Effect of nitrogen rate and water replacement level on leaf biomass production and leaf nitrogen concentration of ten pot-grown blueberry cultivars

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ABSTRACT

Soilless blueberry (*Vaccinium corymbosum* L.) production is an alternative system that is increasing worldwide in surface area. There is little scientific information available as yet on the agronomic management of this cultivation system. The objective of the present study was to evaluate four N rates (0%, 100%, 200%, and 300% of the reference rate) and three water replacement level (70%, 100%, 130%) on leaf biomass production and leaf N concentration previous to winter fall (May and August 2021) in 10 pot-grown blueberry cultivars (Blue Ribbon, Duke, Camelia, Cargo, Last Call, Legacy, Ochlockonee, Suzie Blue, Ventura, and Victoria). An experiment was conducted in south-central Chile (36°55' S) with first-year plants. During the first growing season, results showed interactions between cultivars, N rates, and water replacement levels; there was a synergistic effect between N rates and water replacement levels on leaf biomass production in 'Duke', 'Camelia', 'Ochlockonee' and 'Suzie Blue'. Overall, the highest leaf biomass production in most cultivars was obtained with an N rate ranging from 33.2 to 53.1 g plant^{-1} season^{-1} (100% and 200% N rates, respectively) and 100% and 130% water replacement levels. Water consumption during the season fluctuated between 93.79 a 136.23 L season^{-1}. The highest leaf N concentration in most of the blueberry cultivars was obtained with N rates ranging from 33.2 to 53.1 g plant^{-1} season^{-1} and 70% and 100% water replacement levels. Therefore, agronomic management recommendations for N fertilization and water replacement levels in blueberries produced with this growing system cannot be generalized.

Key words: Blueberries, fertilization, irrigation, leaf production, nitrogen concentration, soilless crop, *Vaccinium corymbosum*.

INTRODUCTION

Container production of blueberries (*Vaccinium* spp.) using soilless substrate is an alternative system that has gained interest from growers in recent years, especially in areas where the climate allows early production, but where soils are unsuitable for blueberry (Voogt et al., 2014). To achieve the maximum potential of blueberry plants, it is necessary to control several soil factors such as organic matter content (Chen et al., 2019), mineral nutrition, and pH (Machado et al., 2014; Caspersen et al., 2016). However, controlling these factors can be challenging for growers because of the great diversity of soil properties (Smrke et al., 2021).

Production using substrates enables better control of environmental factors and harvest time (Asanica et al., 2020), thus promoting nutrient uptake and supporting vigorous growth (Kingston et al., 2020). An advantage of container production of blueberries is that it requires small amounts of substrate. Blueberry production in pots offers better pH control, organic matter, and drainage demands can be more easily maintained by using a selected substrate, controller irrigation, and

rapid harvest (Kingston et al., 2017). This type of pot production enables individual plants to adjust according to plant volume, thus changing plant density. At the same time, pot production can be carried out in areas with inappropriate soil properties, such as soil borne pests, salinity, residual toxic compounds, or poor/infertile soil (Fang et al., 2020).

For blueberry biomass production using a container system, Fang et al. (2020) indicated that studies conducted with 56 to 97 L pots reported a first-year yield ranging from 0.9 kg to more than 2.0 kg per plant based on different cultivars and fertilizer rates. One-year-old southern highbush blueberries (SHB) grown in containers with soil amended with pine bark showed that leaf biomass production measured in autumn was 30% of total plant biomass (Fang et al., 2017). Muñoz et al. (2016) studied first-year blueberry plants grown in 50 L pots filled with sand substrate and fertilized with different N sources at rates of 18 g plant^{-1} season^{-1}; they indicated leaf DM values before autumn of 15.96, 7.10, and 6.65 g plant^{-1} in 'Corona', 'Legacy', and 'Liberty', respectively. In the same experiment, leaf N concentration evaluated in May of each year during the dormant period or close to abscission fluctuated between 1.557% and 1.661% and showed no differences between cultivars.

The most commonly used substrates in container production systems of blueberries are sphagnum peat moss, coconut coir, peat, and perlite (Fang et al., 2020; Schreiber and Nunez, 2021). The substrate influences plant nutrition, irrigation, and the uptake of nutrients such as N, P, Mg, and S (Kingston et al., 2020). However, little information is available on fertilization requirements in container production systems (Wilber and Williamson, 2008). Therefore, research is needed to investigate fertilization and management practices, such as irrigation management for the production of substrate- and container production of blueberries (Schreiber and Nunez, 2021).

The objective of the present study was to determine the effect of four N rates and three irrigation levels on leaf biomass production and leaf N concentration in 10 pot-grown blueberry cultivars, during the first growing season.

MATERIALS AND METHODS

The experiment was established in pots on open field during May 2020 in Chillán (36°35'44.2" S; 72°05'24.5" W), Ñuble Region, Chile. The climate is temperate Mediterranean with a hot, dry summer and a cold, wet winter: 13.4 °C mean temperature (Hirzel et al., 2021); accumulated precipitation during the study period was 391 mm and the information of the evapotranspiration (ET0) of the study period is presented in the Table 1.

The experiment was conducted on 10 blueberry (*Vaccinium* spp.) cultivars; treatments consisted of four N rates applied at different concentrations of ammonium sulfate: 0 (0%), 0.16 (100%), 0.32 (200%) and 0.48 (300%) g plant^{-1} d^{-1} and three irrigation treatments: 70%, 100%, and 130% water replacement levels. The total applied N rates during the growing season were 0, 33.2, 53.1, and 73.7 g plant^{-1} for the 0%, 100%, 200%, and 300% treatments, respectively (Table 2). In addition, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B nutrients were applied through the monoammonium phosphate, potassium sulfate, calcium nitrate, magnesium sulfate, and micronutrients mixture (magnesium oxide, B, Cu, Fe, Mn, Mo, Zn; Fetrilon Combi 2, COMPO EXPERT, Münster, Germany) fertilizers (Table 2).

Table 1. Water consumption of ten pot-grown blueberry cultivars and monthly evapotranspiration.

Cultivars	2020		2021				
	November	December	January	February	March	April	Total
	L mo^{-1}						L season^{-1}
'Blue Ribbon'	11.69	14.53	22.19	22.54	23.03	6.59	100.57
'Duke'	11.69	14.96	23.98	21.95	20.68	8.53	101.79
'Camelia'	11.69	14.53	31.05	30.80	34.34	13.82	136.23
'Cargo'	11.69	15.39	32.59	30.91	27.97	9.99	128.54
'Last Call'	11.69	14.53	23.38	22.26	23.38	8.80	104.04
'Legacy'	11.69	14.96	31.00	29.61	25.22	7.94	120.42
'Ochlokconee'	11.69	14.53	26.46	26.37	28.78	10.48	118.31
'Suzie Blue'	11.69	16.25	30.02	27.89	27.32	11.77	124.94
'Ventura'	11.69	14.96	31.00	29.29	29.00	9.89	125.83
'Victoria'	11.69	14.53	21.82	20.64	19.55	5.56	93.79
Evapotranspiration, mm*	130.61	165.57	160.30	107.28	92.09	55.01	

*Red Agrometeorológica de INIA (www.agrometeorologia.cl).

