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Nutrient omission on growth and leaf contents of blackberry

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Abstract

Cultivation of blackberry has gradually expanded in Brazil, due to their rusticity and low production cost; thus, it stands out as an excellent option for product diversification. There is a need to improve technical information on the management of this crop, especially mineral nutrition. Knowledge of the visual symptoms of nutritional deficiency and foliar contents of the nutrients allows helps farmers to choose the most adequate fertilization for plants. This study aimed at evaluating nutritional aspects and effects of nutrient omission on the growth of BRS Xingu blackberry. Treatments consisted of complete solutions and individual omission of the following nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (C), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn) and iron (Fe). The following variables were evaluated: leaf dry matter, root dry matter, total dry mass, macro- and micronutrients contents in leaves and visual symptoms of nutrient deficiency. Macronutrient and B deficiencies limit growth of blackberry omission led to the highest decrease in plant growth, besides leaf wrinkling, a fact that shows that BRS Xingu is susceptible to its deficiency. Correlation was found between N and Ca, N and P and B and Ca leaf contents. Low leaf contents of N, K, Ca and B were observed when these nutrients were omitted from the solution.

Keywords: macronutrient, micronutrient, missing element, plant nutrition, *Rubus* spp

Introduction

Cultivation of small fruits in Brazil has grown significantly, mainly the cultivation of blackberry (*Rubus* spp.), which has expanded a lot all over the country, especially in Rio Grande do Sul, São Paulo, Minas Gerais, Paraná, Santa Catarina and Espírito Santo states (Antunes et al., 2014). Interest in its cultivation results from its low production cost, rusticity and fast financial return (Pereira et al., 2015; Souza et al., 2020).

In order to increase the area cultivated with blackberry in the country, more technical knowledge of their management is needed, mainly regarding mineral nutrition (Strik & Finn, 2012). Since this berry bush has recently got economic importance in Brazil, there is still shortage of information on its nutritional issues (Strik& Finn 2012; Pereira et al., 2015).

Nutrient deficiency in plants may be observed through symptomatologic signs, which are characteristic of every nutrient. Visual diagnosis enables to characterize deficiency symptoms which are usually more visible in leaves than in other parts of the plant. In order to characterize deficiencies, the missing element technique or diagnosis by omission may be carried out, since it provides information on nutrients that may limit plant development (Barreto et al., 2017; Moschini et al., 2017; Coelho et al., 2020). The use of these techniques enables plants to be grown in a nutritious solution and visible symptoms to be associated with the mineral composition of the plant tissue.

Knowledge of visual symptoms of nutrient deficiency helps farmers to choose the most adequate fertilization for plants (Moschini et al., 2017; Barreto et al., 2017), so as to use it rationally. Studies that help to improve nutrient management of blackberry are needed to make advances in their productivity. Therefore, this study aimed at evaluating nutritional aspects and effects of nutrient

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omission on the growth of BRS Xingu blackberry.

Material and methods

The experiment was carried out in a greenhouse at the Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul state, Brazil (31°40' S and 52°26' W; altitude: 60 m). Plants were grown in a plastic covered transparent polyethylene greenhouse, with anti-insect screens and plastic film curtains on its sides. The experiment had a completely randomized design, with ten treatments and three replicates, i. e., a complete solution and individual omission of N, P, K, Ca, Mg, B, Fe, Mn and Zn in 30 experimental units. Every unit comprised a BRS Xingu blackberry, which was planted in a 5-liter plastic pot with a half-inch drain in the bottom and coarse sand as substrate. Pots were kept on a galvanized iron counter which was 1-meter high.

The nutritious solution was the one proposed by Sarruge (1975), which contains 210.1 mg N, 31 mg P, 234.6 mg K, 200.4 mg Ca, 48.6 mg Mg, 64.1 mg S, 500 µg B, 39 µg Cu, 722 µg Cl, 5000 µg Fe, 502 µg Mn, 12µg Mo and 98 µg Zn per liter. In solutions used in treatments with nutrient omission, concentrations of nutrients were equal to the ones of the complete solution, except the omitted nutrient.

Plants were kept for 121 days, from December 26th, 2017, to April 26th, 2018. In this period, either the nutritious solution or distilled water (250 mL) was alternately applied to the plants every 24 hours. In order to air the sand, pots were drained every 48 hours and then the solution was put back in again. Both the pH and the electrical conductivity of the solution were monitored; the former ranged between 6.5 and 7.0, while the latter varied from 1.8 to 2.3 mS.cm $^{-1}$.

