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RESEARCH ARTICLE

Use of increasing rates of ammonia nitrogen in pot-grown blueberries and its effect on fruit yield and macronutrient concentration in leaves

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ABSTRACT

Due to the smaller size of berries plants and some vegetables, their production in containers has grown worldwide. To understand the effect of increasing doses of ammoniacal N, we evaluated production, firmness and size of fruits and concentration of macronutrients in leaves of four blueberry (*Vaccinium corymbosum* L.) cultivars (Duke, Legacy, Cargo, and Suziblue) in south central Chile. All were grown in pots with substrate during their third growing season. The results showed an interaction of ammoniacal N dose with blueberry cultivar in most of the parameters evaluated, except for firmness and size of fruits. The increasing dose of ammoniacal N allowed higher production per plant in only two of the four cultivars without effects on the quality attributes firmness and size of fruits. Differences in production per plant and fruit firmness were obtained between cultivars, with production values per plant between 86-210, 142-219, 195-203, and 504-979 g in 'Duke', 'Legacy', 'Cargo', and 'Suziblue', respectively, and greater fruit firmness in 'Suziblue'. The concentrations of macronutrients in leaves showed interactions between cultivar and doses of ammoniacal N, which did not allow obtaining a single dose of ammoniacal N that generate nutritional concentration values in leaves associated with greater fruit production in the four blueberry cultivars.

Key words: Fruit firmness, fruit quality, fruit yield, macronutrient, *Vaccinium corymbosum*.

INTRODUCTION

The production of healthy foods in the world has become increasingly important, and one fruit among these is the blueberry (*Vaccinium* spp.) (Brazelton et al., 2019). Although the majority of the global area planted with this fruit tree is found in soil (Brazelton et al., 2019; Fang et al., 2020a), there is a smaller soilless crop area, which allows the incorporation of management techniques such as hydroponics and the use of substrates (Kritzinger, 2014; Voogt et al., 2014; Osorio et al., 2020; Tamir et al., 2021). Many techniques used in traditional soil cultivation are applicable to soilless cultivation, mainly differentiating planting density, water and nutritional management (Kritzinger, 2014; Voogt et al., 2014; Muñoz et al., 2016; Silber and Bar-Tal, 2019; Kingston et al., 2020). Among the nutritional management, the source of N is highlighted stands out, due to its acidophilic or calcifuge nature, and that responds positively to the application of ammoniacal sources (Imler et al., 2019; Leal-Ayala et al., 2021; Tamir et al., 2021; Xu et al., 2021; Yuan-Yuan et al., 2021), and the use of technologies that allow the ammonium ionic species to be kept active within the substrate, such as the use of nitrification inhibitors (Osorio et al., 2020). For the correct nutritional management of blueberries grown in soilless medium, guidelines must be developed (Guo et al., 2021; Schreiber and Nunez, 2021), considering the differences in varietal behavior, both in nutritional needs and nutrient concentrations of plant tissue (Kritzinger, 2014; Strik and Vance, 2015; Muñoz et al., 2016; Fang et al., 2017; Kingston et al., 2020). A widely used tool to evaluate the nutritional status of plants is leaf analysis; however, for blueberry cultivation as well as other fruit trees, changes in the concentration of nutrients in leaves are season dependent (Kritzinger, 2014; Strik and Vance 2015; Hirzel et al., 2020; Kingston et al., 2020). The appropriate sampling moment must be defined, which in the case of blueberry corresponds to the period between 16 to 20 wk post-emergence (Hanson and Hancock, 1986; Strik and Vance, 2015).

Nutritional management both in soil and in soilless medium can affect the quality attributes of blueberry fruit, mainly firmness, size, weight, and total soluble solids (Retamales et al., 2014; Strik et al., 2017; Hirzel et al., 2023; Hirzel, 2024). Considering that the production of blueberries in pots with substrate is a technique in development worldwide and that nutritional management is one of the practices that affects the productivity and nutrition of the plants, the hypothesis of this work is that the use increasing doses of ammoniacal N in different blueberry cultivars will affect yield, quality of fruit, and nutritional concentration in leaves. The objective of this work was to evaluate the effect of four doses of ammoniacal N on the production, firmness and size of fruits, and on the concentration of macronutrients in leaves of four cultivars (Duke, Legacy, Cargo, and Suziblue) of highbush blueberries (*Vaccinium corymbosum* L.)

MATERIALS AND METHODS

Geographical location and climatic conditions

The experiment was established in May 2020 with 9-mo-old blueberries (*Vaccinium corymbosum* L.) plants produced in an in vitro culture system and then developed in 1.5 L mini pots. The location of the experiment was the INIA Quilamapu Experimental Center (36°35'44.2" S; 72°05'24.5" W), Chillán, Chile. The climate is temperate Mediterranean with a hot, dry summer and a cold, wet winter. The evolution of monthly precipitation, monthly minimum and maximum temperatures and monthly evapotranspiration for the development period of the experiment are presented in Figures 1, 2 and 3 respectively. The experiment was designed in 35 L pots, with one plant in each pot and open field conditions, considering a density equivalent to 20 000 plants ha^{-1}. Pots were filled with a 2:2:1 (v/v) mixture consisting of coconut fiber, peat, and perlite. The experiment included 64 plants and a single row for each cultivar, considering cultivars Duke, Legacy, Cargo, and Suziblue.