Table 2. Fertilization application in the experiment.

Fertilizer	Rate	Total rate of applied nutrients during the growing season										
		N	P₂O₅	K₂O	CaO	MgO	S	Fe	Mn	Zn	Cu	B
	g plant⁻¹ d⁻¹	——————————————— g plant⁻¹ season⁻¹ ———————————————										
Ammonium sulfate 0%	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

One-year-old blueberry plants were placed in 35 L pots filled with a 2:2:1 (v/v) mixture consisting of coconut fiber, peat, and perlite with a density of 20 000 plants ha⁻¹. The assay included 480 plants and 1 row for each cultivar. Ten blueberry cultivars were evaluated: 'Blue Ribbon', 'Duke', 'Camelia', 'Cargo', 'Last Call', 'Legacy', 'Ochlockonee', 'Suzie Blue', 'Ventura', and 'Victoria'. Plants were fertilized twice a week with macro- and micronutrient solutions (Table 2) supplemented with one of four different ammonium sulfate concentrations per treatment. Irrigation was conducted with a drip system from October to April. Ten irrigation lines were placed along the rows of plants to independently irrigate each variety. The treatments were applied at different flow rates with two emitters per plant (Supertif PCND, Rivulis, Kibbutz Gvat, Jezreel Valley, Israel): two emitters at 1.1 L h⁻¹ (70%), one emitter at 1.1 and one at 2.2 L h⁻¹ (100%), and two emitters at 2.2 L h⁻¹ (130%). Plants were irrigated daily at a rate of 2 to 3 min every hour (irrigation events) until substrate saturation (onset of leaching) was reached using an irrigation controller (Pro-C support, Hunter, San Marcos, California, USA). The number of irrigation events per day and the duration of each event were adjusted once a week with a water balance to determine a 100% water replacement level. The water balance was calculated for each variety with four 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) on two consecutive days at the same time. 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):

$$ET_i = W_1 - W_2 + I - D$$

where ET_i is water consumption for period i, W_1 is pot weight at the beginning of the period (g), W_2 is pot weight at the end of the period (g), I is pot irrigation during the period (g), and D is the water leachate in the pot plates during the period (g).

Whole plant DM and N concentrations for each treatment were determined. Leaves were collected between May and August 2021 at the dormancy or near abscission stages based on field observations. These samples were oven-dried at 70 °C to constant weight, and each sample was weighed to determine dry weight (g plant⁻¹). A leaf subsample was ground and sieved through a 40 mesh (0.42 mm openings) sieve to measure the total N concentrations by acid digestion, Kjeldahl distillation, and the titration procedure (Sadzawka et al., 2004).

A split-split-plot experimental design was used in which the main plot was the cultivar, the split plot was the N fertilization rate, and the split-split plot was the water replacement level; there were four replicates per treatment. An ANOVA, mean separation test (Tukey), and separation of interactions by contrasts were performed at the 5% significance level with SAS 6.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

There was a very significant effect for all the sources of variation and interactions between these sources for both leaf biomass production ($p < 0.01$) and leaf N concentration ($p < 0.01$); the exception was for the interaction between Cultivar×N rate×Water replacement level for the leaf N concentration, whose effect was significant ($p < 0.05$) (Table 3). Therefore, the effect of N rates and water levels are shown separately for each cultivar in Figures 1 to 10 for 'Blue Ribbon', 'Duke', 'Camelia', 'Cargo', 'Last Call', 'Legacy', 'Ochlockonee', 'Suzie Blue', 'Ventura', and 'Victoria', respectively.

Table 3. Significance analysis of biomass production and N concentration in leaves of 10 blueberry cultivars harvested in the winter of the first growing season (2020-2021).

Source of variation	Leaf biomass production	Leaf N concentration
Cultivar (C)	**	**
N fertilization level (N)	**	**
Water replacement level (W)	**	**
C×N	**	**
C×W	**	**
N×W	**	**
C×N×W	**	*

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level.

Leaf biomass production in 'Blue Ribbon' was affected by the N rate and water replacement level at some N rates; it fluctuated between 18.7 and 155.7 g plant^{-1} (Figure 1A). The highest leaf biomass production was obtained at the 100% N rate, and this value only surpassed the treatment with the 300% N rate ($p < 0.05$). As for water replacement, there was only a difference for the treatments with 100% and 200% N rates; the highest biomass production occurred with the 130% water replacement level ($p < 0.05$) (Figure 1A).

Figure 1. Leaf biomass production (A) and leaf N concentration (B) in 'Blue ribbon' blueberries harvested in the winter of the first growing season (2020-2021).
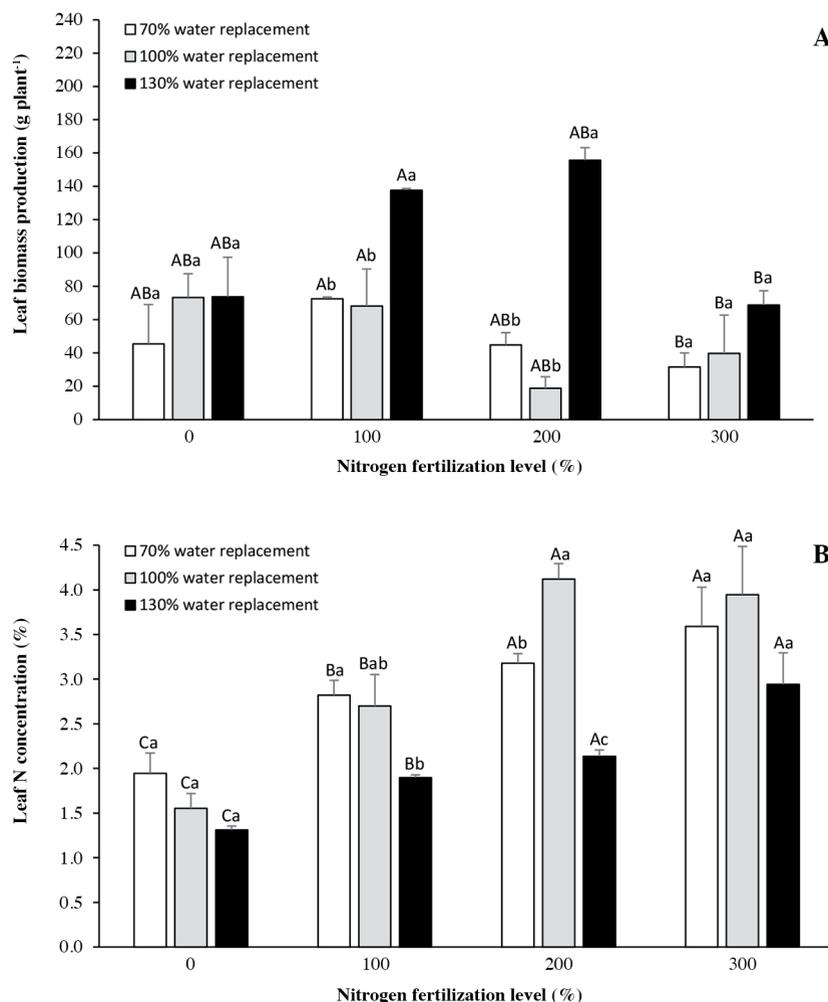
In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test ($p < 0.05$).