In order to register visual symptoms of nutrient deficiency, blackberry was photographed in the studio of the Embrapa Clima Temperado when symptoms emerged. After 121 days, plants were collected, separated into shoots and roots, stored in paper containers and kept in a forced air oven at 65 °C for 72 hours. Dry matter was then determined.

Leaves were separated and ground. Sub-samples of 0.5 g of ground material were submitted to nitro-perchloric acid digestion with $HClO_4$ (1.0 ml) + HNO_3 (6.0 ml) at 190 °C in a digestion block. Phosphorus (P) concentration was determined by UV spectrophotometry (vanadate-molybdate method), while potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn)concentrations were determined by flame atomic absorption spectrometry in the extract. Nitrogen (N) was determined by the micro-Kjeldahl method after digestion of 0.2 g with H_2O_2 (2.0 ml) + H_2SO_4 (5.0 ml) and catalyzing salts at 380 °C. Boron (B) was analyzed after dry digestion of samples in a muffle oven at 550 °C and determined by colorimetry; the reagent was azomethine-H (Tedesco et al., 1995).

Data were submitted to the analysis of variance by the F-test and means were compared by the Scott-Knott test at 5% probability. The statistical analysis was carried out by the Sisvar software program, version 5.6 (Ferreira, 2014). In order to interpret results of correlation among leaf contents, the classification proposed by Dancey & Reidy (2006) was used: positive or negative values below 0.3 show either weak or non-significant correlation; values between 0.3 and 0.7 show moderate correlation; and values above 0.7 show strong correlation.

Results and Discussion

Shoot dry matter of blackberry was decreased by N omission (Table 1). When N was omitted, its concentration in leaves decreased about 4-fold, i. e., from 4.04 g kg^{-1} (complete solution) to 0.96 g kg^{-1} (Table 2). Synthesis of proteins and nucleic acids is affected by lack of N, and leads to decrease in plant growth (Marschner, 2012). Decrease in N in plants may also cause degradation of chlorophylls and, thus, affect photosynthesis and result in decrease in plant growth (Maillard et al., 2015; Taiz et al., 2017). N omission also decreased the aerial part of strawberry plants (Barreto et al., 2017) in a study in which the missing element technique was also applied.

Table 1. Shoot dry matter, root dry matter and total dry matter of blackberry of the cultivar BRS Xingu with nutrient omission.

Treatments	Shoot dry matter		Root dry matter		Total dry matter	
Complete	64.30	a	19.10	a	83.40	a
-N	15.30	d	17.30	a	32.60	d
-K	32.60	b	14.20	b	46.80	c
-P	29.80	b	16.20	b	46.00	c
-Ca	36.80	b	9.40	d	46.20	c
-Mg	23.80	c	16.70	b	40.50	c
-Mn	63.80	a	17.20	a	81.00	a
-Fe	53.70	a	12.50	c	66.20	b
-B	19.50	c	9.70	d	29.20	e
-Zn	59.30	a	17.40	a	76.70	a

Means followed by the same small letter do not differ by the Scott-Knott test at 5% probability.

Table 2. Macronutrient contents (g kg^{-1}) in leaves of blackberry of the cultivar BRS Xingu with nutrient omission.

Treatments	N		P		K		Ca		Mg	
Complete	4.04	a	3.77	b	17.33	a	8.89	b	4.15	b
-N	0.96	b	1.73	c	12.05	b	13.66	a	2.16	c
-K	2.99	a	5.35	a	2.17	c	11.11	b	5.52	a
-P	2.71	a	1.70	c	17.33	a	7.68	c	2.19	c
-Ca	3.87	a	4.47	b	18.31	a	1.62	d	3.44	b
-Mg	3.24	a	5.55	a	20.30	a	7.15	c	1.67	c
-Mn	3.41	a	4.11	b	20.51	a	5.10	c	3.68	b
-Fe	3.24	a	5.72	a	20.33	a	6.03	c	2.84	c
-B	3.12	a	3.88	b	22.71	a	4.69	c	3.78	b
-Zn	3.24	a	2.88	c	19.96	a	4.52	c	3.80	b

Means followed by the same small letter do not differ by the Scott-Knott test at 5% probability.

