Ecophysiology of the Southern Highbush blueberry cv. Biloxi in response to nitrogen fertigation

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Abstract

In Brazil, the nutritional requirements of the blueberry are not sufficiently known, thus requiring further research on the management of mineral fertilization. In this perspective, this work aimed to evaluate physiological attributes of Southern Highbush blueberry plants cv. Biloxi as a function of nitrogen fertigation in Brasília-DF. The experiment was conducted from August 2018 to July 2019, in the Fruit Sector of the Experimental Biology Station of the University of Brasilia (UnB), Federal District. This experiment adopted a randomized block design, with four treatments: 10; 20; 30, and 40 g of N plant^{-1}, 8 replications, and 5 plants per experimental plot. The following variables were measured: net photosynthesis rate (A), transpiration (E), stomatal conductance (gs), internal CO_2 concentration (Ci), instantaneous water-use efficiency (WUE), carboxylation efficiency (CE), SPAD index (SPAD) and leaf nitrogen (N). There was an effect of the different nitrogen doses applied on the physiological attributes. The plants of the blueberry cultivar Biloxi increased their photosynthetic rates at doses up to 30 g of N plant^{-1}. Nitrogen rates did not influence stomatal conductance nor did they provide improvements in the carboxylation efficiency of the blueberry plants. Under the conditions of the present work, the highest N leaf contents were obtained with the application of 30 g N plant^{-1}, and values above this concentration did not correspond to higher net photosynthesis rates, transpiration, and CO_2 concentration in the leaf mesophyll.

Keywords: gas exchange, photosynthetic rate, plant physiology, *Vaccinium* spp.

Introduction

Originated from the woods of North America and Northern Europe, blueberry (*Vaccinium* spp.) is a crop that has significantly increased its agricultural and economic importance in the world over the last few years due to the worldwide increase in consumption (Brazelton, 2015). The world production of this crop surpassed the 211,144 tons in 2000 to 596,813 tons in 2017, led by countries such as the United States and Canada (FAO, 2019).

In Brazil, blueberry cultivation is geographically limited to the states of Rio Grande do Sul, Santa Catarina, Paraná, and to some high-altitude regions in the states of São Paulo and Minas Gerais (Medina et al., 2018). However, the recent introduction of new cultivars with low demand for cold, especially of the "Southern Highbush" group, represents an expansion potential of blueberry cultivation to other regions of the country (Cantuarias-Avilés et al., 2014).

The mineral nutrition of these plants is one of the factors that can contribute the most to the expansion of this crop in Brazil. However, fertilization is nowadays performed based on research results from other traditional producing regions. According to Becker et al. (2009), the fertilization recommendation must take into account the needs of the crop, the growth rate, and the environmental conditions of each region.

Among the nutrients used by the blueberry plants, nitrogen (N) is the most important nutrient to maintain vegetative growth (Pescie et al., 2018). N performs a structural function, participating in various organic compounds vital to the plant, such as amino acids, proteins, proline, among others (Bezerra et al., 2018). Its supply must be performed at ideal rates in each developmental stage of the plant for efficient absorption and to avoid losses that may occur in several ways (Amaral et al., 2018).

Fertilization assays have been performed to determine the optimal nitrogen dose in blueberry (Amaral et al., 2018; Leitzke et al., 2015; Yañez-Mansilla et al., 2014; Bryla et al., 2012; Banãdos et al., 2012). However, information on the N effects on gas exchanges and the chlorophyll content in blueberry plants is still scarce, especially under tropical conditions. Therefore, this work aimed to evaluate the physiological attributes of

plants of the blueberry cv. Biloxi as a function of nitrogen fertigation in the region of Brasília-DF.

Material and Methods

The experiment was performed in the period from August 2018 to July 2019, in the Fruit Farming Sector of the Experimental Biology Station (EEB) (latitude 15° 44' 24''South, longitude 47° 52' 12'' West) of the University of Brasília (UnB), Federal District. The climate of the region, according to the classification by Köppen-Geiger, is Aw (Cardoso et al., 2014), which characterizes this region as Tropical, with dry winter and rainy summer. The data referring to the climatic variables, (temperature, air relative humidity, and precipitation) during the execution of the experiment are listed in Figure 1. The data were obtained from the INMET weather station.

