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Nitrogen fertilizer affects the chemical composition of the substrate, the foliar nutrient content, the vegetative growth, the production and fruit quality of blueberry

A adubação nitrogenada afeta a composição química do substrato, o teor foliar de nutrientes, o crescimento vegetativo, a produção e a qualidade dos frutos de mirtileiro

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Abstract

The objective of this study was to investigate the influence of different nitrogen fertilizer rates on chemical composition of substrate, vegetative growth, yield, fruit quality and leaf nutrient concentration of Misty and O'Neal blueberry cultivars. It was applied five nitrogen rates. In the years 2008 and 2009 were: 0, 5, 10, 15, and 20 g plant^{-1}, whereas, in 2010, due to the growth and development of plants, the rates were: 0; 7.5; 15; 22.5; and 30 g plant^{-1}. The variables analized were: plant height, dry mass of pruning, shoot length, chlorophyll content, yield, fruit mass, pH, titratable acidity, soluble solids, anthocyanin content, antioxidant activity, total phenolics of fruits, leaf nutrient concentration (N, P, K, Ca, Mg, B, Fe, Mn and Zn), and chemical properties of the substrate. Levels of N reduced the pH, exchangeable Ca and Mg and increased the Al content in the substrate. In leaves, there was an increase in N, P and Fe and a decrease in Ca, Zn, Mn and B. The highest yield was obtained with the estimated rate of 7.37 g plant^{-1} of N. Regarding functional compounds, there was a linear increase in antioxidant activity and phenolic compounds. It can be concluded that N fertilization with ammonium sulfate, significantly influenced the chemical composition of the substrate, nutrient content of leaves, vegetative growth, yield, and fruit quality of blueberries.

Additional keywords: ammonium sulfate; mineral fertilization; pH; *Vaccinium*.

Resumo

Este trabalho teve por objetivo verificar a influência de diferentes doses de nitrogênio (N) na composição química do substrato, na concentração foliar de nutrientes, no crescimento vegetativo, na produção e na qualidade dos frutos de mirtileiro, das cultivares Misty e O'Neal. Em 2008 e 2009, foram aplicadas 0; 5; 10; 15 e 20 g de N planta^{-1}; em 2010, devido ao crescimento e ao desenvolvimento das plantas, as doses aplicadas foram 0; 7,5; 15; 22,5 e 30 g de N planta^{-1}. As variáveis analisadas foram: altura de planta, massa seca de poda, comprimento de ramo, teor de clorofila, produção, massa de fruto, pH, acidez titulável, sólidos solúveis, teor de antocianinas, atividade antioxidante, compostos fenólicos totais dos frutos, concentração foliar de nutrientes (N, P, K, Ca, Mg, B, Fe, Mn e Zn) e propriedades químicas do substrato. Com o incremento dos níveis de nitrogênio, houve redução do pH, nos teores de Ca e Mg, e elevação do teor de Al no substrato. Nas folhas, houve incremento do teor de N, P e Fe e diminuição de Ca, Zn, Mn e B. A maior produção foi obtida com a dose estimada de 7,37 g planta^{-1} de N. Em relação aos compostos funcionais, houve incremento linear para a atividade antioxidante e dos compostos fenólicos com o incremento da adubação nitrogenada. Conclui-se que a adubação nitrogenada com sulfato de amônio influenciou na composição química do substrato, no teor foliar de nutrientes, no crescimento vegetativo, na produção e na qualidade dos frutos de mirtileiro.

Palavras-chave adicionais: nutrição mineral; pH; sulfato de amônio; *Vaccinium*.

Introduction

The blueberry belongs to the Ericaceae family, being known as *mirtileiro* in Portuguese or *arándano* in Spanish (Fachinello et al., 2008). It is a native fruit of the Northern Hemisphere, where it is grown commercially on a large scale, especially in the United States of America (USA) and in some European countries. In the Southern Hemisphere, it is grown in Chile and, despite being a culture of little commercial expression in Brazil, the potential for cultivation in the Southern region is promising

(Brackmann et al., 2010). According to Fachinello et al. (2011), the blueberry is one of the main representatives of the group, known as "small fruits", with growing interest in its cultivation by the fruit growers. It has a great diversity of bioactive compounds that can bring benefits to human health, such as polyphenols and anthocyanins, which have high antioxidant activity (Brackmann et al., 2010; Rodrigues et al., 2011).