Besides decrease in growth of the aerial part, N omission caused lower leaf emergence and new budding by comparison with plants grown in the complete solution (Figure 1A and 1B). When deficiency evolves to a more advanced stage, the symptom appears all over the plant (Andrade & Boaretto, 2012), as found in blackberry (Figure 1A). Leaves showed reddish edges in the most advanced stage of N deficiency (Figure 2B), a fact that may occur due to the accumulation of carbohydrates that were not used in the synthesis of nitrogen compounds. Excess of carbohydrates may be used in the synthesis of anthocyanins which bestow the red color to tissues (Nemie-Feyissa et al., 2014). According to Antunes et al. (2014), the red color in leaves of blackberry is typical of anthocyanin accumulation.

Ca omission decreased the radicular system of blackberry (Table 1). Other studies also reported that Ca deficiency in blackberry affects the radicular system (Pereira et al.; 2015; Souza et al., 2015), since it paralyzes radicular growth (Marschner, 2012). It results from the fact that Ca has an important role in cell division and stretching (Jones Jr, 2012). Ca omission decreased the leaf content, by comparison with the other treatments (Table 2), but no visual symptom of Ca deficiency was observed in plants (Figure 1C and 2C).

Besides decreasing radicular growth, B omission in blackberry resulted in low value of total dry matter (Table 1), low content of this element in leaves (Table 3) and drastic wrinkling of the plant (Figure 1D) and its leaves (Figure 2D); it shows that the cultivar BRS Xingu is very sensitive to B deficiency. Small sizes of leaves and plant that result from B omission are due to the fact that its main function is related to cell wall structures (Taiz et al., 2017). Effects of B deficiency are related to decrease in cell extension caused by a signaling pathway with the participation ethylene, auxin and reactive oxygen species production (Camacho-Cristóbal et al., 2015), a fact that leads to cell stress as the result of changes in membrane integrity.

The highest P contents in leaves were found in plants with K, Mg and Fe omission (Table 2). P deficiency led to necrosis on the edges of the oldest leaves and the blade color got opaque (Figures 1E and 2E). Symptoms of P deficiency, similar to the ones observed by this study, were reported in a study of cherry trees (Vieira et al., 2011) grown without this nutrient. Lack of P may affect energy storage and transportation (Marschner, 2012; Taiz et al., 2017), besides carbohydrate transportation in leaf cells (Marschner, 2012). It may also decrease synthesis of nucleic acid and induce accumulation of nitrogen compounds such as anthocyanins that make leaves with P deficiency get purplish.

K content in leaves was lower in the treatment with its omission (Table 2). The aerial part of plants grown in a complete nutritious solution exhibited 17.33 g kg^{-1} K, while the deficient ones had 2.17 g kg^{-1}. Blackberry leaves had dark necrotic spots, similar to burns, besides wrinkled and withered blades (Figures 1F and 2F). The withered aspect may result from the fact that K is closely related to the control of cell turgor and both stomatal opening and closure (Marschner, 2012). Necrotic stains may be related to increase in putrescine concentration, which is quite common in plants with K deficiency (Cui et al., 2020). Putrescine is a polyamine which has been described as a marker of K deficiency and other problems, such as osmotic stress, which are also connected to K (Cui et al., 2020). Pereira et al. (2015) and Souza et al. (2015) carried out a study of K omission in blackberry and also found chlorosis followed by necrosis on leaf edges.

Mg content in leaves was higher in the treatment with K omission in blackberry (Table 2). According to Jones Junior (2012), there is a relation between K and Mg concentrations, since these cations compete for absorption sites and, consequently, the absence of one of them influences the other positively (Epstein& Bloom 2006; Marshner, 2012; Taiz et al., 2017). Mg omission decreased internode length in plants (Figure 1G) and, in older leaves, there was internerve chlorosis (Figure 2G).

Lack of this element causes problems in photosynthesis efficiency, since Mg plays a role in light absorption optimization and energy transference to photosynthetic reaction centers (Farhat et al., 2016).

Zn content in leaves was lower when Zn, P and Mn were omitted (Table 3). Zn omission was found to lead to symptoms of internerve chlorosis in the youngest leaves (Figures 1H and 2H), since this nutrient has low mobility in plants. Leaf chlorosis may show the need for Zn in chlorophyll biosynthesis and this symptom is due to the fact that Zn takes part in photosynthesis, respiration, enzyme activation, synthesis of protein and starch and hormone control (Taiz et al., 2017).

Figure 1. Visual symptoms of deficiency in macronutrients, micronutrients and complete treatment in blackberry of the cultivar BRS Xingu. Photographs by Paulo Lanzetta.

