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Fruit yield and gas exchange in bell peppers after foliar application of boron, calcium, and Stimulate

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ABSTRACT

The objective of this study was to evaluate the effect of the isolated and combined foliar application of boron, calcium, and the plant growth regulator Stimulate on fruit yield and gas exchange in bell peppers. The evaluated treatments were boron, calcium, Stimulate, boron + calcium, boron + Stimulate, calcium + Stimulate, boron + calcium + Stimulate, and control (water). The study was performed in complete randomized block design with three replicates. The applications were performed biweekly on the plant leaves from the beginning of flowering (December 21, 2013) until March 1, 2014. The analyzed gas exchange characteristics were photosynthetic yield, internal CO_2 concentration, and transpiration rate. The evaluated agronomic characteristics were number and yield of marketable and non-marketable fruits, and the average mass, volume, and firmness of commercial fruits. The foliar application of boron from the beginning of flowering increased the photosynthetic yield and the yield of marketable fruits cultivated in the field. The foliar application of calcium and Stimulate did not improve gas exchange and fruit yield. The most common effects of boron were an increase in the number of marketable fruits. Moreover, foliar spraying with calcium from the beginning of flowering increased the firmness of commercial fruits

Keywords: *Capsicum annuum*, leaf fertilizer, photosynthesis.

RESUMO

Produção e trocas gasosas do pimentão em função da aplicação foliar de boro, cálcio e Stimulate

Objetivou-se com este trabalho avaliar o efeito da aplicação foliar isolada e combinada dos nutrientes boro e cálcio e do regulador de crescimento vegetal comercial Stimulate na produção de frutos e nas trocas gasosas do pimentão. Os tratamentos foram boro; cálcio; Stimulate; boro + cálcio; boro + Stimulate; cálcio + Stimulate; boro + cálcio + Stimulate; e testemunha (água), aplicados via foliar. O experimento foi conduzido em delineamento de blocos casualizados com três repetições. As aplicações foliares iniciaram a partir do início do florescimento das plantas (21 de dezembro de 2013), com aplicações quinzenais até 01 de março de 2014. Foram avaliadas as características de trocas gasosas: rendimento fotossintético, concentração interna de CO_2 e taxa de transpiração; e as agronômicas: número e produtividade de frutos não comerciais e comerciais; e a massa média, volume e firmeza de frutos comerciais. A aplicação foliar de boro a partir do início do florescimento aumentou a fotossíntese e a produção de frutos comercializáveis de pimentão cultivado a campo. Não houve efeitos benéficos da aplicação foliar de cálcio e de Stimulate sobre as trocas gasosas e a produção de frutos de pimentão. Os efeitos mais consistentes do boro foram no aumento do número de frutos comerciais. A pulverização foliar com cálcio a partir do início do florescimento aumentou a firmeza dos frutos comerciais de pimentão.

Palavras-chave: *Capsicum annuum*, fertilizante foliar, fotossíntese.

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The application of foliar fertilizers and/or plant regulators may increase the production of fruits and vegetables. Calcium and boron are the nutrients most frequently applied on the leaves of vegetables (Cardozo *et al.*, 2001).

Calcium participates on the formation of plant cell wall (Malinovsky *et al.*, 2014) and is a nutrient transported by the xylem from the roots. At the end of cell division and the beginning of cell growth, small amounts of calcium reach fruit tissues (Hahn *et al.*, 2017). Calcium deficiency leads to the development of apical rot or black rot in fruits (Arruda Júnior *et al.*, 2011; Hahn *et al.*, 2017). Boron acts as a transducer in light-initiated processes, sugar translocation, and cell wall formation (González-Fontes *et al.*, 2008; Zhou *et al.*, 2016) and is essential for the formation of the pollen tube and fertilization and development of fruits (Ganie *et al.*, 2013).