The blueberry plants analyzed in the experiment were in their second productive cycle, cultivated in containers (polyethylene bags), and under direct sunlight. The implantation of the orchard occurred in May 2017, with seedlings originated from the micropropagation of adult plant explants of the cv. Biloxi. Soon after the 6-month hardening process, the seedlings were transferred to the field in polyethylene bags containing 60 dm^3 of the natural rice husk substrate (which did not undergo any burning process). The polyethylene bags were distributed into four distinct rows, spaced 0.40m between each other and 2.5m between rows, with one plant per bag. The physical and chemical characteristics of the substrate are presented in Table 1.
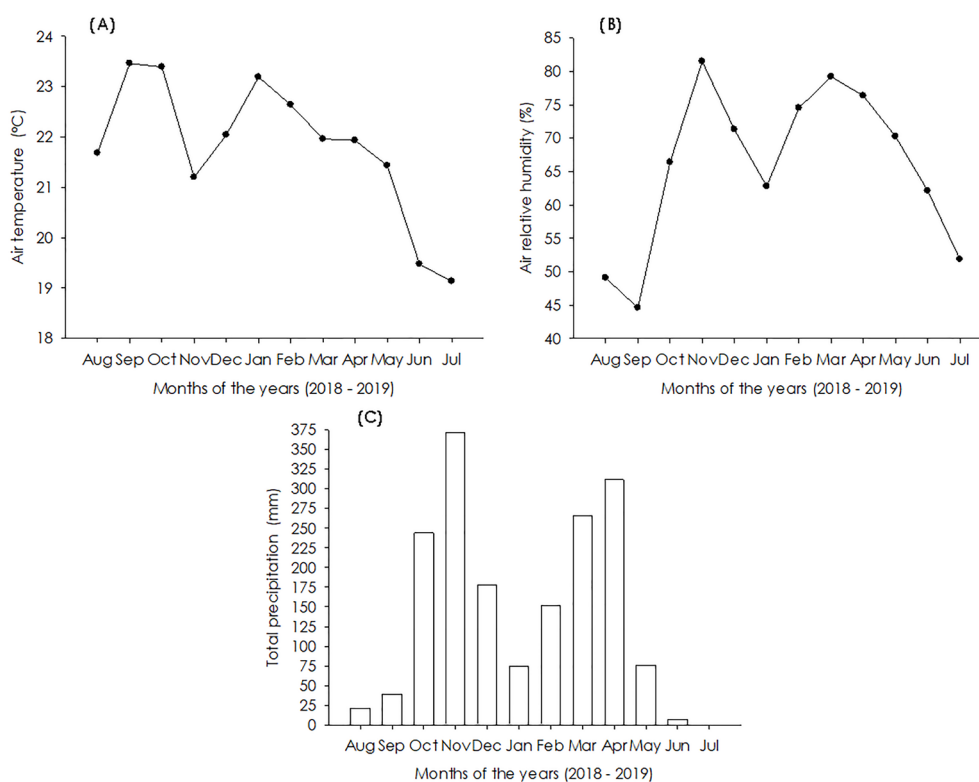
Figure 1. Air temperature (A), air relative humidity (B), and total precipitation (C) during the execution of the experiment.

The experimental design was in randomized blocks (RBD), with four treatments: 10; 20; 30, and 40 g of N plant^{-1}, 8 replications, and five plants per experimental plot, totaling 160 plants. The N doses used in the experiment corresponded to the respective percentages of 50, 100, 150, and 200% of the fertilization recommended for the blueberry crop used by producers of the region. The nitrogen sources used were ammonium sulfate (21% of N) and urea (44% of N), providing a fixed amount of N with the ammonium sulfate N (2.5 g plant^{-1}) for all treatments, and the remainder with urea, according to each dose used.