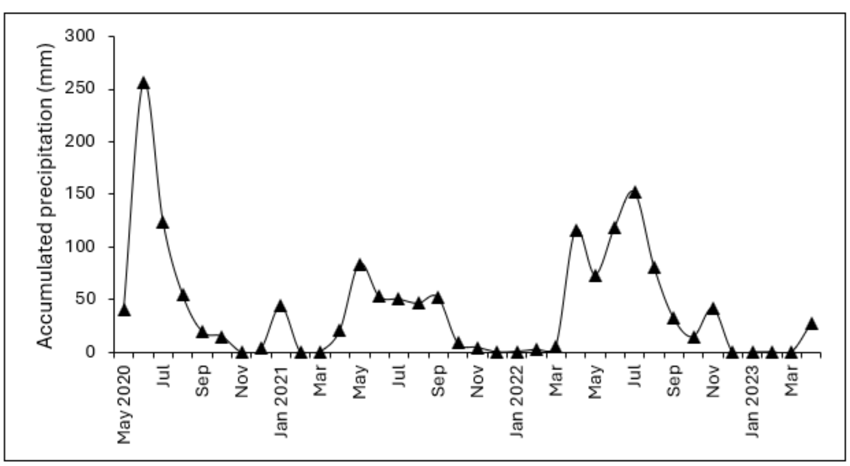

Figure 1. Accumulated precipitation during the evaluation period of four blueberry cultivars with four ammonium sulfate (NH_4-N) rates.

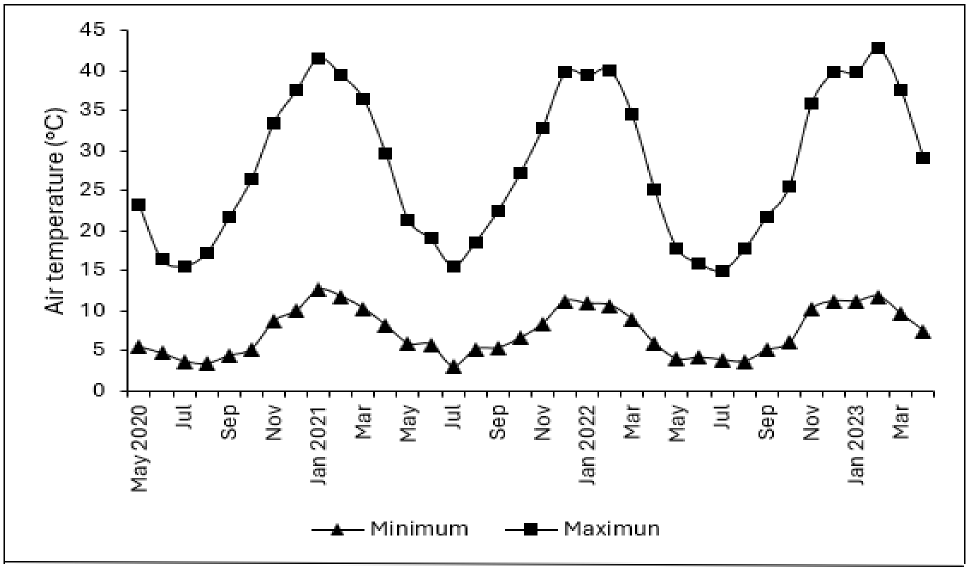

Figure 2. Average monthly minimum and maximum temperature during the evaluation period of four blueberry cultivars with four ammonium sulfate (NH_4-N) rates.

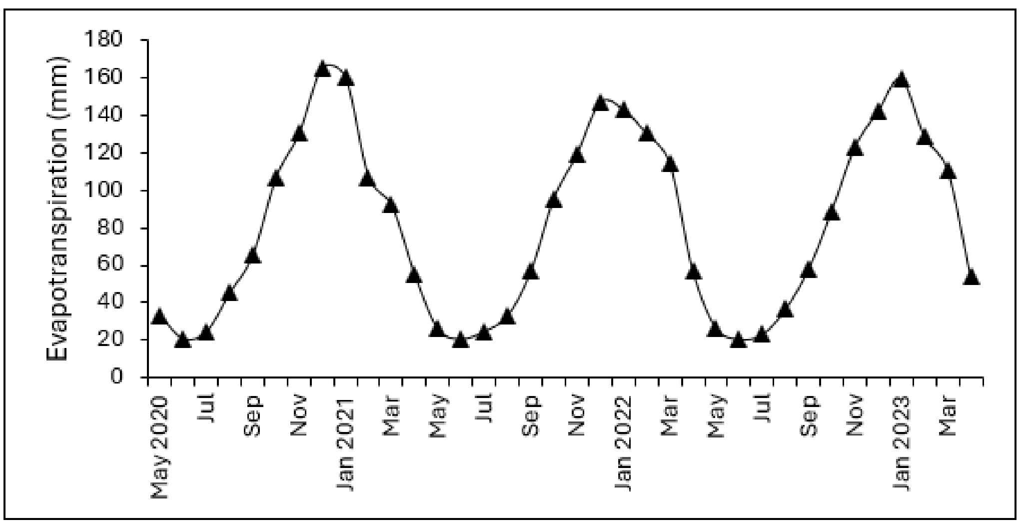

Figure 3. Evapotranspiration (ET_0) during the evaluation period of four blueberry cultivars with four ammonium sulfate (NH_4-N) rates.