Plant regulators are substances that can produce an effect similar to that of plant hormones (Albrecht *et al.*, 2012; Palangana *et al.*, 2012). In agriculture, plant regulators are used for a variety of purposes, including increasing plant development and yield, stimulating rooting of cuttings, promoting dormancy in fruits, stimulating sprouting, slowing or accelerating fruit ripening, and controlling plant development to facilitate cultural treatment and harvest

(Fagan *et al.*, 2016a). Therefore, plant regulators can promote, inhibit, or modify physiological processes in plants (Fagan *et al*., 2016a).

The cultivation of bell peppers is usually difficult, and fruit yield is not always satisfactory. This aspect becomes even more critical in the field cultivation of bell peppers, in which edaphoclimatic conditions during the crop cycle are not always adequate and may limit the productive potential. Therefore, the foliar application of plant regulators and mineral nutrients is a feasible strategy to obtain higher yields, higher quality of the final product, and commercial competitiveness (Palangana *et al.*, 2012; Pérez-Jiménez *et al.*, 2015).

Studies have demonstrated the positive effects of foliar sprays with calcium (Borges *et al.*, 2005), boron (Mashayekhi *et al.*, 2015), and plant growth regulators (Palangana *et al.*, 2012; Pérez-Jiménez *et al.*, 2015) on the vegetative and productive development of fruits and vegetables. Palangana *et al.* (2012) observed that leaf spraying with the plant growth regulator Stimulate at a dose of 150 mL p.c. 100 L^{-1} of spray volume increased the production of bell peppers. However, few studies to date evaluated the foliar application of boron and calcium in bell pepper production or the concomitant use of these nutrients with plant growth regulators.

Bell peppers are susceptible to calcium deficiency (Silva *et al.*, 2017) and boron deficiency (Mello *et al.*, 2002). The spraying of these nutrients, together with plant regulators, on leaves and flowers can increase the rate of gas exchange, promote plant development, and increase fruit yield.

The objective of this study was to evaluate the effect of isolated and combined foliar application of boron, calcium, and the plant regulator Stimulate on bell pepper production.

MATERIAL AND METHODS

This study was carried out at Universidade Estadual do Centro-Oeste (UNICENTRO) located in the municipality of Guarapuava (25°23'01"S; 51°29'37"W; altitude 1100 m), Paraná, Brazil. The local climate is classified as Cfb (humid subtropical) by Köppen classification, with hot summers, high occurrence of frost in winters, 17°C annual mean temperature, and 1,946 mm annual average rainfall (Wrege *et al.*, 2011). The local soil is classified as typical Dystroferric Bruno Latosol, clayey texture.

We used a completely randomized block design, three replicates, and nine plants on each plot. The treatments involved the isolated or combined foliar application of fertilizers Boron Super at a concentration of 0.01% boron by spray volume (H_2O), calcium chloride ($CaCl_2$) at a concentration of 0.04% calcium by spray volume (H_2O), and plant regulator Stimulate [a mixture of kinetin (90 mg L^{-1}), 4-(indol-3-yl)butyric acid (50 mg L^{-1}), and gibberellic acid (as GA_3, 50 mg L^{-1})] at a concentration of 150 mL p.c. 100 L of spray volume (H_2O).

Seedlings were obtained by planting the All Big cultivar (ISLA Sementes®) in 200-cell expanded polystyrene trays containing a pine bark-based commercial biostabilized substrate. Seedlings with four to five leaves were transplanted in the field 49 days after sowing.

The soil was plowed to prepare the experimental area in the field, and a bed shaper was used for preparing beds, 1.0 m width. The soil was corrected with 1.79 tons of calcitic limestone (PRNT 100%) per hectare according to the results of soil analysis to reach a base saturation of 80%. After liming, the soil was tilled, and beds covered with a 3 cm layer of organic compost + corn straw.

Seedlings were transplanted at 1.2×0.4 m spacing and density of 2.08 plants m^{-2}. Plants were staked vertically using bamboo stakes. Drip irrigation was performed according to the water requirement of the crop.

