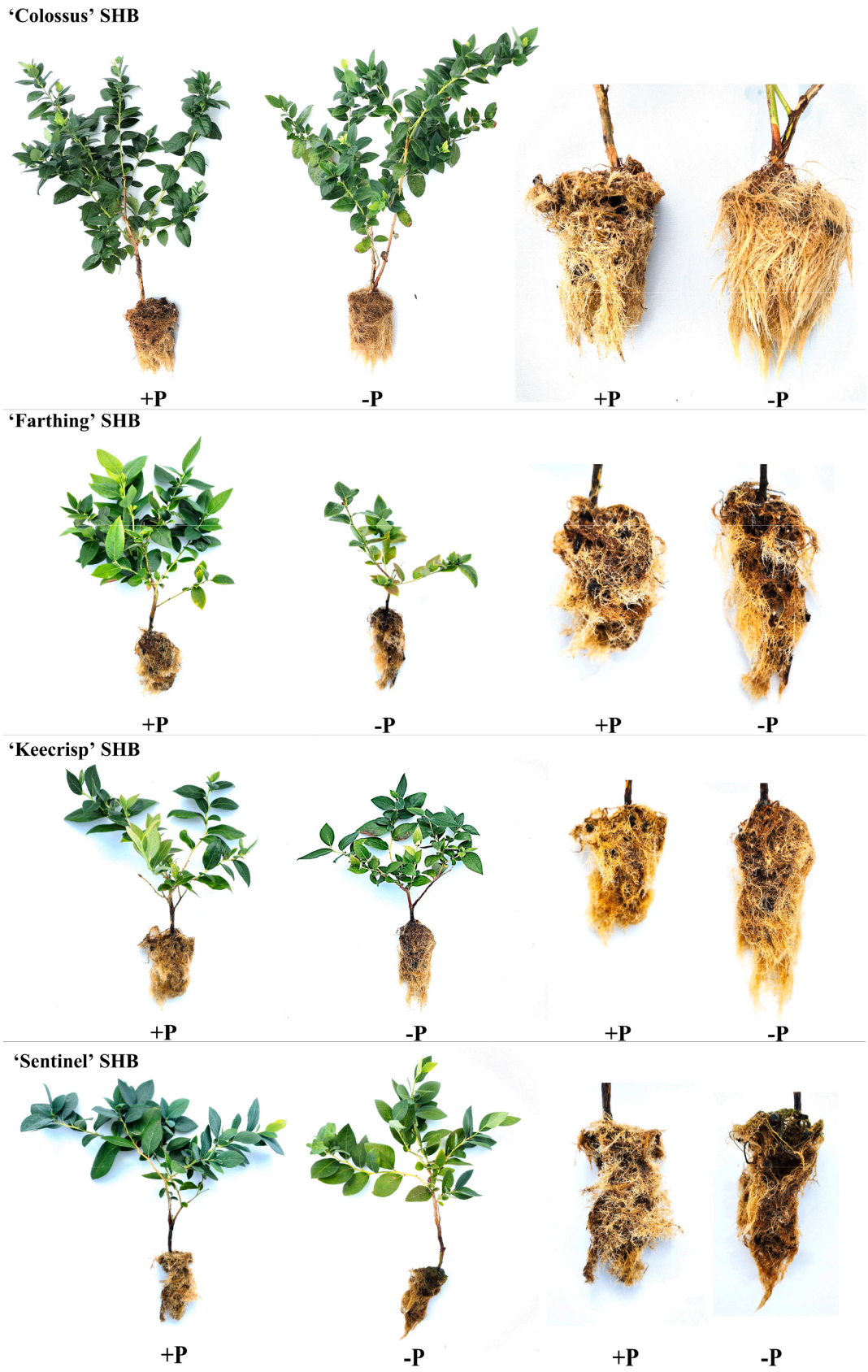

Fig. 4. 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' southern highbush blueberry plants grown in nutrient solutions with 15 mg L^{-1} of phosphorus (+P) or 0 mg L^{-1} of P (-P) for 8 weeks in experiment 2.

Table 3
Average final dry mass of different organs of 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks.

Treatment	Dry mass (g)					
	Roots	Canes	Mature leaves	Young leaves	Total	Root: shoot ratio
Variety (V)						
'Colossus'	5.24 a	13.50 a	7.12 a	15.09 a	41.02 a	0.15 c
'Farthing'	0.61 b	0.37 c	0.40 c	0.41 b	1.78 c	0.51 a
'Keecrisp'	0.55 b	0.95 bc	0.64 c	0.58 b	2.68 c	0.25 b
'Sentinel'	0.84 b	1.79 b	2.48 b	2.29 b	7.41 b	0.14 c
P treatment (P)						
+P	1.94	4.55 a	2.87 a	4.65	13.32	0.22 b
-P	1.68	3.75 b	2.45 b	4.54	13.12	0.30 a
V × P						
'Colossus' +P	5.58	14.88 a	7.43	15.15	40.58	0.13 d
'Colossus' -P	4.90	12.12 b	6.80	15.03	41.47	0.16 cd
'Farthing' +P	0.74	0.48 c	0.54	0.54	2.30	0.42 b
'Farthing' -P	0.47	0.25 c	0.26	0.28	1.26	0.60 a
'Keecrisp' +P	0.52	1.03 c	0.58	0.52	2.37	0.22 cd
'Keecrisp' -P	0.58	0.87 c	0.70	0.64	2.98	0.28 c
'Sentinel' +P	0.92	1.82 c	2.92	2.40	8.05	0.13 d
'Sentinel' -P	0.77	1.77 c	2.05	2.18	6.77	0.14 d
V	***	***	**	**	**	***
P	NS	*	*	NS	NS	**
V × P	NS	*	NS	NS	NS	*