Table 1. Dry density (DS); total porosity (PT); pH; electrical conductivity (EC); organic matter (O.M), and contents of calcium (Ca); magnesium (Mg) and iron (Fe) of the substrate.

DS	PT	pH	EC	O.M	Ca	Mg	Fe
Kg m^{-3}	%	-	mS cm^{-1}		mg L^{-1}		
131.71	85.46	5.56	0.418	224.4	47.4	1.98	0.79

The blueberry cultivar studied in the experiment was the 'Biloxi', which stands out for being a cultivar that does not need cold hours to complete its reproductive cycle (Fall creek, 2018). Also, it is an extremely vigorous and productive variety. At the end of the first production cycle, a drastic pruning was performed in the plants, through which all branches were pruned at a 20 cm height. 20 days after the beginning of sprouting, the thinning of the branches was performed, homogenizing all the bushes for 8 productive branches.

During the execution of the experiment, the application (via foliar spraying) of synthetic auxins was performed. The applications were performed every 15 days, also performing the preventive control of pests and diseases through insecticides, fungicides, and acaricides.

The fertilization and irrigation of the plants were performed via fertigation with products based on macro and micronutrients as to provide, at every year, N: 200 kg ha^{-1}; P_2O_5: 120 kg ha^{-1}; K_2O: 250 kg ha^{-1}; Ca^{2+}: 200 ha^{-1}; Mg^{2+}: 120 kg ha^{-1}, and SO_4^{2-}: 150 kg ha^{-1}, according to the recommendations for the blueberry crop, as used by producers of the region.

Fertigation was performed daily through dripping, using a drip system with two emitters per plant, daily providing a mean water depth of 2.5 liters per plant. Irrigation was automatized, consisting of five watering turns in pre-programmed times (08:00; 12:00; 14:00; 16:00, and 18:00). The monitoring of the pH and the electrical conductivity of the irrigation water and nutritive solution was performed using the handheld measurer HI9814 pH/EC/TDS.

Gas exchanges were measured through an IRGA infrared gas analyzer, model LI-6400XT (LI-COR, Inc., Lincoln, NE, USA). To determine the light saturation, a photosynthetic photon flux density curve of 0, 20, 60, 100, 250, 500, 1000, 2000, 2250, 2500, and 3000 μmol m^{-2} s^{-1} was made. The gas exchange evaluations were performed in mature leaves, in the morning, between 08:00 and 10:00.

Based on the gas exchange analyses, the following variables were obtained: net photosynthesis rate (A) (μmol of CO_2 m^{-2} s^{-1}), transpiration (E) (mmol of H_2O m^{-2} s^{-1}), stomatal conductance (gs) (mol of H_2O m^{-2} s^{-1}), and internal CO_2 concentration (Ci) (μL L^{-1}). With these data, the instantaneous water-use efficiency (WUE) (A/E) (μmol of CO_2 mmol H_2O^{-1}] and the carboxylation efficiency were estimated (CE) (A/Ci) (μmol of CO_2 L m^{-2} s^{-1} μL^{-1}).

The SPAD index, associated with the leaf chlorophyll content, was determined through three readings (base, intermediate part, and apex) in the same leaves and at the same time as the gas exchange analysis, using a handheld chlorophyll measurer, model Minolta SPAD-502. The determination of the leaf nitrogen (N) was performed by collecting the same leaves in which the gas exchange and SPAD index evaluations were performed. The leaves were dried in a forced-air oven at 60ºC until reaching constant weight and processed in a Wiley mill – TE 648 with a 1 mm mesh sieve. The samples were analyzed by the Dumas solid combustion method, through the detection of the thermal conductivity. The N was quantified in a LECO elemental analyzer (model 628CN).

The data were subjected to analysis of variance by the "F" test with a 0.05 significance level. Those variables that presented a significant effect were subjected to the quantitative analysis of polynomial regression, following the recommendations by Banzatto & Kronka (2006), using the statistical softwares Sisvar and SigmaPlot 10.0.