For the development and daily application of fertigation, the recommendations of Trani & Carrijo (2011) were adopted according to the development stage of the crop in clayey soils, using the following nutrient combination 1) first phase (up to 15 days after transplanting), 0.23 kg of nitrogen (N), 0.30 kg of calcium (Ca), 0.51 kg of phosphorus (P), and 0.33 kg of potassium (K) per ha; 2) second phase (16 to 30 days after transplanting), 1.22 kg of N, 1.20 kg of Ca, 0.77 kg of P, and 1.60 kg of K per ha; 3) third phase (31 to 45 days after transplanting), 1.20 kg of N, 1.60 kg of Ca, 2.04 kg of P, and 1.32 kg of K per ha; 4) fourth phase (46 to 60 days after transplanting), 1.43 kg of N, 0.90 kg of Ca, 0.92 kg of P, and 3.45 kg of K per ha; 5) fifth phase (61 days after transplanting to the end of the crop cycle), 2.32 kg of N, 1.30 kg of Ca, 0.27 kg of P, and 2.16 kg of K per ha. We used the fertilizers calcium nitrate, potassium nitrate, and monopotassium phosphate (MPK).

During the crop cycle, only the branches and leaves below the first bifurcation were pruned. Weed control was performed manually. Phytosanitary control was performed preventively with sprays containing thiamethoxam (Actara), copper oxychloride + mancozeb (Cuprozeb), and difenoconazole (Score) according to manufacturers' recommendations for the crop.

Foliar applications were performed using a costal sprayer [with a constant pressure valve (Jacto), 2 kgf cm^{-2} pressure, and a cone-shaped nozzle X2 (2/110)] at 1.05 m s^{-1} speed and 240 L ha^{-1} volume. Plastic curtains were used to avoid contaminating adjacent plots.

Gas exchange was analyzed using a portable photosynthesis system (IRGA, Infrared Gas Analyzer, Li-Cor, LI6400XT) with 1000 μmol photons m^{-2} $s^{-1,}$ 400 μmol mol^{-1} of CO_2, and $\Delta CO_2 + \Delta H_20$ lower than 1%, by measuring the photosynthetic yield or net assimilation (*A*, μmol CO_2 m^{-2} s^{-1}), internal CO_2 concentration (*Ci*, μmol mol^{-1}), and transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}). The three central plants of each plot were evaluated in the third fully expanded leaf from the apex. Measurements were made from 10:00 a.m. to 12:00 p.m. during full blooming (January 20 to 22, 2014) and the beginning of fruiting (February 17 to 19, 2014). All plots were evaluated on three dates in each phase, and one block was evaluated per date in sunny conditions.

Fruits that changed color from green

to bluish-green were harvested from five central plants in each plot on the following dates: February 17, February 27; March 10; and April 1, 2014. Fruits with a length >60 mm and diameter >40 mm were considered marketable and, those <60 mm in length and <40 mm diameter were classified as non-marketable, including those with severe defects, including wilting, deterioration, malformation, disease, or mechanical or pest insect damage, according to Araújo *et al.* (2009).

The agronomic characteristics we evaluated were number of marketable and non-marketable fruits (fruits m^{-2}), production of marketable and non-marketable fruits (g m^{-2}), average mass of commercial fruits (g $fruit^{1}$), volume of commercial fruits (mL) (determined individually in fruits classified by commercial standards based on the displacement of water contained in a 2-L beaker), and fruit firmness (N) [in fruits classified by commercial standards using a digital penetrometer (Instrutherm DD-200) with a 8-mm tip] by compressing two areas in the central region of whole fruits [results expressed in Newton (N)].

The obtained data were tested for normality of residuals, homogeneity of residual variances, and block additivity. After that, analysis of variance was conducted using the F test. When significant, the effect of isolated and combined foliar sprays with boron, calcium, and Stimulate was compared with that of the control treatment using the Dunnett test and the statistical software ASSISTAT version 7.7 at 5% significance level (Silva & Azevedo, 2016). The contrasts of interest for agronomic characteristics (isolated and combined foliar application of boron, calcium, and Stimulate) between the groups were estimated using the Scheffé test and the SISVAR software version 5.6 (Ferreira, 2008).