***, **, *, NS indicate statistical significance at the $P \leq 0.001$, 0.01, and 0.05 level or not significant, respectively based on ANOVA.
For each treatment, means within columns followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 12$ (V) or 24 (P) for main effects; $n = 6$ for interactions).

suggesting that they were P deficient (Fig. 5). Phosphorus concentration and P content in all organs of all varieties was lower in -P plants than +P plants, but the magnitude of change was different among genotypes. -P treatments affected 'Farthing' severely. -P plants exhibited a decrease of 80.0 % and 68.4 % in mature and young leaves P %, respectively, and 60.5 % and 57.4 % decrease in mature and young leaves P content, respectively, compared to +P plants. 'Keecrisp' and 'Sentinel' were similarly affected by -P treatments. 'Keecrisp' plants exhibited a 70.4 % and 69.2 % decrease in mature and young leaves P %, respectively, and 66.9 % and 61.4 % reduction of P content in mature and young leaves, respectively. 'Sentinel' -P plants exhibited 64.5 % and 65.5 % less P % in mature and young leaves, respectively, and 72.3 % and 68.0 % less P content, respectively, compared to +P plants. 'Colossus' -P plants exhibited the smallest reduction in leaf P % and P content under deficiency, as they had 57.9 % and 55.0 % less P % in mature and young leaves, respectively, and 60.5 % and 57.4 % less P content, in mature and young leaves, respectively, than +P plants (Fig. 5).

Significant V × P interaction was measured in PUE and PUtE but not in PUpE where only the main factor variety was significant (Table 4). Phosphorus deficient plants of all varieties except 'Farthing' exhibited higher PUE than their respective +P controls. 'Colossus' and 'Sentinel' exhibited higher PUpE than 'Farthing' and 'Keecrisp'. Phosphorus deficient plants of all varieties exhibited higher PUtE than their respective +P controls.

P treatments occasionally affected gas exchange (Table 5). In week 4, only the factor variety was significant, as 'Colossus', 'Keecrisp', and 'Sentinel' had the highest A_{branch}. However, in week 8, -P plants, regardless of the variety, had 13.3 % less A_{branch} than +P plants. In both periods, R_{root} was higher in -P plants than in +P plants in 'Farthing' and 'Keecrisp'. 'Farthing' had the lowest A_{branch} of all varieties in both periods.

Root architecture traits were affected by P treatments in some SHB varieties (Fig. 6). Fine roots FM was affected only the two main factors. 'Colossus' plants exhibited the highest fine roots FM. Phosphorus deficiency led to higher fine roots FM accumulation. There were significant V × P interactions for the rest of the root architecture traits. 'Colossus' plants exhibited lower woody roots FM and higher root length when grown in -P conditions as compared to +P conditions. Other varieties did not exhibit this response. 'Farthing' plants exhibited higher SRL in -P conditions as compared to +P conditions. Other varieties did not exhibit this response.

P deficiency also led to biochemical changes in SHB roots (Fig. 7). On week 4, 'Farthing' plants in the -P treatment exhibited higher APA than their respective +P controls. This response was not observed in other varieties. On week 8, only the main factor P treatment was significant, and -P plants in all varieties exhibited higher APA than +P plants. When scaled according to total fine roots FM, only 'Colossus' exhibited significantly higher APA activity under P deficit. Root carbon exudation rates were higher in -P plants than in +P plants regardless of variety. 'Colossus' exhibited the highest root carbon exudation rates compared to the other varieties. 'Farthing' exhibited the lowest exudation rate. 'Keecrisp' and 'Sentinel' were intermediate.

Leaf color indexes captured differences between P treatments (Supplementary Table 1). At the start of the treatment period, SPAD was higher in mature leaves than in young leaves in all varieties and there were no differences in ARI1 between treatments After 8 weeks of growth in nutrient solutions with contrasting P concentrations, leaf age and P treatment had interactive effects on SPAD. Mature leaves and leaves of plants in the -P treatment exhibited higher SPAD than their respective controls. Mature leaves in the -P treatment exhibited higher ARI1 than their controls in all varieties except 'Colossus'. Symptom severity varied among leaves (Fig. 8).