Among the difficulties for the expansion of this crop in Brazil, it is cited the high cost of implementation, with correction of fertility and acquisition of seedlings (Coletti et al., 2011; Fischer et al., 2012). In addition, there is a lack of information about its management, particularly with regard to fertilization, which is currently carried out based on research results from other traditional producing regions. Therefore, this aspect is essential for plant development (Li et al., 2012), because according to Becker et al. (2009), the fertilizer recommendation in blueberry should take into account the needs of culture, the growth rate and environmental conditions.

Among the nutrients utilized by plants, nitrogen (N) is the most required (Souza Júnior & Carmello, 2008), being absorbed by the roots as nitrate or ammonium salts. The nitrogen content needed for optimal plant growth varies between 2 and 5% by dry weight thereof (Castaño et al., 2008). Nitrogen fertilizer with ammonium sulfate, depending on the dose, can increase or decrease the growth and development of blueberry fruits. According to Bañados et al. (2012), the application of this N source, at a dose of 16.6 g plant^{-1} N, provided greater growth and fruit development to cultivar Bluecrop. On the other hand, high doses, 37.2 and 55.8 g plant^{-1} N, can negatively influence these parameters.

Losses or export of N by plants should be considered throughout the development cycle. According to Bryla et al. (2012), the 'Bluecrop' blueberry shows losses in the order of 2.18 g plant^{-1} with falling leaves, 0.33 g plant^{-1} with pruning, and 2.34 g plant^{-1} in the fruit harvest. Still, the export of nutrients by plants increases along the growing season, indicating that it is preferable for the application of fertilizer to be performed during the spring. Furthermore, the amount of nutrients absorbed by plants is related to the edaphoclimatic conditions and genetic constitution (Nascimento et al., 2011). Among the edaphoclimatic factors, the availability of nutrients and soil moisture have been considered as the main drivers of changes in nutrient absorption patterns.

Considering the lack of studies and the importance of nitrogen fertilizer in blueberry cultivation, the present study was developed based on the assumption that the use of different nitrogen rates influences the vegeto-productive behavior and the quality of blueberries as well as the chemical composition of the substrate. Therefore, this study aimed to verify the influence of different rates of nitrogen in vegetative growth, production, fruit quality and foliar concentration of the blueberry cultivars Misty and O'Neal, and of the substrate.

Material and methods

The study was conducted in the experimental area of EMBRAPA (Brazilian Agricultural Research Corporation), CPACT unit (Agricultural Research Centre of Temperate Climate), in Pelotas, RS, Brazil (31°40'S latitude, 52°26'W longitude and 57m altitude), during the years 2008, 2009 and 2010. Two blueberry cultivars, Misty and O'Neal, belonging to the *Southern Highbush* group, were planted in March 2008 in plastic pots with 18 liters capacity and maintained in open and drip-irrigated environment. The following mixture was used as substrate: sieved soil (40%), sand (20%), eucalyptus sawdust (30%) and bovine manure (10%; with C/N=20); which had the following chemical composition: 5.3 water pH; 4.4% organic matter (OM); 66.0 mg dm^{-3} of potassium (K); 49.3 mg dm^{-3} of phosphorus (P); 0.4 cmol$_c$ dm^{-3} of aluminum (Al); 2.0 cmol$_c$ dm^{-3} of calcium (Ca); 1.0 cmol$_c$ dm^{-3} of magnesium (Mg); 16.9 mg dm^{-3} of sodium (Na); 0.2 mg dm^{-3} of boron (B); 1.9 mg dm^{-3} of copper (Cu); 23.9 mg dm^{-3} of manganese (Mn); and 5.8 mg dm^{-3} of zinc (Zn).

The treatments tested were different rates of N, using as source the ammonium sulfate $(NH_4)_2SO_4$, manually applied in coverage. In 2008 and 2009, N doses applied were: 0, 5, 10, 15 and 20 g plant^{-1} (Freire, 2006). Applications were made in fertilization maintenance, divided in three times with 30 day intervals from 09-01-2008 and 09-10-2009, respectively. In 2010, also following the recommendations of Freire (2006), depending on the age of the plants and their growth and production, N fertilization rates were increased, being applied: 0; 7.5; 15; 22.5 and 30 g plant^{-1}, divided in three applications at intervals of 30 days from 09/28/2010.