RESULTS AND DISCUSSION

All treatments with isolated and combined application of boron, calcium, and Stimulate increased fruit yield compared with the control treatment,

Table 1. Number and productivity of marketable (M) and non-marketable (NM) fruits and average mass of marketable fruits (AMC) in bell pepper plants, sprayed isolated and combined with boron, calcium and commercial plant regulator Stimulate. Guarapuava, UNICENTRO, 2013/2014.

Treatments	Fruits m^{-2}		Production (g m^{-2})		AMC (g $fruit^{-1}$)
	M[1]	NM[2]	M	NM	
Boron	25.2*	6.9	1095.5*	101.5	130.7
Calcium	19.8	4.6	942.5	72.8*	152.3
Stimulate	24.5	8.1*	941.5	124.6	115.9
Boron + calcium	29.1*	8.3*	1201.4*	108.2	123.7
Boron + Stimulate	27.2*	11.0*	1003.1*	142.1	110.4*
Cálcio + Stimulate	22.9	8.1*	1001.9*	146.1	131.7
Boron + calcium + Stimulate	26.2*	10.0*	1039.7*	120.2	119.5
Control	20.6	4.6	932.5	98.5	136.0
CV (%)	7.4	14.7	5.04	16.1	6.93
Estimating the contrasts of interest					
Boron isolated vs. calcium isolated	5.4⁺	2.3⁺	153.0⁺	28.7⁺	-21.6⁺
Boron isolated vs. Stimulate isolated	0.6	-1.2	154.0⁺	-23.1⁺	14.8
Calcium isolated vs. Stimulate isolated	-4.8⁺	-3.5⁺	1.0	-51.8⁺	36.4⁺
Boron isolated vs. boron + calcium	-4.0	-1.5	-105.9⁺	-6.7	7.0
Boron isolated vs. boron + Stimulate	-2.1	-4.2⁺	92.4⁺	-40.6⁺	20.3⁺
Boron isolated vs. calcium + Stimulate	2.3	-1.2	93.6⁺	-44.6⁺	-1.0
Boron isolated vs. boron + calcium + Stimulate	-1.0	-3.1	55.8	-18.7	11.2
Calcium isolated vs. boron + calcium	-9.4⁺	-3.7⁺	-258.9⁺	-35.4⁺	28.6⁺
Calcium isolated vs. boron + Stimulate	-7.5⁺	-6.4⁺	-60.6	-69.3⁺	41.9⁺
Calcium isolated vs. calcium + Stimulate	-3.1	-3.5⁺	-59.4	-73.3⁺	20.6⁺
Calcium isolated vs. boron + calcium + Stimulate	-6.4⁺	-5.4⁺	-97.2⁺	-47.4⁺	32.8⁺
Stimulate isolated vs. boron + calcium	-4.6⁺	-0.2	-259.9⁺	16.4	-7.8
Stimulate isolated vs. boron + Stimulate	-2.7	-2.9	-61.6	-17.5	5.5
Stimulate isolated vs. calcium + Stimulate	1.7	0.0	-60.4	-21.5⁺	-15.8
Stimulate isolated vs. boron + calcium + Stimulate	-1.7	-1.9	-98.2⁺	4.4	-3.6

*Different from control by Dunnett's level (P<0.05); ⁺significant contrast by Scheffé test, 1% probability.

except for calcium and Stimulate alone (Table 1). Furthermore, boron alone, boron + calcium, boron + Stimulate, and boron + calcium + Stimulate increased the number of marketable fruits compared with the control (Table 1).

The analysis of the contrasts of interest indicated that boron either alone or in combination produced a higher number of commercial fruits than calcium alone. Boron alone produced a higher fruit yield than calcium and Stimulate in isolation; boron + calcium was better than boron, calcium, and Stimulate alone; and boron + calcium + Stimulate was better than calcium and Stimulate alone (Table 1). These results suggest that foliar sprays containing boron were more effective in fixing fruits than the other treatments.

The positive effect of boron on fruit yield may be due to improvements in the physiological processes of plants. Boron is involved in the synthesis, lignification, and composition of the cell wall, sugar transport, and respiration (Fagan *et al.*, 2016b). Moreover, these authors have shown that boron is essential for reproductive growth and development, nitrogen fixation, and cell membrane structuring.