Leaf N concentration in 'Blue Ribbon' varied between 1.31% and 4.12% (Figure 1B); it was affected by both the N rate and water replacement level. The highest leaf N concentration was achieved with the treatments using 200% and 300% N rates (p < 0.05), whereas there were differences in the 100% and 200% N rates for water replacement. The effect of the water replacement level was not consistent between these two N rates (100% and 200%), but overall, the highest N concentration was attained at 100% water replacement (p < 0.05).

In 'Duke', leaf biomass production was affected by both the N rate and water replacement level for some N rates (Figure 2A), and ranged from 13.2 and 65.9 g plant[-1]. The highest leaf biomass production was reached with the 100% and 200% N rates, which was only higher than the control without N (p < 0.05). There were differences for water replacement only at the 0% and 200% N rates (p < 0.05); in both cases, the highest values for leaf biomass were achieved at 70% and 130% water replacement, which is an erratic effect.

The leaf N concentration in 'Duke' only affected the N rate with a directly proportional response to the applied N rate (Figure 2B). Values varied between 1.02% and 2.60%, and the highest N concentration values were obtained at the 200% and 300% N rates.

The leaf biomass production for 'Camelia' was affected by the N fertilization and water replacement levels at only two of the applied N rates (100% and 200%); values ranged from 87.3 to 157.3 g plant[-1] (Figure 3A). For the N rate, the highest leaf biomass production was achieved at the 100% and 200% N rates, which was only higher than the control without N (p < 0.05). There were differences only at the 100% and 200% N rates for the water replacement levels (p < 0.05), and the highest leaf biomass values in both cases were obtained at 130% water replacement.

Figure 2. Leaf biomass production (A) and leaf N concentration (B) in 'Duke' blueberries harvested in winter of the first growing season (2020-2021).
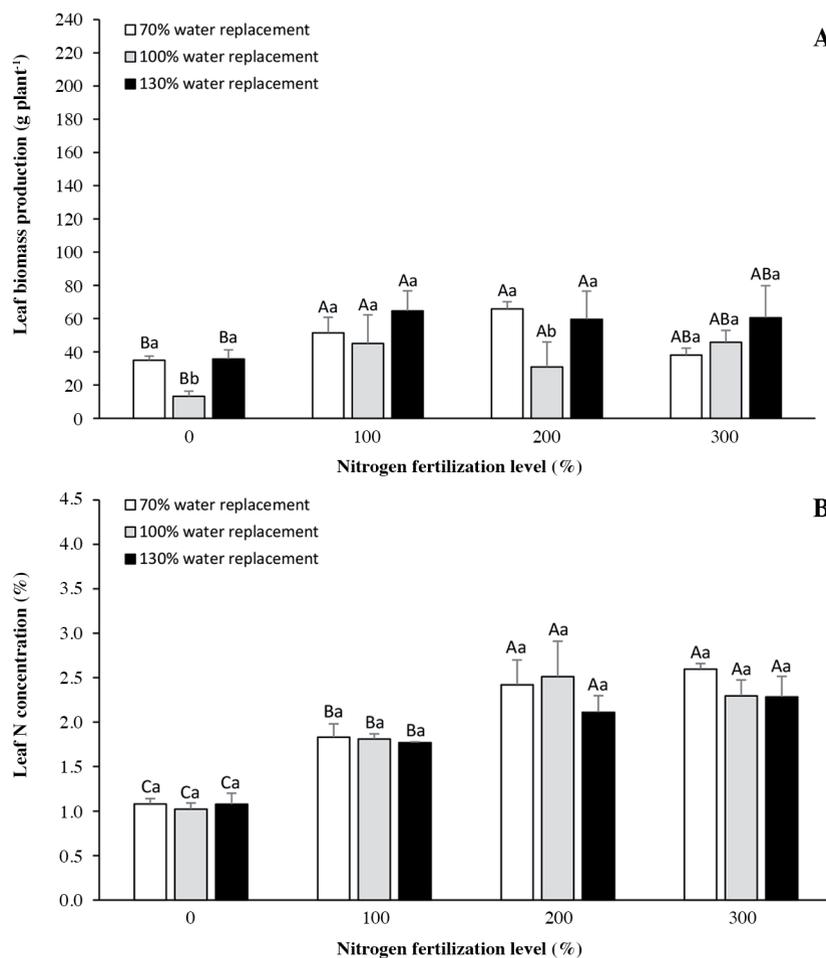
In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