Results and Discussion

Based on the obtained results, there was an effect of the different nitrogen doses on the characteristics associated to gas exchange and chlorophyll content, such as the internal CO_2 concentration (Ci) transpiration (E), instantaneous water-use efficiency (WUE), leaf nitrogen (N) ($p < 0.01$), net photosynthesis rate (A), and SPAD index ($p < 0.05$) (Table 2).

Table 2. Summary of the analysis of variance referring to the net photosynthesis rate (A), internal CO_2 concentration (Ci), stomatal conductance (gs), transpiration (E), instantaneous water-use efficiency (WUE) (A/E), carboxylation efficiency (CE) (A/Ci), SPAD index (SPAD), and leaf nitrogen (N) in plants of the blueberry cv. Biloxi as a function of nitrogen fertigation.

Variation source	A μmol CO_2 m^{-2} s^{-1}	Ci μL L^{-1}	gs mol of H_2O m^{-2} s^{-1}	E μmol of H_2O m^{-2} s^{-1}	(WUE) (A/E) μmol of CO_2 mmol H_2O^{-1}	(CE) (A/Ci) μmol of CO_2 L m^{-2} s^{-1} μL^{-1}	SPAD Spad	N g kg^{-1}
N (F)	5.24*	174.1**	1.01^{ns}	64.02**	27.38**	3.92^{ns}	4.46*	14.56**
MSD	0.58	2.93	0.069	0.37	0.12	0.002	14.06	0.58
CV (%)	7.18	0.40	21.44	3.67	7.20	8.82	10.03	1.07

C.V. = coefficient of variation, MSD = minimum significant difference; ^{ns} = non-significant; ** = significant at a 1% probability level (P<0.01); * = significant at a 5% probability level (P<0.05).

The net photosynthesis rate (A) of the plants of the blueberry cv. Biloxi was significantly affected by the nitrogen doses applied, with a quadratic polynomial adjustment of the data (Figure 2A). It was also observed that the estimated dose of 30.65 g of N plant[-1] resulted in 3.93 µmol of CO_2 m^{-2} s^{-1} as the highest value of A. The result was 16.79% higher compared to the value of A in the treatment with 10 g of N plant[-1]. Corroborating with the results by Yañez-Mansilla et al. (2014), these authors noted a positive effect of the N supply on the photosynthesis of plants of the blueberry cultivar Bluegold. However, these same authors did not observe a positive effect of the application of N on the photosynthetic rates of plants of the blueberry cultivar Legacy with a growing supply of N doses.
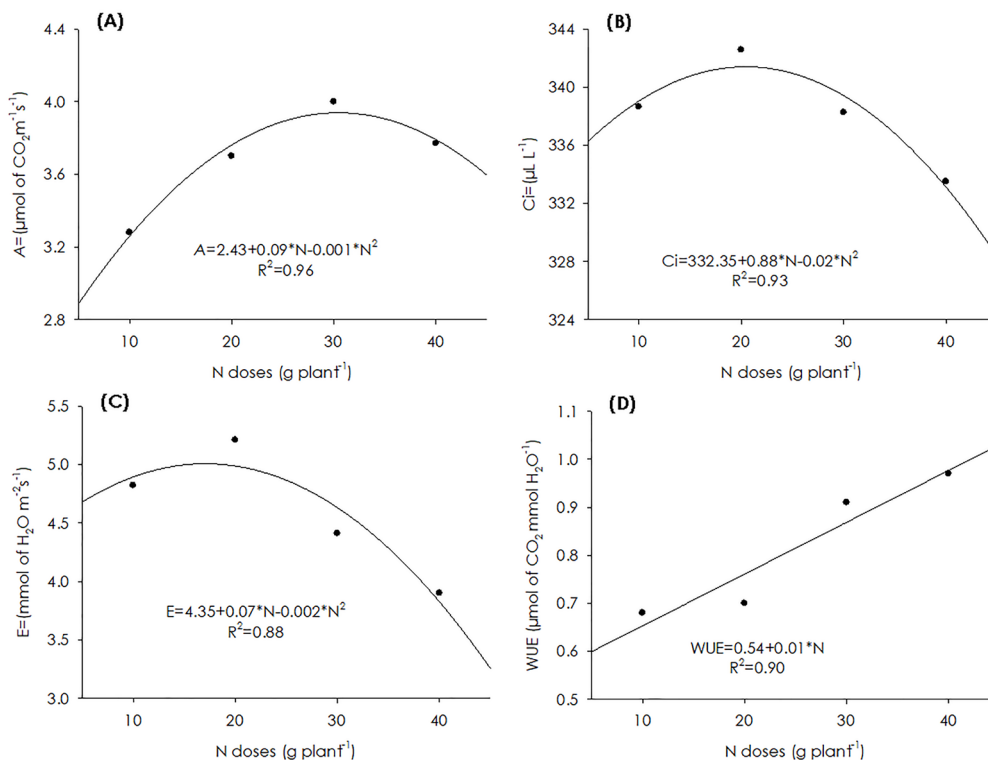
Figure 2. Net photosynthesis rate (A), internal CO_2 concentration (B), transpiration (C), and instantaneous water-use efficiency (D) of the blueberry cv. Biloxi as a function of different doses of nitrogen fertilization via fertigation.