Treatments with isolated and combined foliar sprays were not significantly different from the controls for average mass of commercial fruits, except for boron + Stimulate, which decreased fruit mass (Table 1). This treatment, together with Stimulate alone and boron + calcium + Stimulate, reduced the volume of marketable fruits relative to the control (Table 2).

Calcium alone did not change the characteristics of commercial fruits when compared to the control but decreased the yield of non-commercial fruits relative to the control. The analysis of the contrasts of interest revealed that calcium produced a higher average mass of marketable fruits when compared to boron and Stimulate alone or in combination (Table 1).

A possible explanation for the absence of the effect of calcium on the production of commercial fruits (number and yield of fruits) was that, during the crop cycle, the plants received fertigation containing calcium nitrate. However, calcium spraying decreased the yield of non-commercial fruits (Table 1) and increased fruit firmness (Table 2) compared to the control treatment. With respect to the latter characteristic, calcium in isolation differed from boron and Stimulate either alone or in combination (Table 2).

The greater firmness of fruits from plants treated with calcium compared to the control treatment can be attributed to the structural function of calcium in forming the cell wall of fruits. The binding of calcium to fruit pectins inhibits the solubilization of polyuronides and provides a more rigid structure to the middle lamella (greater firmness) (Glenn *et al.*, 1988). Senescence of tissues is affected to some extent by the degradation of pectic polymers in the cell wall, and fruits with high calcium levels show increased firmness and consequently longer shelf-life (Pereira *et al.*, 2002). The increase in fruit firmness by calcium was also observed in other fruit species, including blueberry (Ochmian, 2012), apple (Rose & Drake, 2008), kiwi (Koutinas *et al.*, 2010), and cherry (Brown *et al.*, 1996).

The spraying of Stimulate increased the number, yield, and average mass of commercial fruits relative to the control only when this product was

Table 2. Volume and firmness of marketable fruits in bell pepper plants with spraying isolated and combined of boron, calcium and commercial plant regulator Stimulate. Guarapuava, UNICENTRO, 2013/2014.

Treatments	Volume (mL fruit^{-1})	Firmness (N)
Boron	282.2	33.4
Calcium	309.9	41.1*
Stimulate	247.7*	30.9
Boron + calcium	269.0	33.9
Boron + Stimulate	226.7*	29.2
Cálcio + Stimulate	281.4	31.8
Boron + calcium + Stimulate	224.1*	28.1
Control	292.9	33.9
CV (%)	6.4	12.62
Estimating the contrasts of interest		
Boron isolated vs. calcium isolated	-27.7	-7.7^{+}
Boron isolated vs. Stimulate isolated	34.5^{+}	2.5
Calcium isolated vs. Stimulate isolated	62.2^{+}	10.2^{+}
Boron isolated vs. boron + calcium	13.2	-0.5
Boron isolated vs. boron + Stimulate	55.5^{+}	4.2
Boron isolated vs. calcium + Stimulate	0.8	1.6
Boron isolated vs. boron + calcium + Stimulate	58.1^{+}	5.3
Calcium isolated vs. boron + calcium	40.9^{+}	7.2^{+}
Calcium isolated vs. boron + Stimulate	83.2^{+}	11.9^{+}
Calcium isolated vs. calcium + Stimulate	28.5	9.3^{+}
Calcium isolated vs. boron + calcium + Stimulate	85.8	13.0^{+}
Stimulate isolated vs. boron + calcium	-21.3	-3.0
Stimulate isolated vs. boron + Stimulate	21.0	1.7
Stimulate isolated vs. calcium + Stimulate	-33.7^{+}	-0.9
Stimulate isolated vs. boron + calcium + Stimulate	23.6	2.8

*Different from control by Dunnett's level ($P<0.05$); $^{+}$significant contrast by Scheffé test, 1% probability.