4. Discussion

Inorganic phosphate is extremely reactive in the soil (Gatiboni et al., 2021; Schubert et al., 2020) and soil microbial communities can affect plant PUpE (Scagel, 2005; Wang and Lambers, 2020). Therefore, hydroponic solutions have been commonly used to study plant P deficiency responses (Neocleous and Savvas, 2019; Niu et al., 2015; Waqas et al., 2023). Here were used P free hydroponic solutions to elicit P deficiency responses in SHB. In both experiments, SHB plants of all varieties tested were P deficient by the end of the treatment period (Fig. 2 and Fig. 5), as evidenced by their foliar P concentrations that were below the minimum critical threshold for highbush blueberry (FDACS, 2011; Hart et al., 2006; Krewer and NeSmith,1999; Phillips and Williamson, 2020). By comparing P deficient and control plants, we produced a detailed understanding of SHB responses and SHB P nutrition.

SHB can remobilize P to maintain growth in P limiting conditions. Phosphorus is a highly mobile nutrient within the plant (Skinner and Matthews, 1989). Phosphorus remobilization from one organ to another is a strategy that allows growth and development to continue under P limiting conditions (Dixon et al., 2020). In addition, responses to P deficiency are age and genotype dependent (Zhu et al., 2020). In Expt. 1, P deficient 'Colossus' SHB plants exhibited lower P concentration in all organs except for young leaves compared to +P plants, suggesting P remobilization from canes and mature leaves to new leaves had occurred (Fig. 2). However, total plant P reserves appear to be important for P plant remobilization capacity. In Expt. 2, where all plants were smaller and contained less P than 'Colossus' from Expt. 1, all P deficient plants