Fertilization and irrigation

The methodology of field experiment has been derived from previous work of Muñoz et al. (2022). The experiment was conducted with four highbush blueberries cultivars. Treatments were four ammonium sulfate rates (hereafter referred to as NH_4-N rates) applied at different concentrations: 0 (0%), 0.16 (100%), 0.32 (200%), and 0.48 (300%) g plant^{-1} d^{-1}, corresponding to 0, 3, 6, and 9 g NH_4-N plant^{-1} for each season, which were obtained and modified according to Kritzinger (2014) for blueberry plant of first season grown in pots with substrate (5.36 g plant^{-1}) (Muñoz et al., 2022).

Plants were fertilized twice a week with macro- and micronutrient solutions supplemented with one of four different ammonium sulfate concentrations per treatment (Table 1). The following nutrients were applied: P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B; nutrients as monoammonium phosphate, potassium sulfate, calcium nitrate, and magnesium sulfate fertilizers, and a micronutrient fertilizer mixture (magnesium oxide, B, Cu, Fe, Mn, Mo, Zn; Fetrilon Combi 2, Compo Expert, Münster, Germany) (Table 1). Irrigation was conducted with a drip system from October to April. Four irrigation lines were placed along the rows of plants to irrigate each cultivar separately. Irrigation was applied with two emitters (Supertif PCND, Rivulis, Kibbutz Gvat, Jezreel Valley, Israel) for each plant at 1.1 and 2.2 $L \cdot h^{-1}$. Plants were irrigated daily at a rate of 2 to 3 min every hour (irrigation events) until substrate saturation (onset of leaching) was reached with an irrigation controller (Pro-C; Hunter Industries, San Marcos, California, USA). The number of irrigation events per day and the duration of each event were adjusted weekly with a water balance to determine a 100% water replacement level. The water balance was calculated for each cultivar with three replicates of plants fertilized with 100% N, assuming that the 24 h mean weight loss of the container would be equal to the daily transpiration rate of the plant. Pots and pot plates containing the leachate were removed and weighed with an electronic balance at an accuracy of 2 to 5 g (0.03 to 0.07 mm); measurements were taken on two consecutive days at the same hour (Muñoz et al., 2022). Pot changes were used to calculate the total water volumes to control the soil moisture content in the pots according to the following formula (Lu et al., 2018):

$$ETi = W1 - W2 + I - D$$

where ETi is water consumption for period i, W1 is pot weight (g) at the beginning of the period, W2 is pot weight (g) at the end of the period, I is pot irrigation (g) during the period, and D is the water leachate in the pot plates (g) during the period.

Monthly water consumption values for the evaluated period are shown in Table 2.

Table 1. Fertilization application in experiment of four pot grown blueberry cultivars with four ammonium sulfate ($N\text{-}NH_4^+$) rates.

		Total rate of applied nutrients during the growing season										
Fertilizer	Rate	N	P_2O_5	K_2O	CaO	MgO	S	Fe	Mn	Zn	Cu	B
	g plant^{-1} d^{-1}	g plant^{-1} season^{-1}										
Ammonium sulfate (0%)	0	0.0										
Ammonium sulfate (100%)	0.16	19.8					22.7					
Ammonium sulfate (200%)	0.32	39.7					45.3					
Ammonium sulfate (300%)	0.48	59.9					68.0					
Monoammonium phosphate	0.052	0.8	1.8									
Potassium sulfate	0.216			11.7			5.0					
Calcium nitrate	0.631	12.6			15.1							
Magnesium sulfate	0.076					1.1	2.1					
Fetrilon Combi 2	0.539						1.9	2.8	2.1	2.8	0.3	1.0

Table 2. Monthly water consumption of four pot grown blueberry cultivars with four ammonium sulfate ($N-NH_4^+$) doses. Agrometeorological Laboratory (AGROMET), Instituto de Investigaciones Agropecuarias (INIA).

	Blueberry cultivars			
Season	Duke	Cargo	Legacy	Suziblue
	L pl^{-1} mo^{-1}			
2020-2021				
November	11.69	11.69	11.69	11.69
December	14.96	15.39	14.96	16.25
January	23.98	32.59	31.00	30.02
February	21.95	30.91	29.61	27.89
March	20.68	27.97	25.22	27.32
2021-2022				
November	10.53	10.37	11.18	16.04
December	22.25	25.81	37.48	47.41
January	36.94	22.63	35.48	58.48
February	24.95	12.91	21.92	54.22
March	22.73	14.69	18.36	46.60
2022-2023				
November	25.16	29.05	19.01	29.48
December	45.58	44.82	50.00	49.46
January	38.45	39.76	27.56	45.49
February	32.08	42.34	23.54	39.96
March	26.14	11.99	20.63	39.53

Production and fruit quality

Fruit production and quality evaluations were carried out during the third season after the experiment began (2022-2023), starting harvest in November 2022 with 'Suziblue', continuing in December 2022 with 'Duke', then 'Legacy', and in January 2023 with 'Cargo'. In general, the four cultivars presented a harvest period of 3 wk, ending in December 2022 with 'Suziblue' and in January 2023 with the other three cultivars. Fruit was collected between 09:00 and 11:00 h in plastic trays and the weight for determined fruit yield was measured in a digital balance (Model 100A-300M, Precisa Gravimetrics AG, Dietikon, Switzerland). In each harvest, fruit firmness and size of 60 fruits from each sample were individually measured with a FirmPro instrument (HappyVolt, Santiago, Chile) and fruit weight for determining fruit yield per plant was determined with a digital balance (Model 100A-300M).