Table 3. Photosynthetic yield (*A*), internal CO_2 concentration (*Ci*), and transpiration rate (*E*) in bell pepper plants with spraying isolated and combined of boron, calcium and commercial plant regulator Stimulate. Guarapuava, UNICENTRO, 2013/2014.

Treatments	*A* (µmol CO_2 m^{-2} s^{-1})		*Ci* (µmol mol^{-1})		*E* (mmol H_2O m^{-2} s^{-1})	
	Fl[1]	Fs[2]	Fl	Fs	Fl	Fs
Boron	22.3*	21.9*	117.6*	112.6*	8.0*	7.1*
Calcium	20.4	19.8	156.7*	167.1	6.3	5.0
Stimulate	20.2	20.2	160.2	162.6	6.9	6.9*
Boron + calcium	22.2*	21.9*	121.6*	107.5*	7.9*	5.8
Boron + Stimulate	19.9	19.9	163.5	171.1	6.1	5.7
Cálcio + Stimulate	20.1	19.6	193.6	165.4	6.6	5.5
Boron + calcium + Stimulate	20.9	20.3	165.2	165.3	6.9	5.6
Control	17.75	19.4	197.9	166.4	5.9	5.8
CV (%)	6.6	6.7	10.6	11.4	9.8	12.0

*Different from control by Dunnett's level ($P<0.05$); [1]full flowering (Fl); [2]fruiting start (Fs).

combined with boron and/or calcium (Table 1), suggesting that Stimulate did not improve the main characteristics in field-cultivated bell peppers. In addition, the positive effects of Stimulate + boron were primarily due to the improvements caused by boron.

Increased fruit yields using Stimulate is reported in the literature for bell peppers (Palangana *et al.*, 2012), cabbage (Zeist *et al.*, 2017b), and soybean (Albrecht *et al.*, 2012). However, in the present study, Stimulate improved only the volume of marketable fruits relative to the control. Plant regulators do not always increase fruit yield, as reported by Ataíde *et al.* (2006) using sprays containing Stimulate and GA_3 in passion fruit.

Boron alone and boron + calcium significantly improved gas exchange relative to the control both in full bloom and at the beginning of fruiting (Table 3). In this respect, Thurzó *et al.* (2010) observed that boron increased the levels of photosynthetic pigments in *Prunus avium*, and Liu *et al.* (2015) found that calcium increased the photosynthetic activity of tomato plants under low temperatures at night.

Boron alone and boron + calcium increased the photosynthetic yield and transpiration rate when compared to the control but resulted in the lowest intracellular CO_2 concentrations (Table 3). The photosynthetic yield is usually dependent on the transpiration rate (Ferraz *et al.*, 2012), and these parameters tend to be inversely correlated with the internal CO_2 concentration (Zeist *et al.*, 2017a,b). The explanation for this result is that, the higher the net assimilation of CO_2 and water absorption and diffusion, the higher is the use of intracellular CO_2 by Rubisco in the Calvin cycle. Dalastra *et al.* (2014) evaluated gas exchange in Sancho melon, 56 days after transplanting, and observed that the photosynthetic yield was increased as the intracellular CO_2 concentration was decreased.

The treatments that improved the agronomic characteristics and photosynthetic yield of bell peppers compared to the control contained boron. Evidence of the increased fruit yield by boron has also been reported for other agricultural crops using boron (Al-Amery *et al.*, 2011) and boron + zinc (Wasaya *et al.*, 2017).

Mello *et al.* (2002) emphasize the importance of boron in the development and yield of bell peppers. These authors observed that boron deficiency might decrease root volume, foliar area, photosynthetic yield, and production of branches and flowers. Moreover, boron deficiency leads to the deficiency of other essential nutrients by affecting their rate of absorption and use.

The results indicate that the biweekly application of boron (0.01%) on the leaves from the beginning of flowering increases the photosynthetic yield and yield of marketable bell peppers cultivated in the field. There were no beneficial effects of the foliar application of calcium and Stimulate on gas exchange and fruit yield. The most common effects of boron were an increase in the number of marketable fruits. Biweekly foliar sprays with calcium (0.01%) from the beginning of flowering increased the firmness of commercial bell peppers.

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