The variables analyzed were: plant height (cm), measured at the end of the growing season, late fall; dry mass of pruning (g plant^{-1}), held at the time of winter pruning; shoot length (cm), measured in five shoots per plant at the end of the growth period, late fall; chlorophyll content, measured through SPAD index by using a SPAD-502 chlorophyll meter (SPAD - *Soil Plant Analysis Development,* Minolta, Osaka, Japão); production (g plant^{-1}); fruit mass (g), obtained by the average weight of 20 fruits per repetitions in each crop; pH, measured with pH meter with automatic temperature correction directly in the juice of the fruit; titratable acidity (%), analyzed accordingly to rules of Adolfo Lutz Institute (Pregnolatto & Pregnolatto, 1985); soluble solids (°Brix), determined on portable analog refractometer; anthocyanin content (mg 100 g^{-1}), quantified by Fuleki & Francis (1968) adapted methodology; antioxidant activity (μmol trolox g^{-1} fresh mass), determined through adapted methodology of Brand-Williams et al. (1995); total phenolic compounds of fruit (mg 100 g^{-1}), determined by adapted methodology of Swain & Hillis

(1959); foliar nutrient content (N, P, K, Ca, Mg, B, Fe, Mn and Zn) and chemical properties of the substrate (pH, Al, O.M., Ca, Mg, P and K), determined by the Plant Nutrition Lab of EMBRAPA/CPACT.

The experimental design was randomized block with four replications of five plants, following the factorial 2 x 5 (two cultivars and five rates of N). The results were submitted to variance analysis, and variables with significant differences in the qualitative factor had their means compared by Tukey test at 5% error probability, while variables with significant differences in the quantitative factors were submitted to regression analysis. Statistical analyzes were performed using the statistical program Winstat 2.1 (Machado & Conceição, 2003).

Results and discussions

Nitrogen fertilizer significantly influenced the chemical composition of the substrate (Figure 1).