Figure 3. Leaf biomass production (A) and leaf N concentration (B) in 'Camelia' blueberries harvested in the winter of the first growing season (2020-2021).
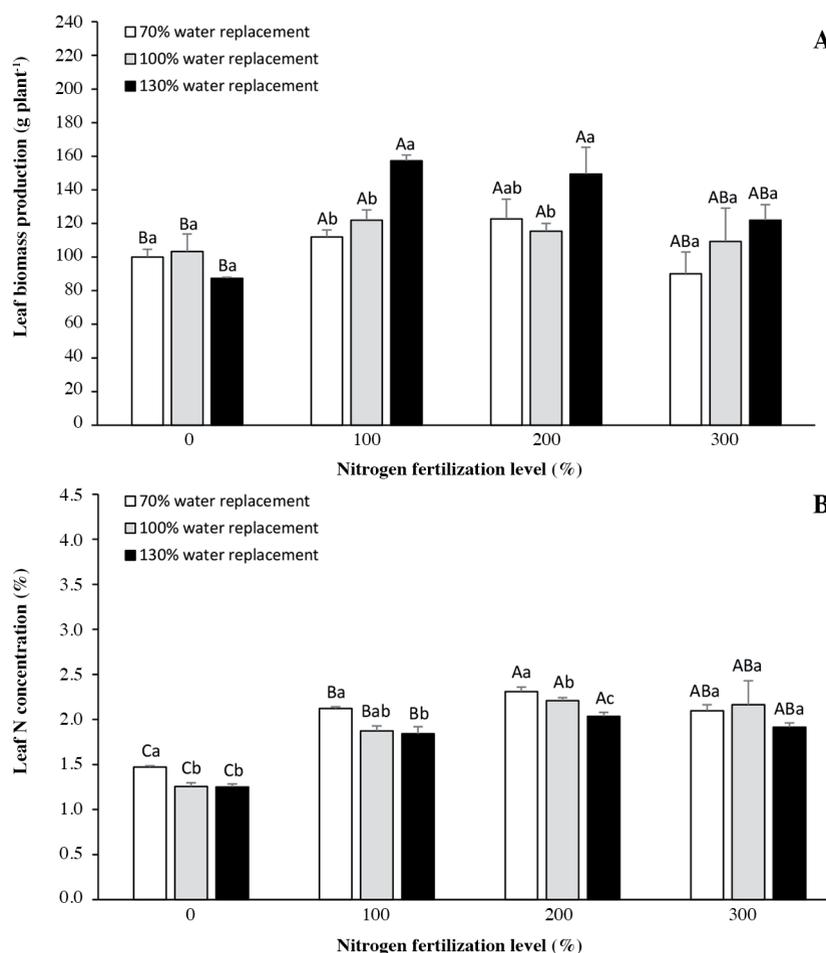
In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

The leaf N concentration values in 'Camelia' (Figure 3B) varied between 1.25% and 2.31%, and there was a directly proportional effect to the applied N rate up to the 200% rate. The highest leaf N concentration was attained at the 200% N rate, which was similar to the value obtained at the 300% rate (p < 0.05). There were differences for water replacement at the 0%, 100%, and 200% N rates; overall, the highest N concentration was reached at 70% water replacement.

The leaf biomass production in 'Cargo' was affected by the fertilization and water replacement levels at only two of the N rates (0% and 300%), whose values fluctuated between 46.7 and 96.7 g plant^{-1} (Figure 4A). For the N rate, the highest leaf biomass production was obtained at the 100% rate, which was only higher than the control without N (p < 0.05). There were differences in the 0% and 300% N rates for the water replacement levels (p < 0.05). For the control without N, the highest leaf biomass production was reached with 130% water replacement (p < 0.05), while the highest biomass production at the 300% N rate was obtained with 100% and 130% water replacement (p < 0.05).

The leaf N concentration values in 'Cargo' (Figure 4B) ranged from 1.09% to 1.89%, and there was a directly proportional effect to the N rate. The highest leaf N concentration was achieved at the 300% N rate, which was similar to the value obtained at the 200% N rate (p < 0.05). As in the case of 'Camelia', there were differences in the 0%, 100%, and 200% N rates; the highest N concentration was reached with 70% water replacement.

The leaf biomass production in 'Last Call' varied between 44.0 and 130.7 g plant^{-1} (Figure 5A), and was not affected by the N fertilization level (p < 0.05). Meanwhile, the water replacement level only affected the 300% N rate, reaching the highest leaf biomass production with 100% water replacement and surpassing only the 70% water replacement level (p < 0.05).

Figure 4. Leaf biomass production (A) and leaf N concentration (B) in 'Cargo' blueberries harvested in the winter of the first growing season (2020-2021).
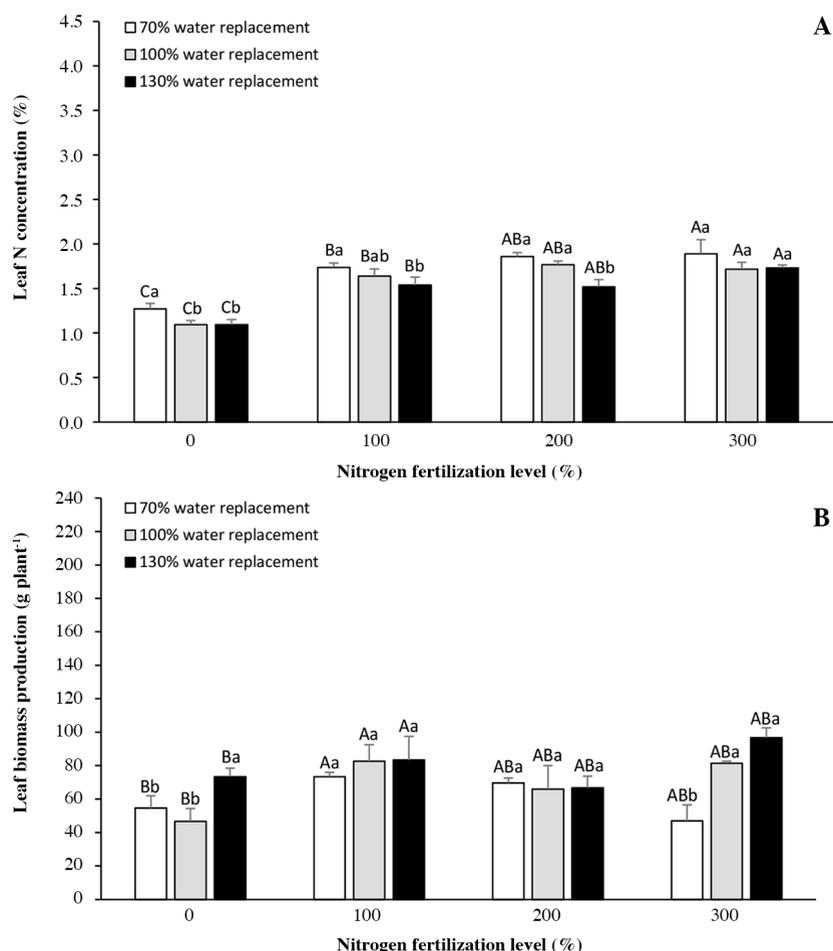
In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

The leaf N concentration values in 'Last Call' fluctuated between 0.97% and 2.35% (Figure 5B), and the highest values for the N concentration were obtained at the 100%, 200%, and 300% N rates (p < 0.05). Water replacement only affected the 0% and 300% N rates (p < 0.05); overall, the highest leaf N concentration was attained with 70% water replacement.