One of the positive implications of N on the photosynthetic rate of the plants is the consequence of the greater stimulation in the synthesis of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which is the main enzyme related to the assimilation of carbon dioxide and its conversion into organic acids (Taiz & Zeiger, 2013). The amount of this enzyme can drastically affect the photosynthetic rate of the plants (Ibrahim et al., 2018). According to Makino (2011), the increase in the amount of nitrogen in the leaf tissue leads to a greater allocation of N for the Rubisco. Li et al. (2013) reported that the greater N supply is usually associated with a greater content of Rubisco in the leaves of the plants.

The low photosynthetic rate at the dose of 10 g N plant[-1] might have occurred because the plants cultivated with a low nitrogen supply usually have a significant increase in the concentration of starch in the chloroplasts (Braun et al., 2016), and this accumulation is normally followed by mechanical damage in the thylakoids of the chloroplast (Walter et al., 2015). Furthermore, high amounts of starch in the chloroplast may contribute to the decrease of the CO_2 diffusion rate from the intercellular spaces to the carboxylation sites (Cruz et al., 2007), reducing the photosynthetic rate of the plants under these conditions.

N doses above 20 g plant[-1] did not provide any increment in the internal concentration of CO_2 (Ci), and there was a decrease in the Ci as the N doses were raised (Figure 2B). The reduction in the Ci with the increase of nitrogen fertilization might have been the result of greater stomatal closure, decreasing the input of CO_2 and limiting the A (Tominaga et al., 2018). However, no significant effects of the N doses were verified on stomatal conductance (gs) (Table 2), thus suggesting that the reduction of the Ci and A at a 40 g N plant[-1] dose might be more related with damages in the photosynthetic apparatus or the enzymatic system of CO_2 fixation than

with stomatal limitations related to the input of CO_2 in the leaf tissue.

It was also observed that the transpiration rate (*E*) was lower for the plants cultivated with the highest N doses (Figure 2C). As a consequence, these plants presented greater water-use efficiency (Figure 2D), that is, greater carbon gain per amount of transpired water. These results suggest that the plants of the blueberry cultivar Biloxi, when cultivated under high nitrogen concentrations, presented a stomatal adjustment to minimize water loss. Lima et al. (2010) reported that the stomatal behavior determines the transpiration demand to which the leaves are potentially subject, controlling their loss of H_2O for the environment in the form of steam.

A (*A/E*), which represents the instantaneous water-use efficiency (*WUE*) and quantitatively promulgates the momentary behavior of gas exchanges in the leaf, presented a growing linear response (Figure 2D). The best results of *WUE* when providing 40 g of N plant[-1], even with the reduction of the value of *A* under this dose of N (Figure 2A) is possibly a result of the low *E* that the plants presented when cultivated with 40 g of N plant[-1]. These results corroborate with Melo et al. (2009), who reported the *WUE* as a process that besides the environmental conditions can be also highly influenced by the nutritional management of nitrogen to which the plants are subjected.