Tissue analysis

Leaf sampling for nutritional analysis was carried out at the end of January 2023, collecting leaves from the middle third of the annual twig. To determine leaf nutrient concentrations, these samples were oven-dried at 70 °C to constant weight. Leaf samples were ashed in a ceramic crucible at 500 °C for 7 h; the ash was dissolved in 10 mL boiling 2 M HCl for 5 to 10 min and filtered through filter paper (Whatman N°5 paper, Merck KGaA, Darmstadt, Germany). The evaluated nutrients were N, P, K, Ca and Mg using the methodologies indicated by Sadzawka et al. (2007). Total N was determined by the macro-Kjeldahl procedure (Vadupest, Gerhardt, Germany) and total K, Ca, and Mg by atomic emission (K) and atomic absorption (Ca and Mg) spectrophotometry following dry ashing at 500 °C and acid digestion (2 M HCl)

(Thermo Spectronic Helios, Thermo Electron Corporation, Sittingbourne, UK). Colorimetry was used to measure P in the same extracts according to the molybdate ascorbic acid method.

Experimental design

The experimental design corresponded to a split plot with four replicates, where the main plot was the cultivar and the split-plot was the N fertilization rate (4 cultivars × 4 ammonium sulfate rates × 4 replicates = 64 experimental units). An ANOVA and both mean separation test and separation of interactions were performed with Tukey test (Keselman and Rogan, 1977) at the 5% significance level with statistical software SAS version 6 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Fertilization management was constant throughout the experiment (Table 1), and only suspended when electrical conductivity indicated an increase in values higher than 1.5 dS m^{-1} (data not shown), as a reference guideline for the fertilization management for species sensitive to salinity in soilless cultivation (Silber and Bar-Tal, 2019). Water replacement increased between seasons associated with the increase in biomass production, and as expected with differences between cultivars associated with their difference in biomass, highlighting the greater water need during the second and third evaluation season in 'Suziblue' (Table 2).

The significance analysis showed effects of the cultivar for all the parameters evaluated, except for fruit size (Table 3). The dose of ammoniacal N (NH_4-N) had no effect on fruit quality attributes but affected the concentration of all the nutrients analyzed in the leaves (Table 3). The interaction Cultivar × NH_4-N dose affected fruit yield and the concentration of all nutrients evaluated in the leaves (Table 3), therefore the effects of these interactions are presented in Figure 4 for fruit yield, and for leaves nutrients concentration in Tables 4, 5, 6 and 7 for 'Duke', 'Legacy', 'Cargo', and 'Suziblue' respectively. The effect of the NH_4-N dose on fruit firmness is presented in Figure 5.

Table 3. Significance analysis for fruit yield, firmness and size and leaf nutrient concentration in experiment of four pot grown blueberry cultivars with four ammonium sulfate doses (%).

Evaluated parameter	Cultivar (C)	N rate (N)	C × N interaction
Fruit yield	0.0001	0.07	0.05
Fruit firmness	0.0001	0.13	0.11
Fruit size	0.19	0.15	0.34
N concentration	0.0003	0.0001	0.013
P concentration	0.0028	0.0001	0.0002
K concentration	0.0001	0.0001	0.0001
Ca concentration	0.0001	0.0001	0.002
Mg concentration	0.0001	0.0001	0.014

The highest fruit production was obtained with 'Suziblue' ($p < 0.05$), followed by 'Cargo', without differences between 'Duke' and 'Legacy' ($p > 0.05$) (Figure 1). Fruit production per plant in 'Cargo' and 'Suziblue' were 3.1 and 5.3 times higher respectively than the production obtained in 'Duke' (Figure 4). Regarding the effect of increasing NH_4-N doses in each cultivar there were only differences in 'Duke' and 'Cargo', and in both there was greater fruit production per plant with the NH_4-N dose of 100% ($p < 0.05$) (Figure 4), while for 'Suziblue' and 'Legacy' there weren't response to the N application.

Respecting fruit firmness, the highest value was obtained in 'Suziblue' ($p < 0.05$) which in general was 16.6% higher than the average value of the other cultivars (Figure 5), which did not present a significant difference between them ($p > 0.05$).