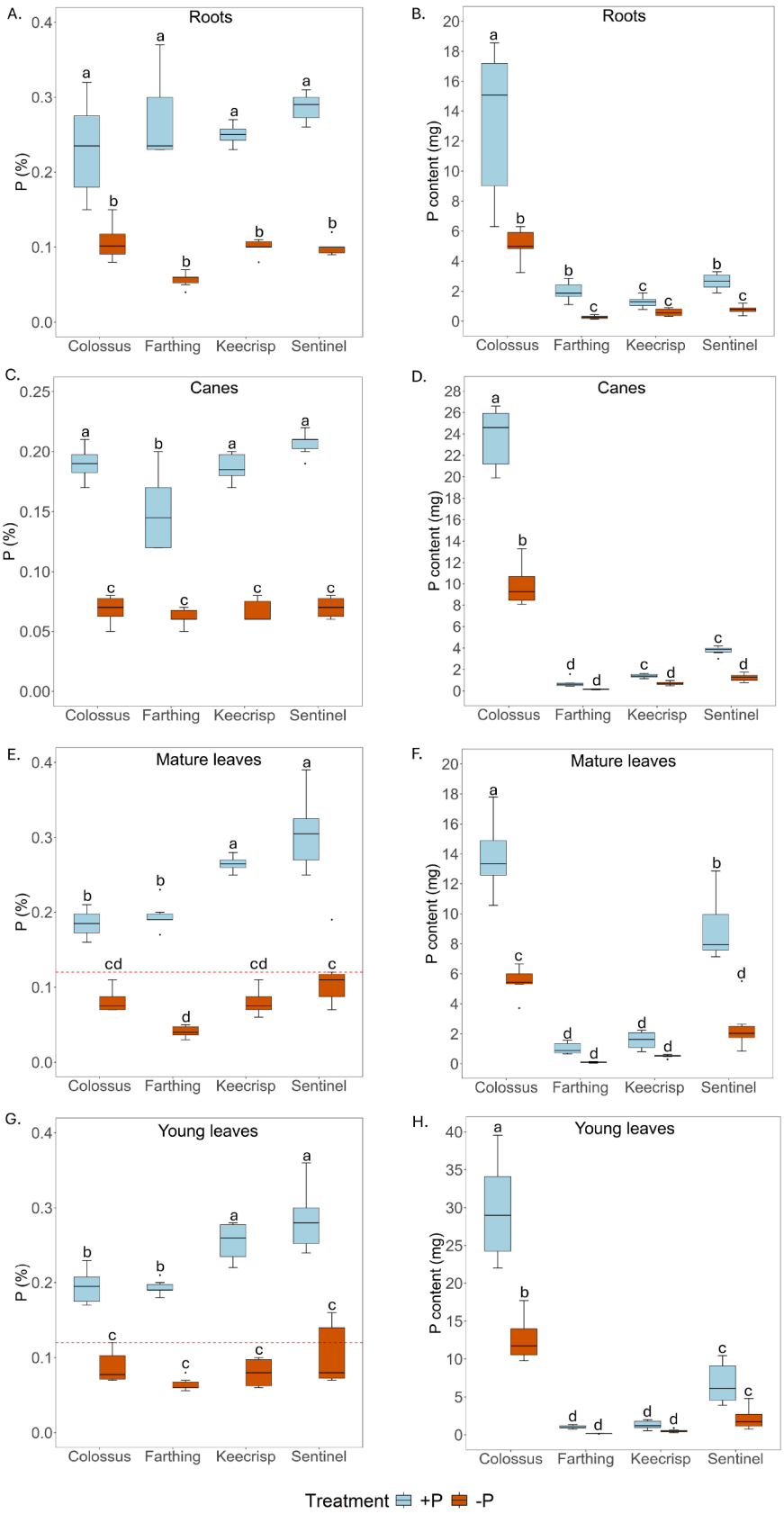

Fig. 5. Phosphorus concentration (P %) (A, C, E, G) and P content (B, D, F, H) of different organs of 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks. Data from each organ was analyzed separately. Red dotted line indicates baseline for P deficiency in leaves. For each organ, boxes topped by the same letter were not significantly different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 6$). Dots represent outliers.

Table 4
Phosphorus use efficiency (PUE), P uptake efficiency (PupE), and P utilization efficiency (PUtE) of 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks in experiment 2.

Treatment	PUE (g TDM g^{-1} P applied)	PUpE (mg P g^{-1} P applied)	PUtE (g TDM mg^{-1}·P)
Variety (V)			
'Colossus'	190.24 a	214.01 a	0.91 b
'Farthing'	7.17 c	8.52 c	1.14 a
'Keecrisp'	12.98 c	15.28 c	0.89 bc
'Sentinel'	32.88 b	50.60 b	0.73 c
P treatment (P)			
+P	34.16 b	75.70	0.43 b
-P	87.46 a	68.50	1.40 a
V × P			
'Colossus' +P	104.04 b	218.01	0.48 d
'Colossus' -P	276.44 a	210.02	1.33 bc
'Farthing' +P	5.91 d	12.29	0.49 d
'Farthing' -P	8.43 d	4.76	1.80 a
'Keecrisp' +P	6.07 a	15.65	0.40 d
'Keecrisp' -P	19.89 d	14.92	1.38 b
'Sentinel' +P	20.64 d	56.86	0.37 d
'Sentinel' -P	45.11 c	44.30	1.10 c
V	***	***	***
P	***	NS	***
V × P	***	NS	***

***, **, *, NS indicate statistical significance at the $P \leq 0.001$, 0.01, and 0.05 level or not significant,
respectively based on ANOVA.
For each treatment, means within columns followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 12$ (V) or 24 (P) for main effects; $n = 6$ for interactions).
PUE deviates from the product of PUtE x PUpE due to rounding and averaging of raw data.

had lower P concentration and P content in all organs than control plants (Fig. 5). Growth continued throughout the experiment despite lack of P in the nutrient solution. Since P uptake was not possible in the -P treatment, -P plants must exhibit high PUtE (Table 4). Nevertheless, P remobilization was not enough to prevent P deficiency in young leaves of the small plants. While research related to P remobilization under P deficiency is lacking in blueberry, P remobilization from reserves in perennial stems and roots has been previously reported in other woody perennial crops such as grapevine (Schreiner et al., 2006) and citrus (Zambrosi et al., 2012).

These findings have important implications for SHB farming. Our results suggest that detection of P deficiency may be more accurate in mature leaves than in younger leaves (Fig. 2 and Fig. 5). However, current manuals recommend sampling youngest fully expanded leaves for plant nutrient assessment (FDACS, 2011; Hart et al., 2006; Krewer and NeSmith,1999; Phillips and Williamson, 2020). Where young leaves are sampled, the P concentration may be at or above the critical level, masking the true P nutritional status of the plant and leading to a misdiagnosis for fertilization purposes. Since older, mature leaves are the most informative part of the foliage, we propose that these are the leaves should be sampled for assessing the P nutritional status of SHB.

Responses were different among SHB varieties. Our results suggest that there were differences in P use efficiency (PUE) among the tested SHB varieties (Table 4). PUE is the capacity of a genotype to function adequately under low available P conditions (Shenoy and Kalagudi, 2005). A genotype with high PUE is one that has a high ability to absorb P from the soil [P uptake efficiency (PUpE)] and/or a high efficiency of remobilization of P among plant organs for sustaining biomass production [P utilization efficiency (PUtE)] (Sandaña, 2016; van de Wiel et al., 2016). Phosphorus deficient 'Colossus', 'Keecrisp', and 'Sentinel' plants continued growing (Table 3) even when their P concentration and P content decreased (Fig. 5). There are several reports of PUE in young plants of other fruit species such as peach (Menegatti et al., 2021), citrus (Tu, 2018; Zambrosi et al., 2013), and grapevine (Gautier et al., 2018). However, to the best of our knowledge, there is no research on PUE in SHB. Our findings indicate that a combination of morphological and biochemical adaptations (see below) contribute to PUE in SHB. Also, the diversity documented in this very small sample of varieties suggests that

Table 5
Branch photosynthetic rates (A_{branch}) and root respiration rates (R_{root}) of 'Colossus', 'Farthing', 'Keecrisp', and 'Sentinel' southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) at week 4 and 8 of treatment period.