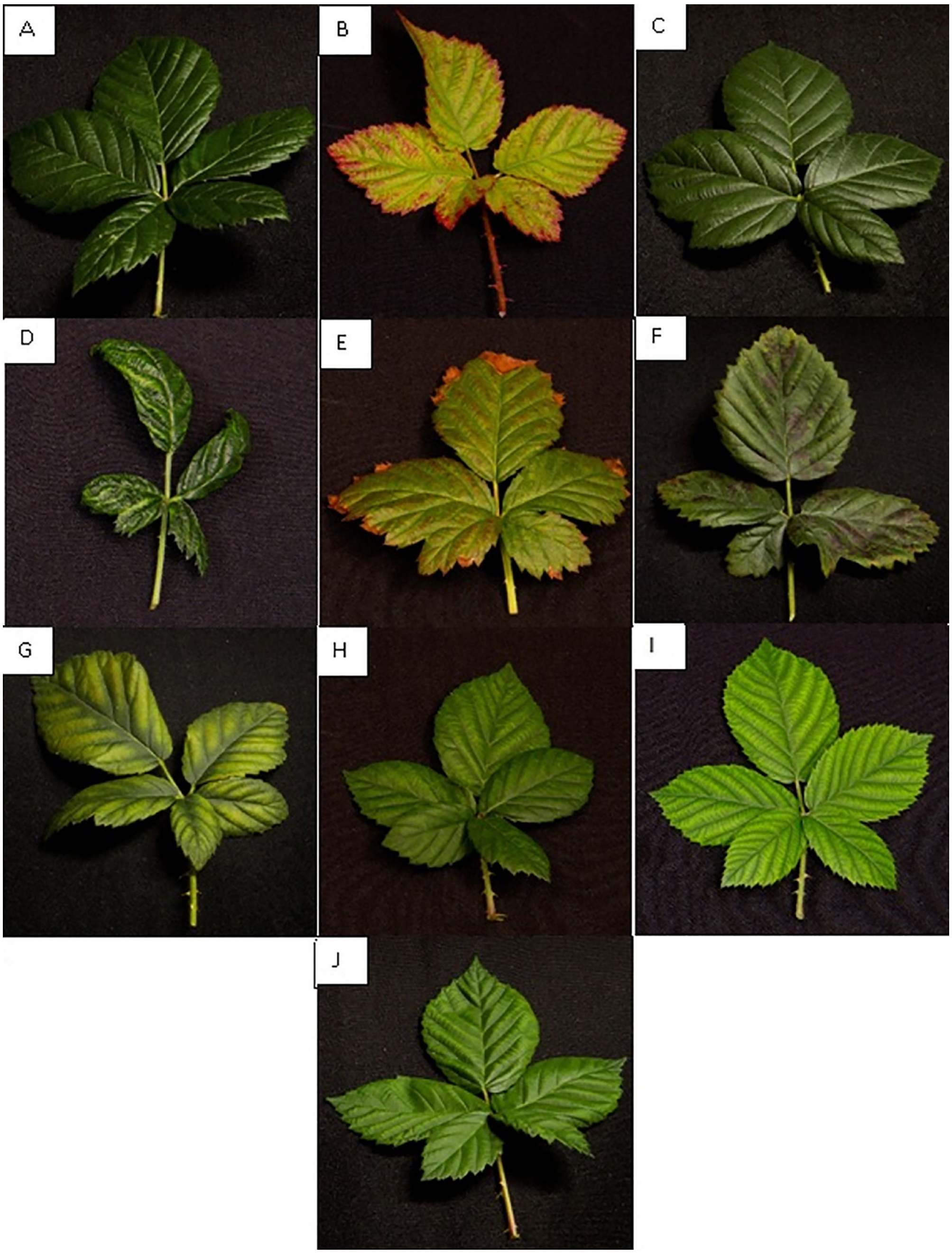

Figure 2. Visual symptoms of deficiency in macronutrients, micronutrients and complete treatment in leaves of blackberry of the cultivar BRS Xingu. Photographs by Paulo Lanzetta.

Table 3. Micronutrient content (mg kg^{-1}) in leaves of blackberry of the cultivar BRS Xingu with nutrient omission.