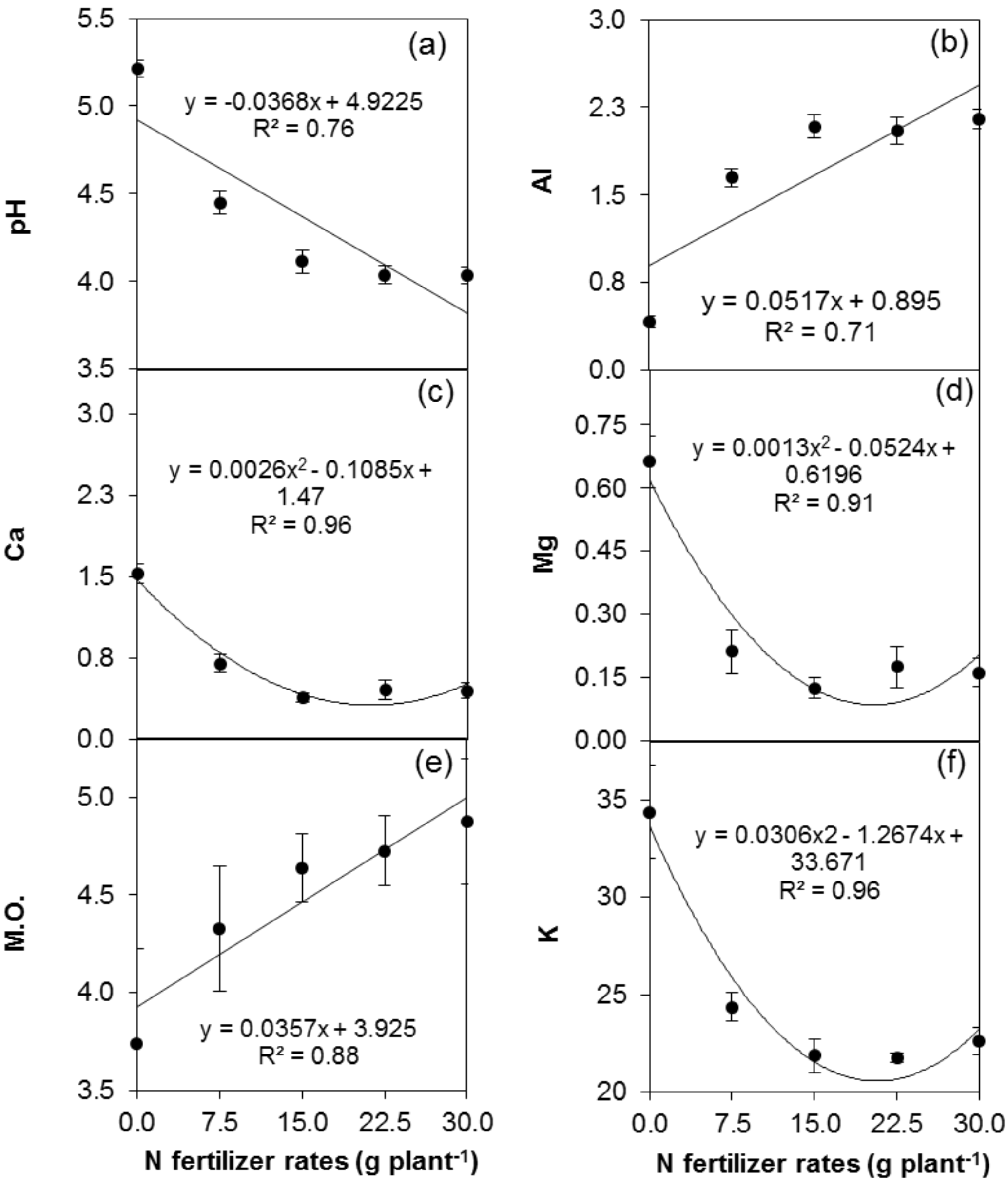

Figure 1 - Changes on pH and Al, organic matter (M.O.), Ca, Mg, P, and K substrate contents in response to N fertilizer rates at 2010.

As the N levels increased, there was a decrease in the pH of the substrate, which went from 5.20, when not fertilized, to 4.04 in plots that received 30 g plant^{-1} N (Figure 1a). According to Bañados et al. (2012), the pH reduction may have occurred due to the use of ammonium sulfate as a source of N, because this fertilizer has a high amount of sulfur (S), which acts as acidifying.

The decrease in pH value influenced the concentration of various nutrients in the substrate, being observed a linear increase of the Al concentration (Figure 1b) and the organic matter (M.O.; Figure 1e), and reduction of Ca (Figure 1c), Mg (Figure 1d) and K (Figure 1f). Similar results were obtained by Ristow et al. (2009), evaluating different substrates. These authors found that in the substrates with low pH, Al was present in higher concentrations. Although, the blueberry has adaptation to acid soils (Ristow et al., 2009; Paal et al., 2011), this species is highly sensitive to high concentrations of Al (Reyes-Díaz et al., 2011), which is a toxic element harming the development of the root system (Ristow et al., 2009; Antunes et al., 2012.). Furthermore, reducing the availability of Ca, Mg and K affects productive aspects, especially related to fruit quality (Miranda et al., 2008). Regarding cultivars, there was no significant difference for pH, Ca, P and K, however, M.O. and Al were higher in the cultivar O'Neal, which had lower Mg (Table 1).

Table 1 - Influence of blueberry cultivars 'Misty' and 'O`Neal' on plant height, shoot length, dry mass of pruning, chlorophyll, yield, fruit quality, and foliar nutrient content.