Treatment	4 weeks		8 weeks	
	A_{branch} (μmol CO_2·m^{-2}·s^{-1})	R_{root} (μg CO_2·g of $roots^{-1}$·min^{-1})	A_{branch} (μmol CO_2·m^{-2}·s^{-1})	R_{root} (μg CO_2·g of $roots^{-1}$·min^{-1})
Variety (V)				
'Colossus'	3.17 a[i]	1.68 b	2.80 a	1.86 c
'Farthing'	1.44 b	3.58 a	1.69 b	4.13 ab
'Keecrisp'	2.84 a	3.78 a	3.12 a	5.06 a
'Sentinel'	3.13 a	3.27 a	3.08 a	3.88 b
P treatment (P)				
+P	2.75	2.26 b	2.86 a	2.62 b
-P	2.54	3.89 a	2.48 b	4.85 a
V × P				
'Colossus' +P	3.48	1.13 d	2.63	1.19 e
'Colossus' -P	2.86	2.23 bcd	2.97	2.53 de
'Farthing' +P	1.36	2.13 cd	2.21	2.57 de
'Farthing' -P	1.51	5.02 a	1.17	5.68 ab
'Keecrisp' +P	3.10	2.70 bc	3.35	3.48 cd
'Keecrisp' -P	2.58	4.85 a	2.88	6.63 a
'Sentinel' +P	3.05	3.08 bc	3.25	3.22 cd
'Sentinel' -P	3.22	3.46 b	2.90	4.53 bc
V	**	**	**	**
P	NS	**	*	**
V × P	NS	**	NS	*

***, **, *, NS indicate statistical significance at the $P \leq 0.001$, 0.01, and 0.05 level or not significant, respectively based on ANOVA.
For each treatment, means within columns followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 12$ (V) or 24 (P) for main effects; $n = 6$ for interactions).