Treatments	B		Fe		Mn		Zn	
Complete	102.98	b	112.50	b	154.71	a	43.91	b
-N	188.20	a	265.45	a	226.34	a	51.60	b
-K	144.89	a	78.76	c	399.67	a	47.62	b
-P	103.19	b	81.35	c	118.58	a	32.81	c
-Ca	72.00	b	130.23	b	286.67	a	85.36	a
-Mg	77.14	b	98.03	c	391.89	a	81.75	a
-Mn	76.48	b	72.12	c	29.88	b	39.09	c
-Fe	51.31	b	65.28	c	73.92	b	51.12	b
-B	23.04	c	115.30	b	34.08	b	56.45	b
-Zn	65.75	b	58.11	c	45.33	b	28.53	c

Means followed by the same small letter do not differ by the Scott-Knott test at 5% probability.

Fe contents in leaves decreased when Zn, Fe, Mn, K, P and Mg were omitted (Table 3). Fe omission led to the lowest Fe contents but did not differ from the omission of other nutrients that relate to chlorophyll (K, P, Zn, Mn and Mg), i. e., Fe absorption tends to relate to nutrients that influence photosynthesis. Plants grown in the treatment with Fe omission exhibited leaves with uniform internerve chlorosis in the whole blade of their youngest leaves (Figures 1I and 2I), a fact that infers that this nutrient has low mobility in plants. Fe takes part in chlorophyll biosynthesis (Marschner, 2012) and enzyme systems related to energy production, respiration and N assimilation (Jones Jr., 2012). Symptoms of Fe deficiency, such as internerve chlorosis in the youngest leaves and fine reticulation, had already been found by another study of blackberry (Souza et al., 2015).

In the treatment with Mn omission, there was decrease in its leaf content (29.88 mg kg^{-1}), by comparison with the complete treatment (154.71 mg kg^{-1}) (Table 3). However, there was no difference between both treatments regarding shoot dry matter and root dry matter (Table 1). Plants grown with no Mn exhibited light internerve chlorosis and rugosity in their youngest leaves (Figures 1J and 2J). This symptom is due to the fact that Mn takes part in processes related to photosynthesis and energy transference (Marschner, 2012).

Strong correlation was found between N and Ca (-0.7270), N and P (0.7071) and B and Ca (0.8222) leaf contents (Table 4). Negative linear correlation between N and Ca may be because of dilution since N promotes plant growth by diluting Ca in the aerial part. Another factor that may have influenced is the competitive ionic interaction between Ca^{2+} and NH_4^+ (Fernandes et al., 2018), because NH_4^+ is used to supply N in the nutritious solution with Ca omission. The main function of P in plant metabolism is notably energy storage and transference (EST); thus, plants grown with either adequate or high P levels exhibit increase in N absorption and accumulation (Agren et al., 2012).

Table 4: Linear correlation among macro and micronutrients contents in leaves of blackberry of the cultivar BRS Xingu with nutrient omission.

	N	Ca	Mg	K	P	Fe	Mn	Zn	B
N	1.0000								
Ca	-0.7270	1.0000							
Mg	0.3236	-0.0583	1.0000						
K	0.3518	-0.5879	-0.3686	1.0000					
P	0.7071	-0.3603	0.4208	-0.0185	1.0000				
Fe	-0.6038	0.4309	-0.2604	-0.1781	-0.2917	1.0000			
Mn	0.0234	0.3939	0.0203	-0.4527	0.2386	0.2471	1.0000		
Zn	0.2575	-0.1987	-0.2274	0.0968	0.4616	0.2600	0.5198	1.0000	
B	-0.5997	0.8222	-0.0411	-0.6176	-0.3161	0.5318	0.5618	-0.1068	1.0000

Positive effect on correlation between Ca and B may be due to synergistic physiological effects between them since they are structural components of cell walls. According to Bénédicte et al. (2019), the matrix of cell walls of superior plants contains mainly polysaccharides, such as cellulose, hemicellulose and pectin, that affect wall resistance and flexibility and represent a physical barrier against adverse environmental conditions. Thus, Ca^{2+} connects to acid groups of membrane lipids and to cross-linking between pectin, mainly in the middle lamella that separates cells that have been recently divided. B has similar effects, since it not only promotes cross-linking of RG II (rhamnogalacturonan II, a pectic polysaccharide) in cell walls, but also cell extension (Taiz et al., 2017).

Conclusions

Macronutrient and B deficiency is the main factor that is responsible for limitation of blackberry growth.B omission decreases blackberry growth drastically, a fact that shows that the cultivar BRS Xingu is sensitive to its deficiency. Low N, K, Ca and B contents in its leaves are found when these nutrients are omitted from the solution.

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