Variables	Cycle	Cultivar		F-test value	C.V. (%)
		Misty	O`Neal		
Vegetative Growth	--	--	--	--	--
Plant height (cm)	2008	75.93 a	69.59 b	23.90**	5.71
Shoot lenght (cm)	2008/09	25.71a	22.86 a	0.16ns	38.34
Dry mass of pruning (g plant^{-1})	2009	286.16 a	213.94 b	15.36**	21.81
Chlorophyll (SPAD index)	2009	51.61 a	40.20 b	232.14**	5.41
Yield and fruit quality	--	--	--	--	--
Yield (g plant^{-1})	2010	414.12 a	180.63 b	58.79**	32.38
Fruit mass (g)	2010	1.20 b	1.60 a	130.09**	8.01
pH	2010	3.58 b	4.05 a	59.84**	4.99
Titratable acidity (%)	2010	0.26 a	0.18 b	59.10**	15.02
Soluble Solids (°Brix)	2010	10.46 a	10.68 a	0.95ns	6.58
Anthocyanins (mg 100 g^{-1})	2010	447.21 a	303.59 b	55.51**	14.06
Antioxidant Activity (µg g^{-1})	2010	12389.96 a	11667.21 a	2.11ns	11.32
Total phenolic compounds (mg 100 g^{-1})	2010	1017.42 a	944.38 b	4.63*	9.47
Foliar nutrient content	--	--	--	--	--
Nitrogen (g kg^{-1})	2010	18.3 a	1.75 b	8.76**	5.04
Phosphorus (g kg^{-1})	2010	1.3 a	0.12 a	1.05ns	19.9
Potassium (g kg^{-1})	2010	7.5 a	0.56 a	0.67ns	108.65
Calcium (g kg^{-1})	2010	4.5 b	0.67 a	88.86**	13.67
Magnesium (g kg^{-1})	2010	2.4 a	0.26 b	7.01*	10.36
Boron (mg g^{-1})	2010	17.15 a	14.35 b	9.74**	18.01
Iron (mg g^{-1})	2010	94.35 a	75.95 b	58.81**	8.91
Manganese (mg g^{-1})	2010	420.35 a	331.15 b	7.12*	28.12
Zinc (mg g^{-1})	2010	11.40 a	10.10 b	4.34*	18.34
Chemical properties of substrate	--	--	--	--	--
pH (water)	2010	4.40 a	4.35 a	2.14ns	2.47
O.M. (%)	2010	4.12 b	4.80 a	13.20**	13.27
Al (cmol$_c$ dm^{-3})	2010	1.61 b	1.73 a	4.57*	10.62
Ca (cmol$_c$ dm^{-3})	2010	0.73 a	0.69 a	0.75ns	20.65
Mg (cmol$_c$ dm^{-3})	2010	0.30 a	0.24 b	4.35*	36.81
P (mg dm^{-3})	2010	22.13 a	25.71 a	4.18ns	23.1
K (mg dm^{-3})	2010	24.95 a	25.05 a	0.01ns	12.11