The leaf nitrogen contents were affected by the doses of N, with a quadratic polynomial adjustment of the data and observing the highest value of leaf N with the application of 30 g of N plant[-1] (Figure 3A), a similar adjustment to that observed by Vargas (2015) in a study with the nitrogen fertigation of the 'Bluecrop' blueberry. However, the means found in the present work varied within the amplitude from 24.02 to 25.21 g kg^{-1}, results above the range from 17.6 to 20.10 g kg^{-1} described by Hart et al. (2006) as the adequate N content in the leaf tissue of the blueberry plants.
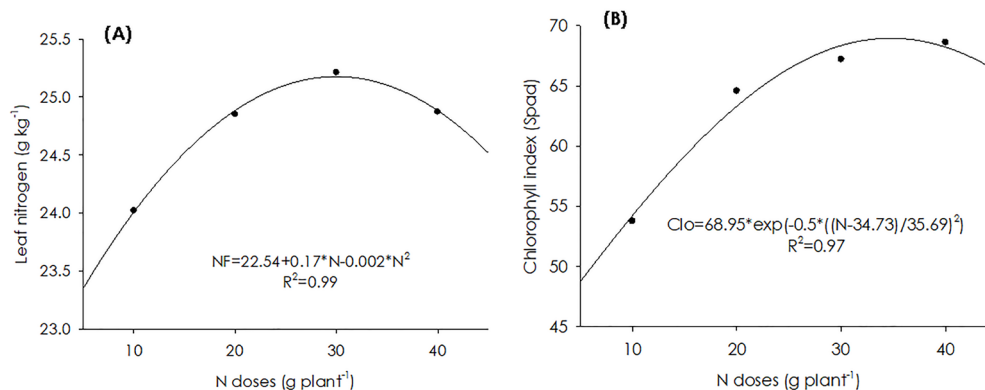
Figure 3. Leaf nitrogen (A) and chlorophyll index (B) of the blueberry cv. Biloxi as a function of different doses of nitrogen fertilization via fertigation.

The relative chlorophyll index in the leaves of the blueberry 'Biloxi' was significantly influenced by the nitrogen doses, varying from 53.75 to 68.60 SPAD units, verifying a progressive increase of the chlorophyll indexes as a function of the nitrogen doses applied (Figure 3B). These results are in accordance with those by Moura (2013), in a study with nitrogen fertilization in plants of the blueberry cv. Powderblue who, such as in the present study, found higher chlorophyll relative indexes with a dose of 40 g of N plant[-1]. Leitzke et al. (2015) observed a maximum chlorophyll index in plants of the blueberry cultivar Misty, when providing 12.1 g of N plant[-1].

The minimum nitrogen dose provided to the blueberry plants resulted in the lowest chlorophyll relative index. These results must be related to the reduction of the leaf nitrogen contents at the dose of 10 g of N plant[-1]

(Figure 3A), since this nutrient is one of the main precursors of the chlorophyll synthesis in the plants (Mógor et al., 2013). The low chlorophyll relative index must be another factor that contributed to the low net photosynthesis rate of the blueberry plants under this dose of N, as a consequence of the importance of these pigments for the absorption of photons and release of electrons (Kume et al., 2018).

Conclusions

The plants of the blueberry cultivar Biloxi increased the photosynthetic rates at doses of up to 30 g of N plant[-1].

The nitrogen doses did not influence the stomatal conductance, as well as did not provide improvements in the carboxylation efficiency of the blueberry plants.

In the conditions of the present work, the highest leaf N contents were obtained with the application of 30 g of N plant[-1], and values above this concentration did not

correspond to higher net photosynthesis rates, transpiration, and concentration of CO_2 in the leaf mesophyll.

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