As in the case for 'Last Call', leaf biomass production in 'Legacy' was not affected by the N fertilization level (p < 0.05) (Figure 6A) and the water replacement level only affected the 300% N rate, reaching the highest leaf biomass production at 100% and 130% of water replacement and surpassing only the 70% replacement level (p < 0.05). The leaf biomass production values ranged from 97.0 to 157.3 g plant^{-1} (Figure 6A).

The leaf N concentration in 'Legacy' varied between 1.12% and 2.37% (Figure 6B). For the N fertilization levels, the highest leaf N concentration was obtained at the 100%, 200%, and 300% rates (p < 0.05). Water replacement only affected the 200% and 300% N rates (p < 0.05), and the highest N concentration was usually reached with 70% water replacement.

The leaf biomass production in 'Ochlockonee' was affected by the N rate and the water replacement level (Figure 7A). Leaf biomass production ranged from 130.0 to 234.0 g plant^{-1}, reaching the highest values at the 100%, 200%, and 300% N rates (p < 0.05). The highest biomass production for water replacement was at the 130% water replacement level at the 100% N rate, while for the 200% and 300% N rates, it was higher at the 100% and 130% water replacement levels, respectively (p < 0.05).

Figure 5. Leaf biomass production (A) and leaf N concentration (B) in 'Last Call' blueberries harvested in the winter of the first growing season (2020-2021).
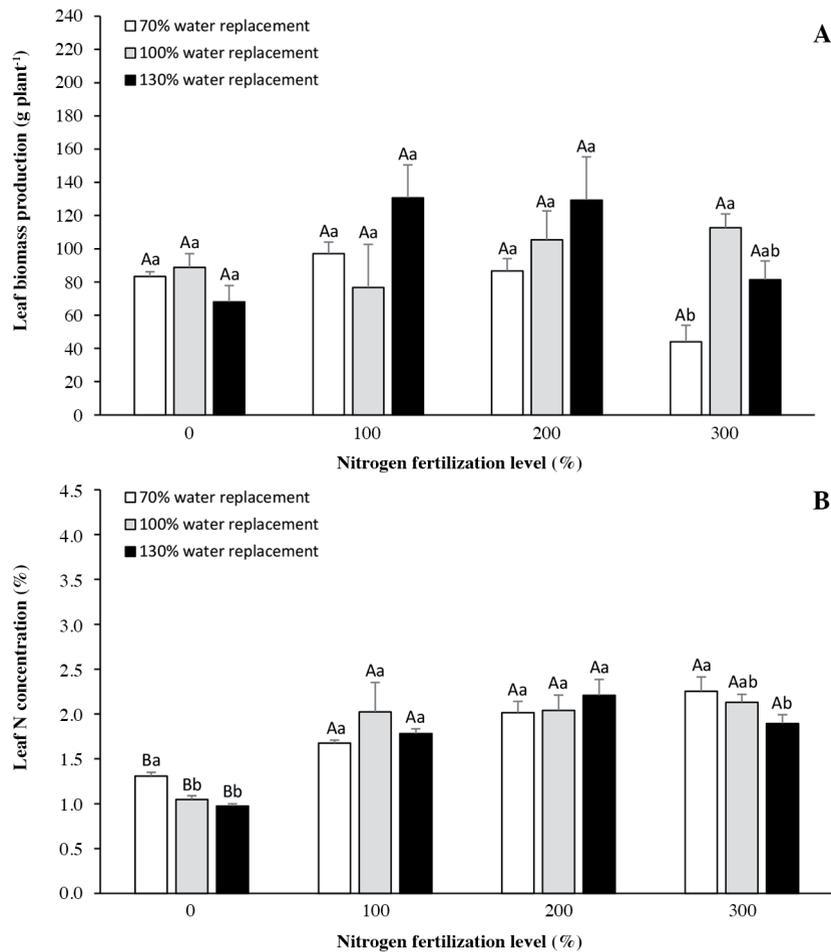
In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

The leaf N concentration values in 'Ochlockonee' varied between 1.21% and 2.65% (Figure 7B), and it was affected by N fertilization levels and water replacement. The highest leaf N concentration was obtained at the 200% and 300 % N rates (p < 0.05), while the effect of water replacement was only observed at the 0%, 200%, and 300% fertilization levels; overall, the highest N concentration was reached with the 70% water replacement level (p < 0.05).

The leaf biomass production in 'Suzie Blue' was affected by the N rates and the water replacement level (Figure 8A) with values between 67.3 and 164.0 g plant^{-1}. The highest leaf biomass production was obtained at the 100%, 200%, and 300% N rates (p < 0.05). Water replacement affected leaf biomass production at each N rate (p < 0.05); overall, it was higher at the 130% water replacement level.

The leaf N concentration in 'Suzie Blue' ranged from 1.21% to 2.65% (Figure 8B). The highest leaf N concentration values were achieved at the 200% and 300% N fertilization levels. The water replacement level affected leaf N concentration at the 0%, 100%, and 200% N rates (p < 0.05), which was higher with the 70% water replacement level at each of the N rates (p < 0.05).

The leaf biomass production in 'Ventura' was not affected by the N rates as were 'Last Call' and 'Legacy', and there was an effect of water replacement level only at the 200% and 300% N rates (Figure 9A). Leaf biomass production varied between 50.7 and 174.7 g plant^{-1}, reaching the highest values with the 130% water replacement level at the 200% N rate and the 100% and 130% water replacement level at the 300% N rate (p < 0.05).