F statistic values followed by * and ** are significant at 1 and 5%, respectively. ns - not significant. Different lower case letters indicate significant differences at 5% error probability by Tukey test.

In addition to the influence on chemical characteristics of the substrate, N rates provided significant effect ($p<0.05$) on the foliar nutrient content, where increases in N, P, and Fe (Figure 2a, b, f), as well as decreases in Ca, B, Mn and Zn (Figure 2c, e, g, h), were observed.

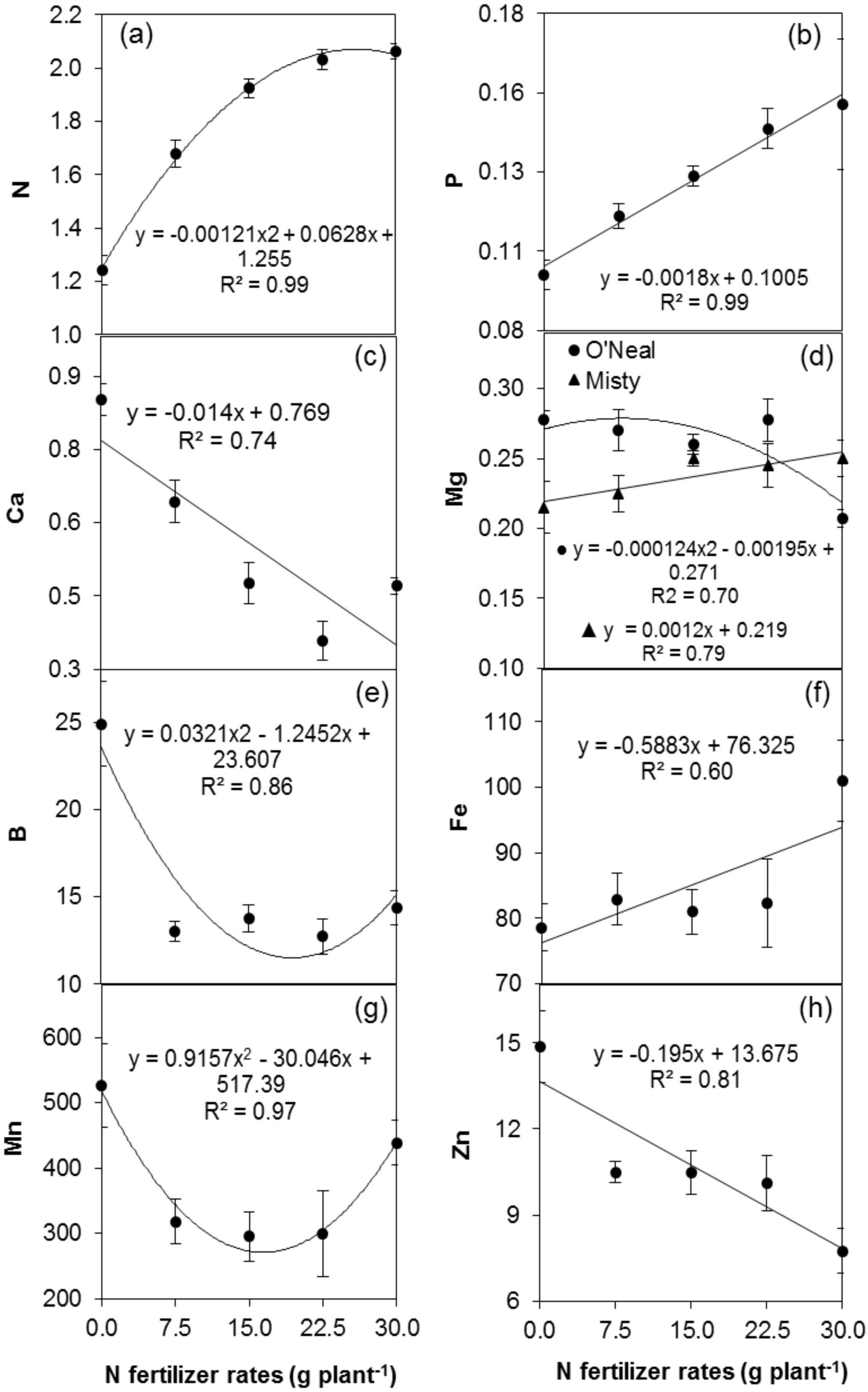

Figure 2 - Change in foliar concentration of N, P, Ca, Mg, B, Fe, Mn, and Zn in blueberry leaves due to the increase of N fertilizer rates, 2010.

Studying the effect of increasing doses of N in blueberries, Bryla et al. (2012) observed similar effects for N, P and Fe, and opposite for Ca and Mn. Foliar N increased until the estimated dose of 25.95 g plant^{-1} N (Figure 2a), while P and Fe presented linear increase (Figure 2b, f). On the other hand, foliar decrease observed in Ca, B and Mn occurred to the estimated doses of 22.95, 19.40, and 16.41 g plant^{-1} N, respectively (Figure 2c, e, g). Still, there was an interaction between the factors for Mg, presenting linear increase for 'Misty', and for 'O'Neal', an increase until the dose levels of 7.88 g plant^{-1} N (Figure 2d).

Among the cultivars, 'Misty' had the highest foliar concentration of N, Mg, B, Fe, Mn, and Zn, while 'O'Neal' was higher in Ca, with no differences for P and K (Table 1). The response variables plant height and dry mass of pruning were influenced by the interaction between dose and cultivar factors (Figure 3).