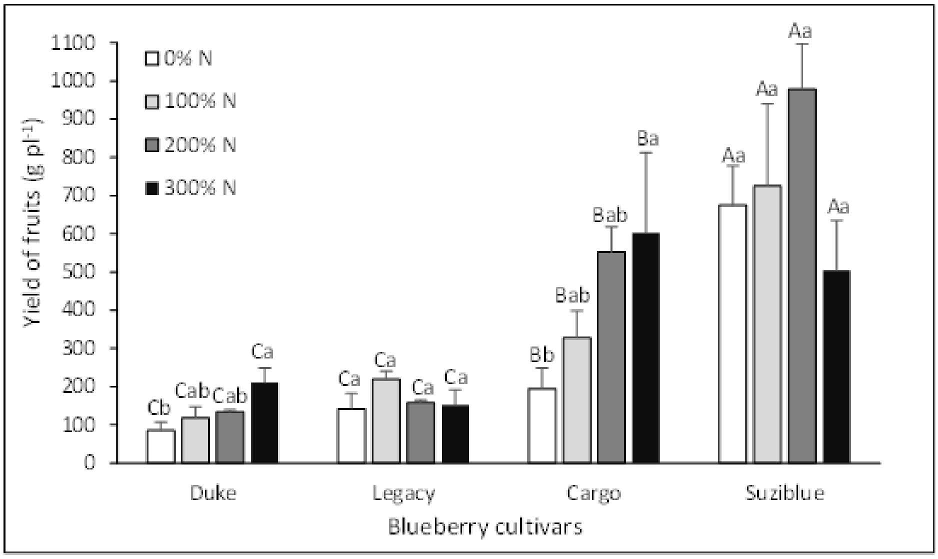

Figure 4. Yield of fruits of four blueberry cultivars grown in pots with four ammonium sulfate (NH_4-N) rates. Uppercase letters indicate significant difference between blueberry cultivars and lowercase letter indicate significant difference between N fertilization level as ammonium sulfate, in according to Tukey's test ($p < 0.05$). Bars over the columns indicate the standard error.

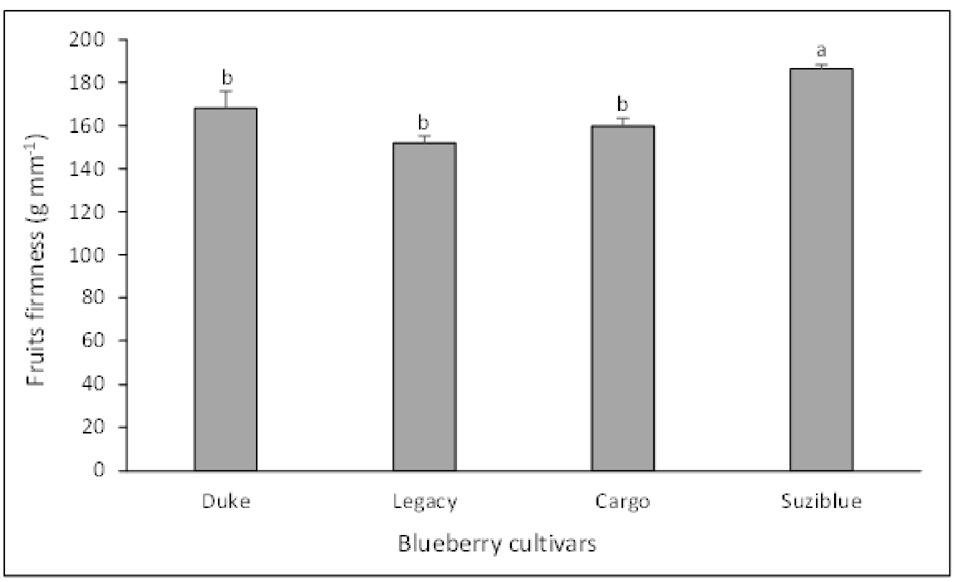

Figure 5. Fruit firmness of four blueberry cultivars grown in pots, as average of four ammonium sulfate (NH_4-N) rates (0, 100, 200 and 300%). Different letters indicate significant difference between blueberry cultivars in according to Tukey's test ($p < 0.05$). Bars over the columns indicate the standard error.

In relation to the effect of increasing NH_4-N doses on the concentration of macronutrients in leaves of each cultivar, in ‘Duke’ (Table 4) there was a significant increase in the concentration of N and P in leaves up to the dose of 200% NH_4-N, with a drop in the concentration of P in leaves when using the highest NH_4-N dose. The concentration of Mg in leaves was also increased with the NH_4-N doses, but only the highest dose used was different from the control without NH_4-N application ($p < 0.05$). For its part, the concentration of K in leaves was negatively affected by the NH_4-N application ($p < 0.05$), with no differences between the different doses used ($p > 0.05$), while the concentration of Ca was not affected by NH_4-N application ($p > 0.05$) (Table 4).

In ‘Legacy’ (Table 5) the effects of increasing NH_4-N doses on the concentration of macronutrients in leaves were similar to those described for ‘Duke’ (Table 4), however the NH_4-N dose that allowed achieving the highest concentration of P and Mg were lower than those obtained in ‘Duke’, and the effect on the K concentration in leaves was generally inversely proportional to the NH_4-N doses used (Table 5).

Table 4. Leaf nutrient concentration in blueberry ‘Duke’ grown in pots with four ammonium sulfate (NH_4-N) rates. Different letters in the same column indicate significant differences between N rates according to Tukey’s test ($p < 0.05$). Mean ± standard error.