Figure 6. Leaf biomass production (A) and leaf N concentration (B) in 'Legacy' blueberries harvested in the winter of the first growing season (2020-2021).
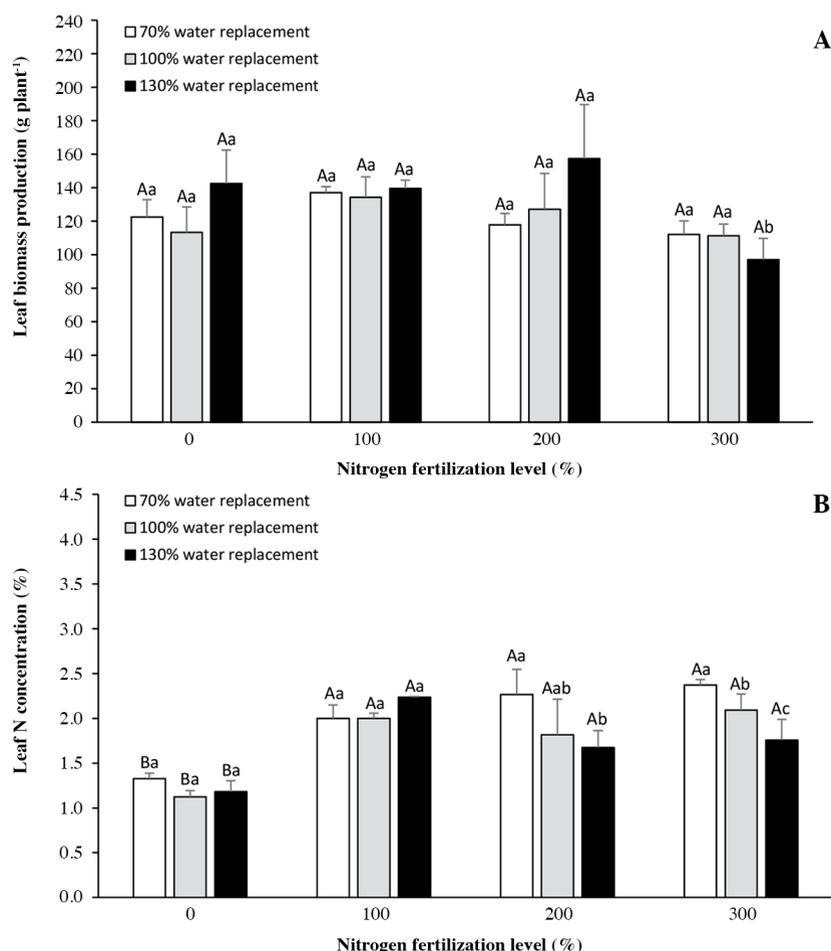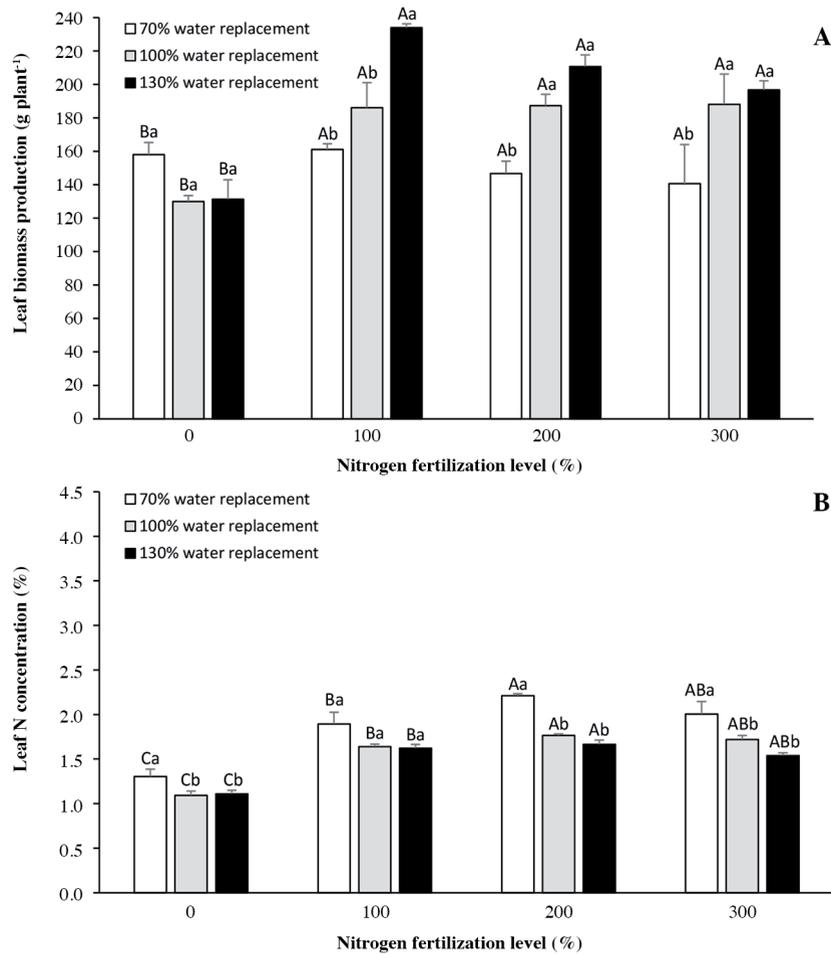

In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test ($p < 0.05$).

The leaf N concentration in 'Ventura' was affected by the N concentration and by the water replacement level at the 0%, 200%, and 300% N rates (Figure 9B). Leaf N concentration fluctuated between 1.23% and 2.65%, and was higher at the 200% and 300% N rates ($p < 0.05$). A higher leaf N concentration was obtained with the 70% water replacement level ($p < 0.05$).

The leaf biomass production in 'Victoria' was affected by the N rate and only at the 100% N rate for the water replacement level (Figure 10A) with values between 91.3 and 162.0 g plant[-1]. The highest biomass production was obtained at the 200% N rate, reaching similar values at the 0% and 100% N rates ($p < 0.05$). At the 100% N rate, the highest leaf biomass production was attained with the 130% water replacement level ($p < 0.05$).

The leaf N concentration in 'Victoria' ranged from 1.08% to 2.31% (Figure 10B). Only the N rates affected the leaf N concentration, given that there were no differences compared with water replacement. The increase in N rate produced an increase in the leaf N concentration; the highest value was reached at the 300% N rate, and there were no differences between the 100% and 200% rates ($p < 0.05$).

Figure 7. Leaf biomass production (A) and leaf N concentration (B) in 'Ochlockonee' blueberries harvested in the winter of the first growing season (2020-2021).
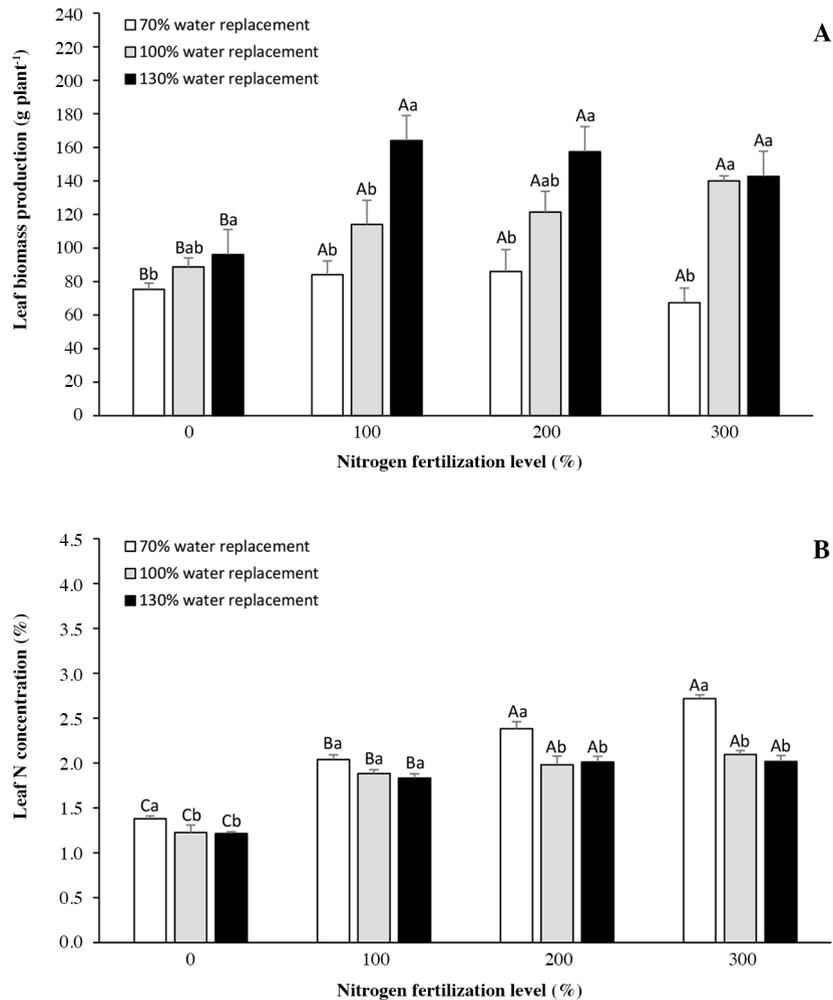