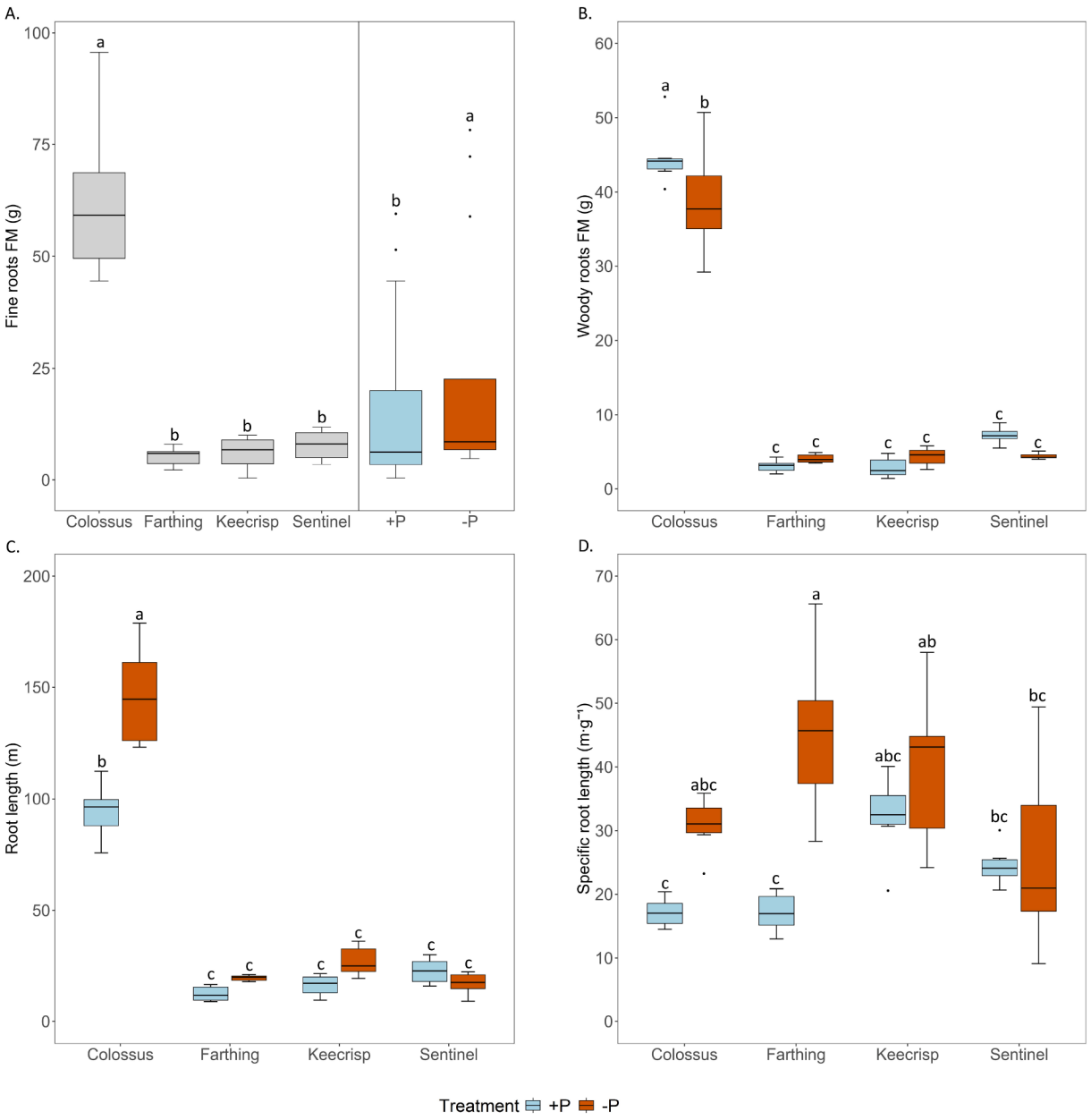

Fig. 6. Root architecture traits of 'Colossus' (A-D), 'Farthing' (E-H), 'Keecrisp' (I-L), and 'Sentinel' (M-P) southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks. For each root trait, boxes topped by the same letter were not significantly different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 12$ (variety) or 24 (P treatment) for main effects; $n = 6$ for interactions). Dots represent outliers.

breeding for P-efficient SHB might be possible.

Our results provide a detailed description of P deficiency symptoms in SHB. Since P is a highly immobile nutrient in the soil (Ibrahim et al., 2022), a commonly observed morphological response to P deficiency is to explore a larger area in the soil by producing long, thin, and branched roots at the lowest possible energetic cost (Gao et al., 2023; Maillard et al., 2015; Sun et al., 2015; Tang et al., 2020; Wissuwa et al., 2005). Long, thin roots are less expensive (in terms of energy and resources) to produce than woody roots. Phosphorus deficient SHB plants exhibited higher fine root mass than control plants (Fig. 3 and Fig. 6). 'Farthing' plants exhibited higher SRL under P deficiency conditions. These rapid changes in root architecture suggest that SHB responded to low P conditions by maximizing the area of soil explored by its roots. Low SRL indicate that the investment in biomass is relatively low for the high volume of roots produced (Ostonen et al., 2007).

In addition to morphological changes, we also documented biochemical responses to P deficiency (Fig. 3 and Fig. 7). Most varieties exhibited increased APA and all varieties exhibited increased root C exudation under P deficiency. Acid phosphatase enzymes catalyze the hydrolysis of organic P to increase its bioavailability to plants (Malhotra et al., 2018). The release of root exudates has been observed at later stages of P deficiency (Carvalhais et al., 2011). Exuded compounds include carboxylic acids such as acetic, citric, gluconic, malic, and succinic acids (Pandey et al., 2014) that modify the rhizosphere chemistry to help release the P fixed in the soil (Minemba et al., 2019; Ramaekers et al., 2010). Therefore, our results suggest that SHB plants were actively modifying the rhizosphere to improve their PUpE.

P deficiency symptoms were also observed in leaves. Leaf pigmentation was assessed by SPAD and ARI1 measurements (Suplementary table1). SPAD is an indicator of chlorophyll content. Phosphorus deficient leaves display a darker green color due to a high chlorophyll content (Li et al., 2006) caused by anatomical changes in the leaf mesophyll (Chiera et al.,