NH_4-N rates	N	P	K	Ca	Mg
%			%		
0	1.04 ± 0.06[c]	0.07 ± 0.006[c]	1.73 ± 0.12[a]	0.74 ± 0.03[a]	0.23 ± 0.01[b]
100	1.72 ± 0.12[b]	0.10 ± 0.010[b]	0.77 ± 0.06[b]	0.85 ± 0.05[a]	0.31 ± 0.03[ab]
200	2.03 ± 0.04[a]	0.12 ± 0.006[a]	0.91 ± 0.06[b]	0.97 ± 0.11[a]	0.34 ± 0.05[ab]
300	2.10 ± 0.15[a]	0.10 ± 0.009[b]	0.87 ± 0.02[b]	0.84 ± 0.02[a]	0.38 ± 0.03[a]

Table 5. Leaf nutrient concentration in blueberry ‘Legacy’ grown in pots with four ammonium sulfate (NH_4-N) rates. Different letters in the same column indicate significant differences between N rates according to Tukey’s test ($p < 0.05$). Mean ± standard error.

NH_4-N rates	N	P	K	Ca	Mg
%			%		
0	0.97 ± 0.04[c]	0.07 ± 0.003[b]	1.33 ± 0.05[a]	0.53 ± 0.02[a]	0.18 ± 0.01[b]
100	1.86 ± 0.05[b]	0.12 ± 0.007[a]	0.84 ± 0.02[bc]	0.50 ± 0.02[a]	0.19 ± 0.01[b]
200	2.21 ± 0.06[a]	0.13 ± 0.008[a]	0.97 ± 0.02[b]	0.66 ± 0.08[a]	0.27 ± 0.01[a]
300	2.09 ± 0.09[a]	0.12 ± 0.010[a]	0.79 ± 0.04[c]	0.63 ± 0.05[a]	0.24 ± 0.02[ab]

In ‘Cargo’ (Table 6) the N concentration in leaves was only increased with the lower NH_4-N dose ($p < 0.05$), without additional effect of the higher doses. Unlike what was obtained in ‘Duke’, ‘Legacy’, in ‘Cargo’ (Table 6) the concentration of P in leaves was not affected by the increasing NH_4-N dose, while the concentration of K was significantly increased by the higher NH_4-N dose used. For their part, the concentrations of Ca and Mg showed an increase with 100% and 200% NH_4-N doses compared to the control ($p < 0.05$), but a drop with the highest dose applied compared to the 200% dose ($p < 0.05$).

In ‘Suziblue’ (Table 7) there was a response of increased N concentration in leaves compared to the control with the three NH_4-N doses used ($p < 0.05$), but without difference between the three doses used ($p > 0.05$). The concentration of P had a significant increase when increasing the NH_4-N doses up to 200% ($p < 0.05$), with a decrease in concentration when using the dose of 300% compared to the value obtained with 200% NH_4-N doses ($p < 0.05$). The concentrations of K and Ca were not affected by the different NH_4-N doses ($p > 0.05$), while the concentration of Mg in leaves presented a significant increase with the highest NH_4-N dose ($p < 0.05$).

Table 6. Leaf nutrient concentration (%) in blueberry 'Cargo' grown in pots with four ammonium sulfate (NH_4-N) rates. Different letters in the same column indicate significant differences between N rates according to Tukey's test ($p < 0.05$). Mean ± standard error.

NH_4-N rates	N	P	K	Ca	Mg
%	%				
0	0.94 ± 0.11^{b}	0.09 ± 0.009^{a}	0.61 ± 0.15^{b}	0.64 ± 0.07^{c}	0.20 ± 0.02^{c}
100	1.82 ± 0.04^{a}	0.11 ± 0.002^{a}	0.76 ± 0.05^{ab}	0.98 ± 0.03^{b}	0.36 ± 0.01^{ab}
200	1.97 ± 0.04^{a}	0.11 ± 0.002^{a}	0.92 ± 0.02^{ab}	1.22 ± 0.05^{a}	0.44 ± 0.02^{a}
300	1.85 ± 0.08^{a}	0.10 ± 0.005^{a}	0.99 ± 0.04^{a}	0.96 ± 0.03^{b}	0.34 ± 0.03^{b}

Table 7. Leaf nutrient concentration (%) in blueberry 'Suziblue' grown in pots with four ammonium sulfate (NH_4-N) rates. Different letters in the same column indicate significant differences between N rates according to Tukey's test ($p < 0.05$). Mean ± standard error.

NH_4-N rates	N	P	K	Ca	Mg
%	%				
0	0.96 ± 0.03^{b}	0.06 ± 0.001^{c}	1.29 ± 0.03^{a}	0.64 ± 0.04^{a}	0.20 ± 0.01^{b}
100	2.06 ± 0.08^{a}	0.12 ± 0.003^{b}	1.10 ± 0.05^{a}	0.72 ± 0.04^{a}	0.23 ± 0.02^{ab}
200	2.22 ± 0.05^{a}	0.13 ± 0.003^{a}	1.27 ± 0.07^{a}	0.70 ± 0.10^{a}	0.28 ± 0.03^{ab}
300	2.07 ± 0.09^{a}	0.11 ± 0.006^{b}	1.20 ± 0.12^{a}	0.85 ± 0.07^{a}	0.32 ± 0.03^{a}

As described, the interactions between NH_4-N doses and cultivar were analyzed. Taking in consideration that the effect of cultivar was significant on the concentration of nutrients and for the purposes of useful technical information in the nutritional diagnosis of those four cultivars it is important to note the following: As an average of the NH_4-N doses used, the highest concentration of N and Mg in leaves was obtained in 'Suziblue' and 'Cargo', higher than 'Legacy' and 'Duke' ($p < 0.05$) (data not shown), while the highest concentration of P in leaves was obtained in 'Cargo' greater than 'Suziblue' and 'Legacy' ($p < 0.05$) and in turn greater than 'Duke' ($p < 0.05$) (data not shown).