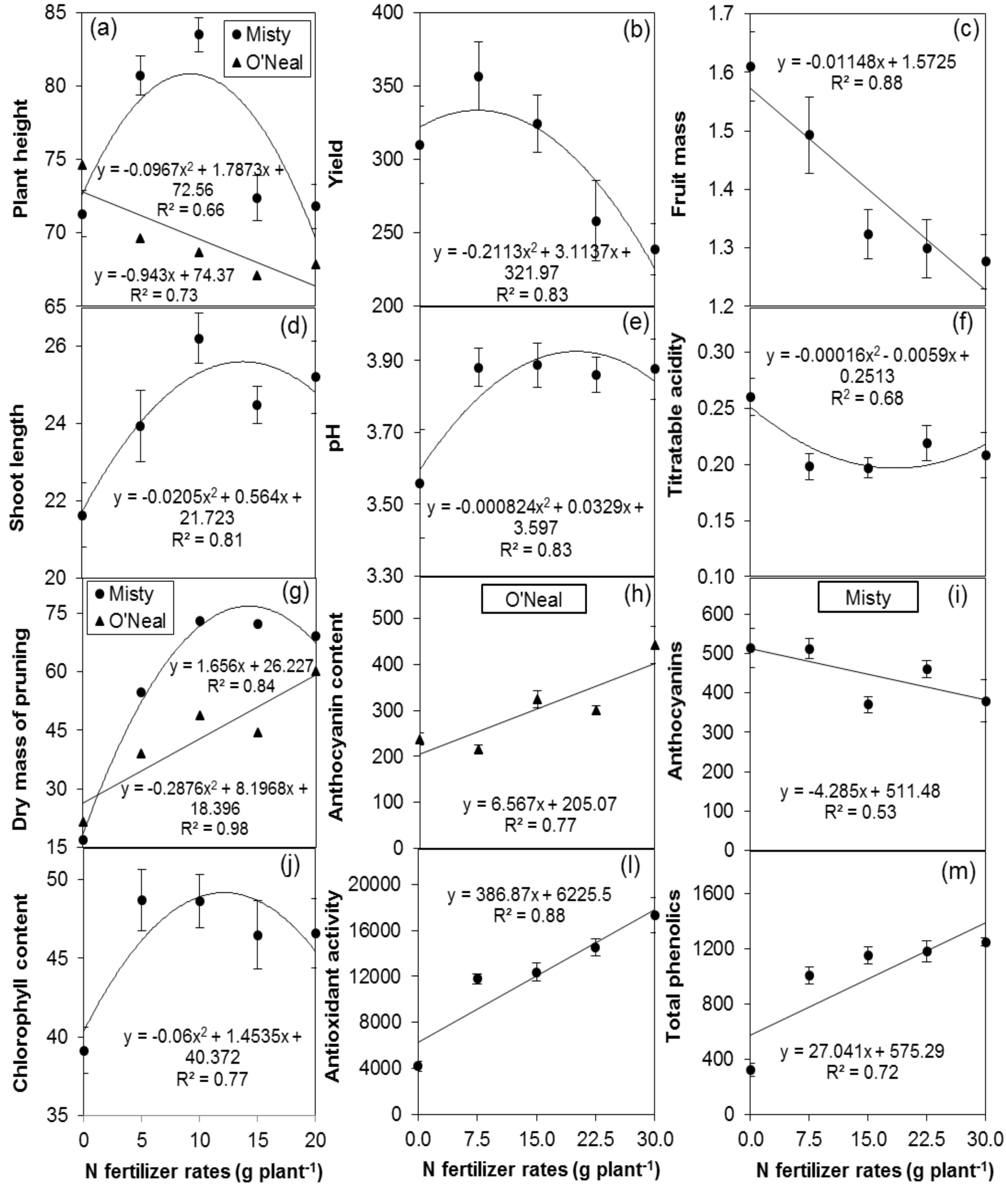

Figure 3 - Influence of nitrogen rates on plant height, dry mass of pruning, shoot length, chlorophyll content, yield, fruit mass, pH, titratable acidity, anthocyanin content, antioxidant activity, and total phenolics of blueberry fruits.

On the other hand, there was isolated effect for variables shoot length and chlorophyll content (Figure 3d, j, respectively). For plant height, 'Misty' showed a quadratic response to nitrogen rates (9.24 g $plant^{-1}$ N point of maximum), while 'O'Neal' had a negative linear response (Figure 3a). Similar behavior was observed in the variable dry mass of pruning, in which 'Misty' again presented quadratic effect (14.25 g $plant^{-1}$ N point of maximum), while for 'O'Neal' there was linear effect, ie, with the increase of N rates there was also increased dry mass of pruning (Figure 3g). In relation to the shoot length, it was observed that the fertilizer levels induced growth up to a dose of 13.75 g $plant^{-1}$ N (Figure 3d). A similar response was observed for chlorophyll content, which increased the dose until 12.11 g $plant^{-1}$ N (Figure 3j).

In general, it was found that the increase of nitrogen fertilizer, to a limited extent, provided increase in the vegetative growth. Probably, these results are related mainly to the N element, which also showed an increase of its concentration in leaves in response to its nutrient doses (Figure 2a). Results alike were observed by Pereira et al. (2013), in a study conducted with ammonium sulfate in blackberries. It is also believed that the stoppage of vegetative growth, at higher doses, has close relationship with the increase of Al content in the substrate. The results obtained in this study corroborate with Tamada (1997), which found that the increase in Al concentration in soil cultivated with blueberry decreases the dry weight of plants.

The cultivar Misty was highlighted in the vegetative parameters, once the highest values were observed regarding plant height, dry mass of pruning and chlorophyll content, besides presenting trend towards greater shoot length, compared to 'O'Neal' (Table 1).