In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

DISCUSSION

The leaf biomass production and leaf N concentration were different among varieties, as reported by Muñoz et al. (2016) in an experiment with pot-grown 'Corona', 'Liberty', and 'Legacy' blueberries. However, leaf biomass production for first-year plants in almost all the cultivars of our experiment, except 'Duke', was higher than values indicated by Muñoz et al. (2016). Likewise, leaf biomass production in most of the evaluated cultivars was higher than values mentioned by Machado et al. (2014) for first-year pot-grown 'Bluecrop'.

There is little information available in the literature on water replacement levels and their effect on leaf biomass production and leaf N concentration in pot-grown blueberries. However, it has been reported that during the production period of pot-grown 'Bluegold' water stress negatively affected fruit weight and production per plant, while the number of fruits per plant and fruit diameter were not affected (Lepaja et al., 2019). Ribera-Fonseca et al. (2019) grew 'Legacy' blueberries in 50 L pots with two water replacement levels (60% and 100%) and indicated that the decrease in water replacement increased the soluble solids content and fruit firmness, while yield per plant and other fruit quality attributes such as fresh weight, caliber, and percentage citric acid were not affected by the water replacement level. Bryla and Strik (2007) showed that high-density blueberry cultivation, such as in the present study, increased water demand and total DM production.

Figure 8. Leaf biomass production (A) and leaf N concentration (B) in 'Suzie Blue' blueberries harvested in the winter of the first growing season (2020-2021).
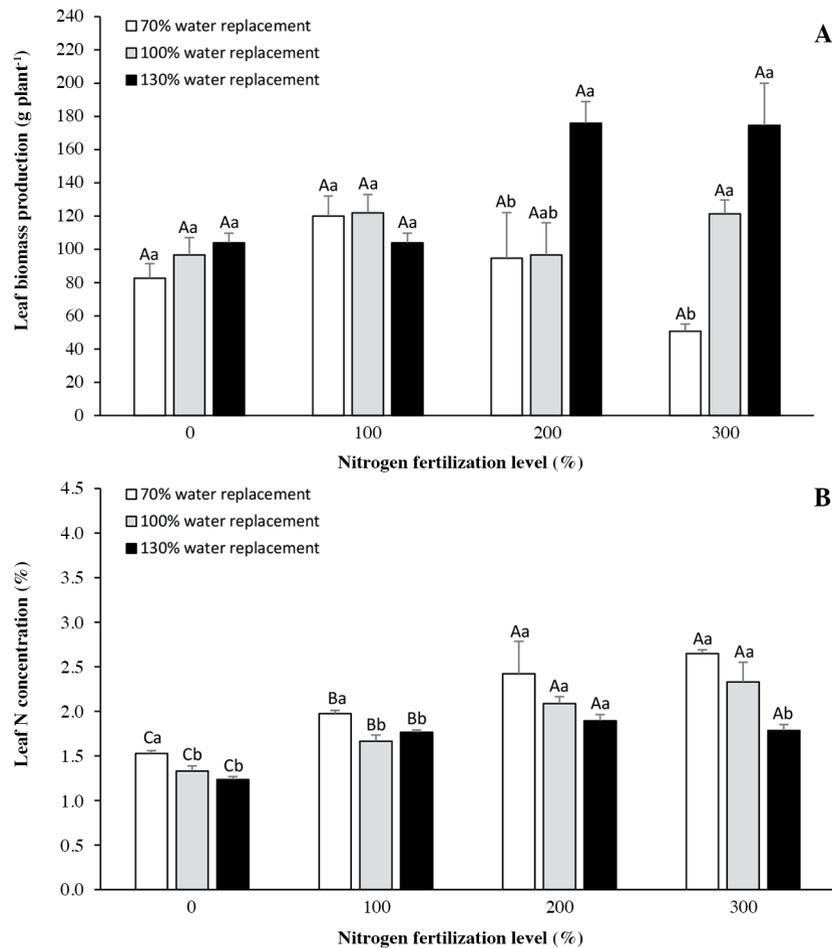

In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test ($p < 0.05$).

The increase in applied N rates generated a directly proportional response in leaf biomass production in most of the evaluated cultivars, but only up to the 100% or 200% N fertilization levels; leaf biomass production decreased in most cultivars at the 300% N fertilization level. Machado et al. (2014) studied first-year plants grown in 8 L pots and reported that 'Bluecrop' exhibited an inversely proportional effect in leaf biomass production when increasing N (ammonium sulfate) rates were applied. This leaf biomass production response up to a determined N rate in different cultivars is explained by genetic and functional factors of this nutrient in the plant, as described by Mengel and Kirkby (2001) and Marschner (1995). This difference in response among cultivars does not allow us to generalize agronomic management recommendations for N fertilization of blueberries produced in this growth medium.

There was an erratic response effect in some cultivars for the water replacement level at different N rates. This could be explained by the fact that the substrate was prepared and mixed manually, which could produce differences in the proportion of the substrate components in some pots, thus causing an erratic response in some plants. Kingston et al. (2017; 2020) determined that the selected substrate, its components, and the proportion of these components influence moisture retention and nutrient availability to pot-grown blueberry plants.