The highest concentration of K in leaves was obtained in 'Duke', followed by 'Cargo' and 'Suziblue' ($p < 0.05$) and in turn greater than 'Legacy' ($p < 0.05$) (data not shown). Finally, the highest concentration of Ca in leaves as an average of the NH_4-N doses used was obtained in 'Suziblue' and 'Cargo', which only surpassed 'Duke' ($p < 0.05$) (data not shown).

Regarding the nutritional concentrations in leaves that allowed the highest fruit production per plant in the third growing season (Figure 4), the following can be noted: For 'Duke' concentrations of N, P, K, Ca and Mg in leaves sampled in late January fluctuated between 1.60%-1.84%, 0.09%-0.11%, 0.71%-0.83%, 0.80%-0.90%, 0.28%-0.34%, respectively (Table 4). In 'Legacy' concentrations of N, P, K, Ca and Mg in leaves fluctuated between 0.93%-1.91%, 0.07%-0.12%, 0.82%-1.38%, 0.50%-0.53%, 0.18%-0.19%, respectively (Table 5). For 'Cargo' concentrations of N, P, K, Ca and Mg in leaves fluctuated between 1.78%-1.86%, 0.10%-0.11%, 0.71%-0.81%, 0.95%-1.01%, 0.35%-0.37%, respectively (Table 6). In 'Suziblue' concentrations of N, P, K, Ca and Mg in leaves sampled in late January fluctuated between 0.93%-2.14%, 0.06%-0.12%, 1.05%-1.32%, 0.60%-0.76%, 0.19%-0.25%, respectively (Table 7).

DISCUSSION

The nutritional management of soilless crops requires constant evaluation and dosage changes depending on the crop species, genotype, stage of development, biomass and fruit production (Silber and Bar-Tal, 2019), as also reported in the production of different cultivars of blueberries (Kritzinger, 2014; Muñoz et al., 2022). This effect was also observed in our experiment, highlighting the greater need for water replacement of 'Suziblue', associated with its greater fruit production per plant and the biomass necessary to achieve this production. In addition, the water reposition was different between seasons associated to the increase of biomass production and the differences between precipitation and evaporation of each season.

The effect of increasing doses of ammoniacal N (NH_4-N) on fruit production observed in our experiment during its third season has also been reported in blueberries by other authors (Imler et al., 2019; Fang et al., 2020b; Osorio et al., 2020; Xu et al., 2021; Yuan-Yuan et al., 2021), both in fruit and biomass production or as an increase in indicators of physiological activity in plants (leaf photosynthetic rate, stomatal conductance, evaporation rate, and the lowest intercellular CO_2 concentrations and superoxide dismutase activity). The differences in yield per plant between the evaluated cultivars are attributed to the yield potential of each cultivar, whether in soil or substrate conditions (Kritzinger, 2014; Strik and Vance, 2015; Fang et al., 2020b; Bryla et al., 2021). However, in the experiments carried out in soil that have compared the 'Duke' and 'Legacy', the highest yield per plant has been obtained in 'Legacy' (Strik and Vance, 2015; Bryla et al., 2021); however, in our experiment there was no difference between these cultivars, probably because in the third season of development the yield potential of these varieties is still low and does not allow detectable differences. In addition, the doses used per plant associated with the equivalent density of 20 000 plants per ha are equivalent to field doses of 0, 396, 794, and 1198 kg N per ha in each treatment, which decreases the possibility of finding differences in yield or fruit quality attributes with the higher doses. As a reference in commercial production conditions in pots with hydroponics, the doses used in full production fluctuate between 400 and 700 kg N ha^{-1}. In turn, the presence of peat in the substrate also generates an N supply that was not evaluated in this experiment.

We describe that increasing NH_4-N doses on the firmness and size of the fruit of the four cultivars had no effect, differing from results of Fang et al. (2020b) in their field experiment with increasing doses of N (75% ammoniacal and 25% nitric) in 'Emerald' and 'Farthing', where an inversely proportional and significant effect was obtained on fruit size (correlation coefficient -0.64) and an increase in firmness only with the highest dose of N used (336 kg N ha^{-1} with respect to dose 0, 42, 84 and 168 kg N ha^{-1}). Although the condition of the experiment and the cultivars evaluated by Fang et al. (2020b) are different from those of our experiment, the drop in fruit size observed by these authors responds to the increase in yield obtained with increasing doses of N in their experiment, which was observed only in two of the cultivars evaluated in our experiment with no effect on both firmness and fruit size.

Differences in fruit production and quality attributes such as firmness between cultivars of blueberries for the same growing condition has been reported (Kritzinger, 2014; Strik and Vance, 2015; Fang et al., 2020b; Bryla et al., 2021), explained by the production potentials and differentiating characteristics in the fruit of each cultivar. However, for those experiments carried out in field conditions in which the performance of 'Duke' and 'Legacy' has been compared, the highest performance has always been obtained in 'Legacy' (Strik and Vance, 2015; Bryla et al., 2021), unlike of the results obtained in our experiment.