For the variable production, doses of N induced quadratic response, with maximum yield at the estimated dose of 7.37 g $plant^{-1}$ (Figure 3b); lower than that obtained by Li et al. (2012), who found the best results from 28 g $plant^{-1}$ of the nutrient. However, these authors worked in the field, distinctly of this work, in which higher doses are generally required due to losses. Oppositely, the reduction of yield observed at high doses of N was also found by Bañados et al. (2012). The initial increase in yield is justified by the increase in foliar content of N as a function of nitrogen fertilization (Figure 2a). In contrast, the later decrease in yield, observed at high doses, has possibly occurred due to the nutritional imbalance checked. As an example of this imbalance, one can cite the increasing in levels of P and Fe (Figure 2b, f, respectively) and a decrease in Ca, B and Zn (Figure 2c, e, h, respectively). Similar results were reported by other authors, who also observed changes in foliar concentration of some elements in response to increasing doses of N (Spiers & Braswell, 2002; Castaño et al., 2008; Bañados et al., 2012; Bryla et al., 2012). However, the main cause of nutritional imbalance was the change in the chemical characteristics of the substrate as a function of N levels (Figure 1a); having the decrease of pH as probable responsible factor, once it induced the increase in Al content (Ristow et al., 2011), and the decrease in Ca, Mg and K (Figure 1c, d, f, respectively). According to Spiers (1990), lowering the pH associated with increased aluminum available to plants can result in damage to the growth and production of blueberry plants. Coinciding results involving pH change due to fertilization with N and presenting impact on production have been reported by Bañados et al. (2012) and Li et al. (2012). Abrupt changes in pH and/or the Al content, caused by high concentration of soluble fertilizers such as ammonium sulfate, as occurred in the present experiment, may also adversely affect the survival of mycorrhizal fungi which live in symbiosis with the blueberry and assist in absorption of nutrients (Montalba et al., 2010; Paal et al., 2011), influencing aspects of growth, production and fruit quality.

The fruit mass decreased with increasing N (Figure 3c). A comparable response was observed by Yadong et al. (2009) by testing the following concentrations: 0; 14; 28 and 42 g $plant^{-1}$ N. Probably, this decrease is a result of increased number of fruits, because the production did not have the same behavior. A result that would be comprehensive, since there were reduced levels of the important elements in the formation and size of the fruit, such as Ca, Mg, and K.

The cultivar 'Misty' produced 414.12 g $plant^{-1}$, being significantly higher than 'O`Neal', 180.63 g $plant^{-1}$ (Table 1). Similar trend was verified by Barrau et al. (2006), studying the same cultivars in Spain, found the highest production of 'Misty' in relation to 'O`Neal'. Possibly, the highest production presented by 'Misty' is related to high production of flower buds (Raseira & Franzon, 2012). In general, the low production of the cultivars, especially 'O'Neal', in the conditions on which this study was conducted, can be attributed to growing in pots, a situation that limits the yield potential of the crop. In contrast, 'O`Neal' showed higher fruit mass (1.60 g) than 'Misty' (1.20 g) (Table 1), results that differ from the ones obtained by Barrau et al. (2006), which found no differences between these cultivars.

In the variables related to fruit quality, it was verified isolated effect of dose and cultivar, except for anthocyanins, where there was interaction between the factors. Regarding the pH of the fruits, it was observed that the nitrogen fertilizer led to an increase of it, compared to the treatment without N (Figure 3e). The titratable acidity presented reverse effect, ie, there was a decrease in acidity in the treatments with N (Figure 3f). As for the content of anthocyanins in the fruits, there was interaction between factors, and in cultivating O'Neal increasing N rates induced an increase in concentrations of these compounds (Figure 3h), unlike cultivar Misty, which presented reverse effect (Figure 3i). The increase in N concentrations induced linear increase in antioxidant activity and total phenolic compounds (Figure 3m). Results which, added to increased anthocyanin levels in O`Neal fruit, may be associated with stress, induced by conditions such as high leaf concentrations of N, excessive decrease of pH and increase of toxic Al in the substrate. In this context, plants subjected to abiotic and/or biotic stress may present increasing functional properties of fruit (Sperdouli & Moustakas, 2012; Sánchez-Rodríguez et al., 2012).

Regarding the factor cultivar, the fruit of 'O`Neal' showed higher pH, and the fruit of 'Misty', higher titratable acidity (Table 1). 'Misty' still presented variation in the content of anthocyanins and total phenolic compounds; the first, 47% higher than 'O'Neal'. Differences in anthocyanins and phenolic compounds contents have been reported (You et al., 2011; Wang et al., 2012; Scalzo et al., 2013), and cultivars with higher concentrations of these compounds have greater commercial appeal since this characteristic is directly related to the nutraceutical value. However, in this study there was no significant difference between genotypes for antioxidant activity.

Conclusions

The nitrogen rates influence the chemical composition of the substrate, the foliar nutrient content, the vegetative growth, and the quality and yield of 'Misty' and 'O'Neal' blueberry; regardless of the cultivar, the estimated dose of 7.37 g plant^{-1} of N presented the highest yield per plant. The ammonium sulfate as a nitrogen source lowers the pH and raises the Al content of the substrate; stress generated by application of high doses of ammonium sulfate induces increased functional compounds in blueberry fruit.

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