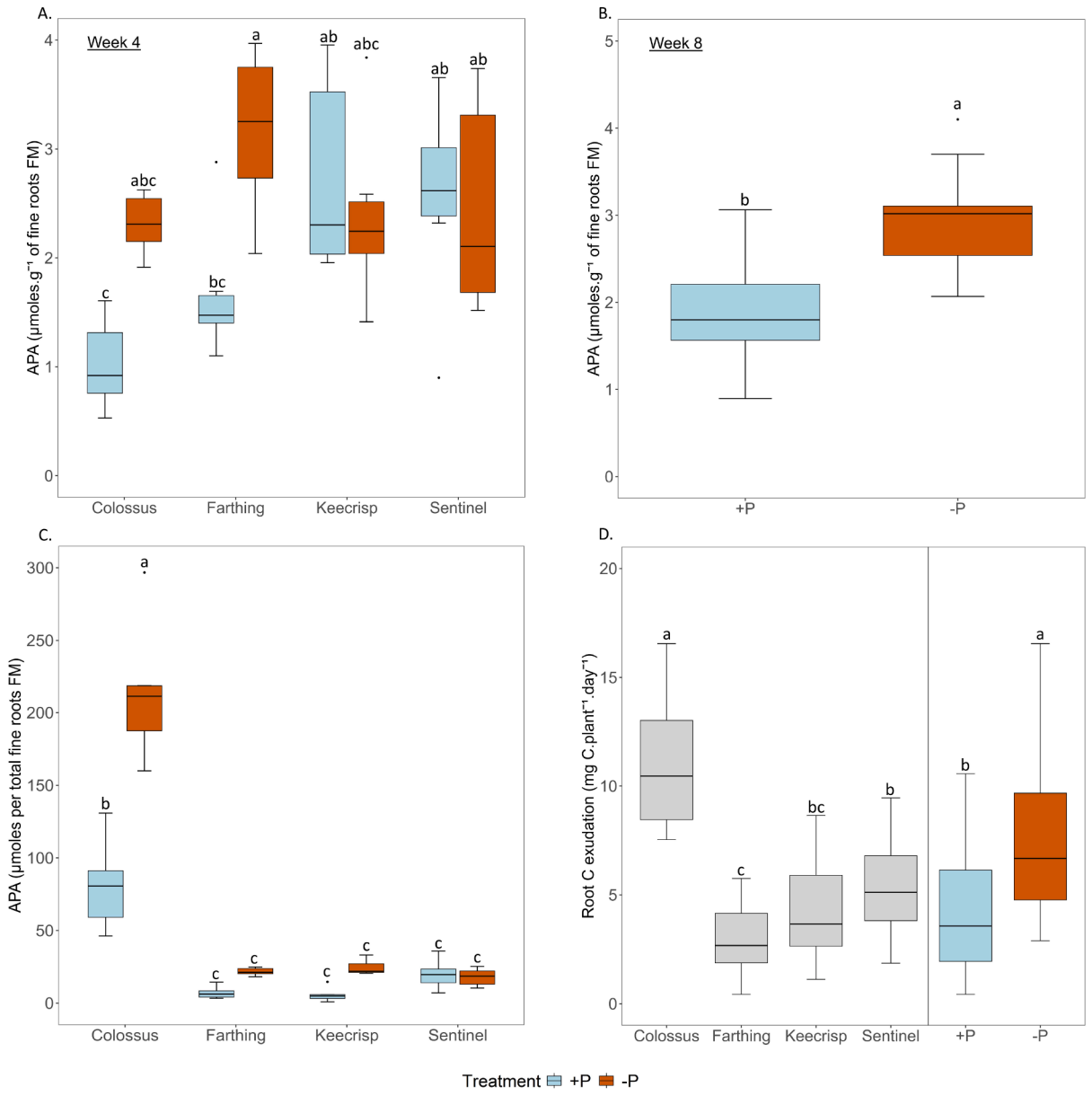

Fig. 7. Acid phosphatase activity of a root sample, total root system acid phosphatase activity, and root carbon exudation rate of 'Colossus' (A-C), 'Farthing' (D-F), 'Keecrisp' (G-I), and 'Sentinel'(J-L) southern highbush blueberry grown in nutrient solutions with 15 mg L^{-1} (+P) or 0 mg L^{-1} phosphorus (-P) for 8 weeks. For each response variable, boxes topped by the same letter were not significantly different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 24$ for main effect; $n = 6$ for interactions). Dots represent outliers.

2002). ARI1 is an indicator of anthocyanin content. Anthocyanin accumulation leads to the characteristic purple or reddish coloration in P-deficient plants (Henry et al., 2018; Jezek et al., 2023; Zheng et al., 2020). Anthocyanins also play photoprotective, antioxidant, and senescence roles in the leaf (Landi et al., 2015; Lo Piccolo et al., 2018; Pei et al., 2021). In this research, the appearance of leaf symptoms in P deficient plants depended on the variety. Leaves of 'Colossus', 'Keecrisp', and 'Sentinel' did not exhibit coloration changes when sampled 4 weeks after the start of the experiment. On the other hand, 'Farthing' leaves were dark green and reddish at this point. During the final sampling point, mature leaves in all -P plants were darker green, but only 'Farthing' and 'Sentinel' contained more anthocyanins. The heterogeneity in foliar symptoms development further emphasizes the importance of appropriate sampling for tissue analysis.

P deficiency affected the plant C balance. Findings in other species suggest that P deficiency reduces photosynthetic rates by inhibiting ATP synthase activity (Carstensen et al., 2018), reducing rubisco amount and activity (de Bang et al., 2021; Xu et al., 2007), reducing stomatal conductance, reducing the efficiency of photosystem II (PSII), and increasing the production of free radicals that damage the photosynthetic apparatus (Poudyal et al., 2021). Here, P deficiency occasionally reduced C assimilation (Tables 2 and 5). Additionally, P deficiency led to higher respiration rates in root tips (Table 2) or whole root systems (Table 5) in most varieties. These results support previous findings in other crops that indicate that when P is scarce, the proportion of C allocated to root growth and root respiration increases (Walk et al., 2006). In other species, root respiration can consume up to 40 % of daily photosynthates in P deficient plants (Funayama-Noguchi et al., 2021; Postma and Lynch., 2011). Additionally, root exudation represents a C sink in the root system. Therefore, our results suggest that P deficiency creates a challenging C scenario for SHB plants. Stable or decreased photosynthesis and increased root respiration and exudation cause lower daily C gain under P limiting conditions in SHB. Lower daily C gain, in turn, affects growth rates leading to smaller plants, explaining