The firmness values of the fruits of 'Duke' and 'Legacy' were slightly lower than those reported for 'Duke' by Retamales et al. (2014) and for 'Legacy' by Strik and Davis (2022); however, both authors used the FirmTech instrument for the determination of fruit firmness, which is different from the FirmPro used in our experiment. In relation to fruit size, the values obtained in our experiment for 'Duke' and 'Legacy' were lower than those reported by Retamales et al. (2014) and Strik and Davis (2022), which may be due to growing conditions, which in our case is in a pot and high density, reducing the amount of carbohydrate reserves per plant and affecting the fruit load/size ratio of fruits. In this respect, several fruit load regulation experiments have shown that by reducing fruit load, increases in fruit size are achieved (Muñoz-Vega et al., 2017; Karimi et al., 2019; Hirzel et al., 2023).

The increases in macronutrient concentration in leaves obtained in our experiment as an effect of increasing doses of ammoniacal N has also been reported for blueberries. Bañados et al. (2012) and Strick and Vance (2015) reported a directly proportional effect on N concentration. Leal-Ayala et al. (2021) reported an increase in the concentrations of N, P and Mg. The higher concentration obtained in some macronutrients when applying increasing rates of ammoniacal N can be associated with the nutritional functions of N in the plant and its effect on metabolic, structural, enzymatic and electron transport activities of the other nutrients (Marschner, 2002; Maathuis, 2009). In general, the concentration of K in leaves decreased with the increasing dose of ammoniacal N in 'Legacy' and 'Duke', which can be explained by a nutritional dilution effect that is generated with the increase in plant biomass production in response to the application of a higher dose of N (Bañados et al., 2012; Fang et al., 2017).

In some cultivars there was no change in concentration in several of the macronutrients evaluated against the increasing NH_4-N dose. This effect can be explained by the genotypic and phenotypic differences of the cultivars evaluated, as has been pointed out for blueberries by different authors (Bañados et al., 2012; Strik and Vance, 2015; Bryla et al., 2021; Leal-Ayala et al., 2021).

In general, the concentrations of macronutrients in leaves obtained in the four cultivars evaluated in our experiment with the same N dose used by Kritzinger (2014) for 'Emerald' and 'Snowchaser' grown in 35 L pots with peat moss, coconut fiber and perlite substrate in a proportion of 7:2:11, were similar to the published data, except for N and Ca, whose values were higher in our experiment. In turn, the concentration of Mg in leaves in 'Duke' and 'Cargo' was higher than the values reported by Kritzinger (2014).

The values of macronutrients concentration in leaves of the four cultivars evaluated in our experiment, although they cannot be considered as a reference standard, they contribute to the development of comparative information for the production of these cultivars of blueberry in pot conditions with substrate. This study using increasing NH_4-N doses is still preliminary to determine doses of N that maximize production per plant of cultivars evaluated at different production ages, our results allow us to have more information than that reported to date for similar growth conditions (Kritzinger, 2014; Kingston et al., 2020).

CONCLUSIONS

The application of increasing doses of ammoniacal N (NH_4-N) for blueberries growing in pots allowed higher production per plant in only two of the four cultivars evaluated, without effects on the quality attributes as firmness and size. Differences in production per plant and fruit firmness were found between cultivars. For the conditions of our experiment, in 'Duke' and 'Cargo' the adequate dose of N was 100%, while for 'Suziblue' and 'Legacy' this dose was found between 0% and 100%, therefore it requires more precise studies. The concentration of macronutrients in leaves showed interactions between cultivars and different NH_4-N doses. The nutritional concentrations in leaves sampled in late January that allowed the highest fruit production per plant in the third growing season were the next: 'Duke' concentrations of N, P, K, Ca and Mg of 1.60%-1.84%, 0.09%-0.11%, 0.71%-0.83%, 0.80%-0.90%, 0.28%-0.34%, respectively; 'Legacy' concentrations of N, P, K, Ca and Mg of 0.93%-1.91%, 0.07%-0.12%, 0.82%-1.38%, 0.50%-0.53%, 0.18%-0.19%, respectively; 'Cargo' concentrations of N, P, K, Ca and Mg of 1.78%-1.86%, 0.10%-0.11%, 0.71%-0.81%, 0.95%-1.01%, 0.35%-0.37%, respectively; 'Suziblue' concentrations of N, P, K, Ca and Mg of 0.93%-2.14%, 0.06%-0.12%, 1.05%-1.32%, 0.60%-0.76%, 0.19%-0.25%, respectively. Nevertheless, we cannot define a single NH_4-N dose that generates nutritional concentrations values in leaves associated with greater fruit production in the four cultivars of blueberries evaluated in their third season of development.

Author contributions

Conceptualization: J.H., V.M. Methodology: J.H., V.M., H.U., O.L., E.M. Software: C.B., J.H. Validation: J.H. Formal analysis: J.H., V.M. Investigation: J.H., V.M. Resources: J.H. Data curation: J.H., V.M. Writing-original draft: J.H. Writing-review & editing: J.H., E.M. Visualization: J.H. Supervision: J.H. Project administration: J.H., V.M. Funding acquisition: J.H. All co-authors reviewed the final version and approved the manuscript before submission.

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