Figure 9. Leaf biomass production (A) and leaf N concentration (B) in 'Ventura' blueberries harvested in the winter of the first growing season (2020-2021).
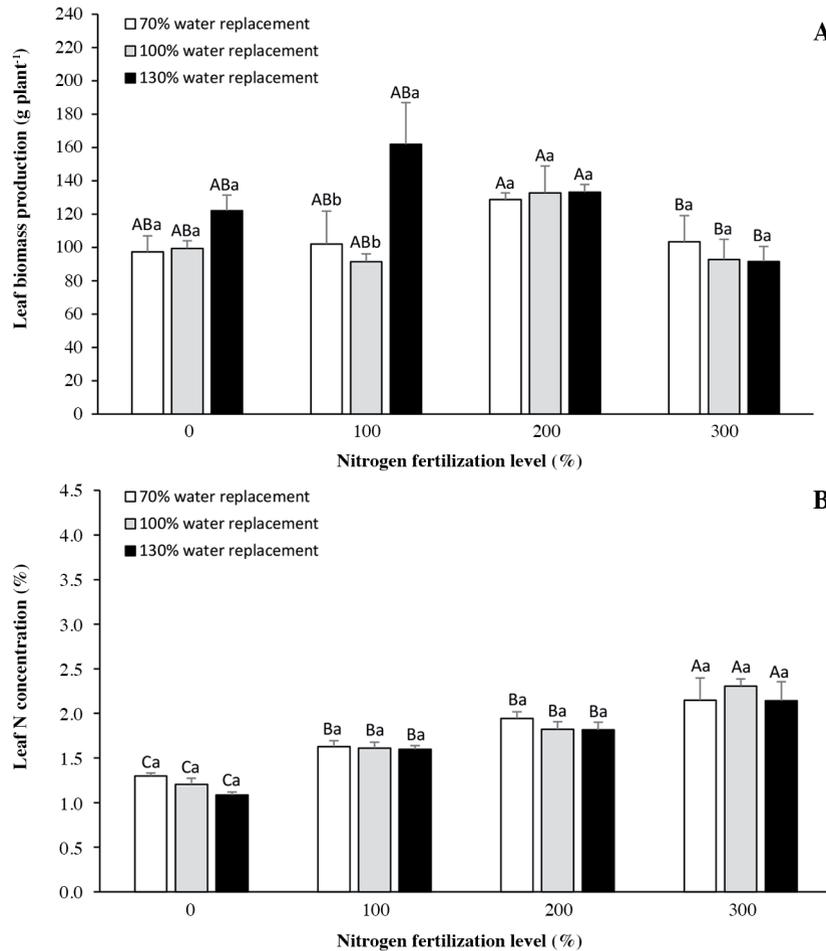

In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test (p < 0.05).

The leaf biomass production was higher in some cultivars when the water replacement level was increased at each increasing N rate, indicating a synergistic effect of water and N use by plants. This effect concurs with findings reported for tomatoes by Wang et al. (2019), who indicated that there is a positive correlation between the irrigation level and N rate; this generates a higher total root volume, fruit production, and total DM in the plants. Similarly, the response to the increasing water replacement level in some cultivars was only up to a certain N rate. This is explained by a limited response to the combination of these production factors at increasing levels, and there could be other factors not evaluated in the present study that could increase the productive response. Guo et al. (2021) showed that for 'Brightwell' rabbiteye blueberry, only moderate fertilization and water levels increased growth and improved some physiological characteristics. Likewise in jujube (*Ziziphus jujuba* Mill.), only a reasonable N fertilization level was able to increase both yield and water use efficiency, while high N rates decreased water use efficiency without significantly increasing yield (Dai et al., 2019). These results concur with reports of findings for other species such as wheat (Zhang et al., 2018) and sorghum (Sudan grass) (Kaplan et al., 2019).

For the effect of increasing N fertilization levels on leaf N concentration, there was a positive correlation in all the evaluated cultivars; this concurs with findings reported by other authors (Fang et al., 2017; Strik et al., 2019; Osorio et al., 2020). However, for most cultivars, applying increasing N increased leaf N concentration only up to the 200% N rate because values were usually equal to those obtained at the 300% N rate. The exceptions were 'Legacy' and 'Last Call' in

Figure 10. Leaf biomass production (A) and leaf N concentration (B) in 'Victoria' blueberries harvested in the winter of the first growing season (2020-2021).

In each figure, uppercase letters indicate a significant difference between the N fertilization level and lowercase letters indicate a significant difference between the water replacement levels at the same N rate according to Tukey's test ($p < 0.05$).

which leaf N concentration showed no differences between the different N applications, and it was only higher than those obtained when N was not applied (0% N). Therefore, there was a differential response among cultivars, which does not allow us to generalize an agronomic management recommendation for all the blueberry cultivars produced in this growth medium. Spiers (1983) reported increased leaf N concentration in 'Tifblue' rabbiteye blueberry grown in 19 L pots with sand for rates ranging from 25 to 50 mg N L^{-1} and the absence of response in concentrations of 100 mg N L^{-1}. In addition, for first-year 'Emerald' blueberries produced in 57 L pots with pine bark, it has been indicated that leaves in autumn concentrate approximately 50% of N absorbed by the plant (Fang et al., 2017). These values of N distribution in blueberry leaves concur with those reported by Kritzinger (2014) for first-year 'Snowchaser' blueberries grown in 20 L pots.

The leaf N concentration in some cultivars decreased when the water replacement level increased; this is explained by the dilution effect of this nutrient in the plant tissue (Marschner, 1995; Mengel and Kirkby, 2001) due to a higher leaf biomass production.

The leaf N concentration values obtained it the present experiment concur with those found in other studies (Muñoz et al., 2016; Strik et al., 2019), except for the values in 'Blue Ribbon' that were higher compared with the other evaluated cultivars and those described in the literature (Doyle et al., 2021). 'Blue Ribbon' showed susceptibility to increasing N rates and exhibited toxicity effects that caused leaf necrosis and defoliation in plants subjected to treatments at 200% and 300% N rates; an excess of this nutrient can cause physiological alterations in plants (Liu and von Wirén, 2017).

CONCLUSIONS

The use of different N rates and combinations with water replacement levels in the 10 evaluated blueberry cultivars produced different responses for leaf biomass production and leaf N concentration; a synergetic effect between the N rate and water replacement level was observed in some cultivars. Overall, the highest leaf biomass production in most of the blueberry cultivars was obtained when the N rate varied between 33.2 and 53.1 g plant^{-1} season^{-1} (100% and 200% N rate, respectively) and the 100% and 130% water replacement levels. The highest leaf N concentration in most of the blueberry cultivars was attained at N rates fluctuating between 33.2 and 53.1 g plant^{-1} season^{-1} and with the 70% and 100% water replacement levels. Therefore, it is not possible to generalize agronomic management recommendations for N fertilization and the management of water replacement in blueberries produced in this growing system.

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