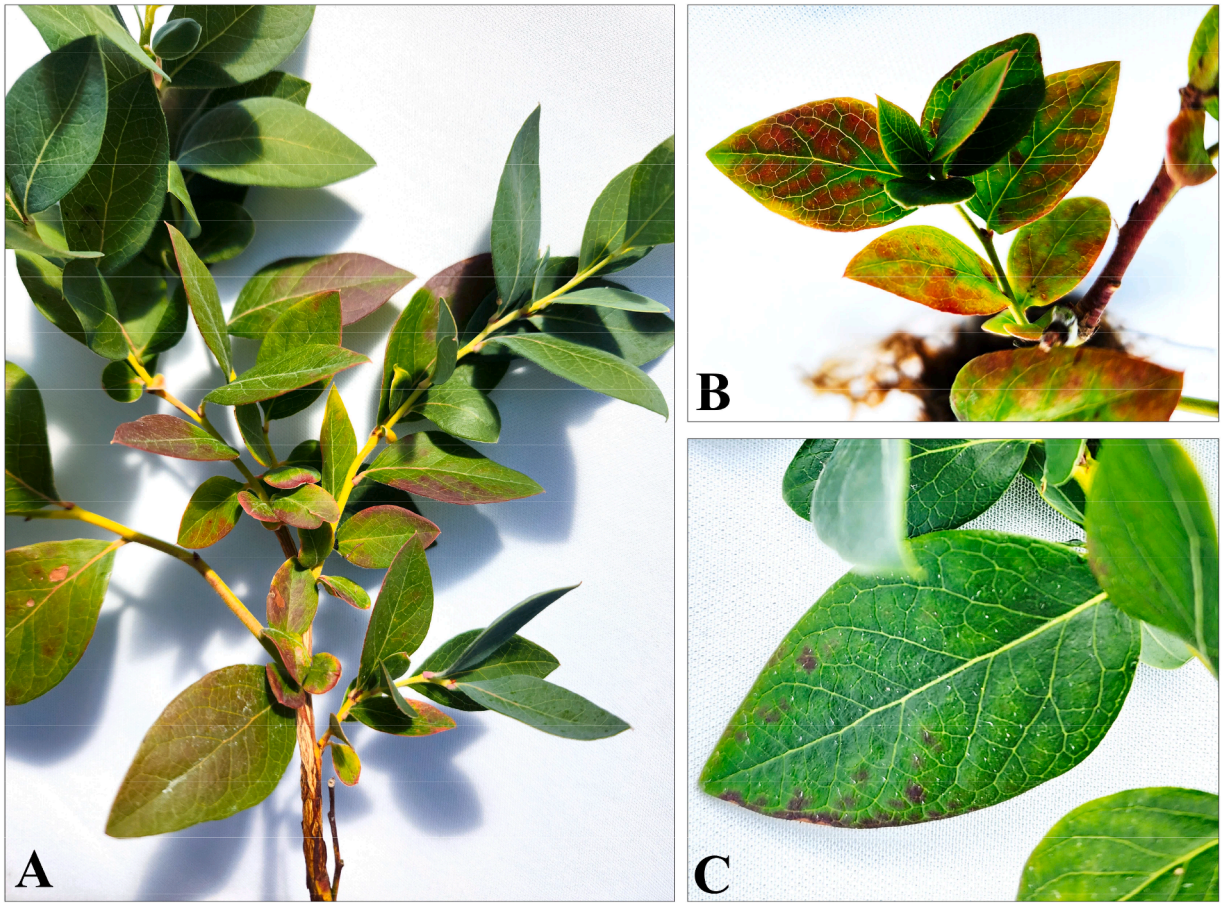

Fig. 8. Phosphorus (P) deficiency symptoms on leaves of 'Sentinel' (A), 'Farthing' (B), and 'Keecrisp' (C) southern highbush blueberry plants grown in nutrient solution with 15 mg L^{-1} (+P) or 0 mg L^{-1} P in experiment 2.

the stunting response commonly observed in farms (Muneer et al., 2024; Wissuwa et al., 2005). Our gas exchange, biomass accumulation, and root exudation data provide a glimpse into the mechanism through which P deficiency leads to diminished growth in SHB.

5. Conclusion

SHB plants exhibit morphological and biochemical responses to P deficiency. Phosphorus remobilization from storage organs helped maintain growth under P-limiting conditions. SHB plants produced more fine roots and greater root length, increased their APA, and exudated more C from their roots under P limiting conditions. Phosphorus deficiency led to visual symptoms in all leaves, but older leaves were most informative when diagnosing plant P status. Overall plant C balance was affected by P deficiency due to a reduction of C assimilation and increases in root respiration and C exudation rates.

CRediT authorship contribution statement

Marlon Retana-Cordero: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Gerardo H. Nunez:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Gerardo H. Nunez reports financial support was provided by Florida Department of Agriculture and Consumer Services. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114057.

Data availability

Data will be made available on request.

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