Physiological and nutritional responses in two highbush blueberry cultivars exposed to deficiency and excess of boron

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Abstract

Boron (B) is an essential micronutrient required for physiological and biochemical processes in fruit crops such as highbush blueberry (*Vaccinium corymbosum* L.), well adapted species to acidic soils ($\text{pH}_{\text{water}} \leq 5.5$) with relatively low B availability. However, an optimal range of B supplies is quite narrow, particularly among cultivars, making it easy to under- or over-fertilize which could lead to boron toxicity. Under controlled conditions, cultivars Brigitta and Legacy were grown in a peat moss substrate (pH 4.5) with five B concentrations (0, 50, 200, 400 and 800 μM). Plant biomass, nutrient concentration, water potential (Ψ_{leaf}), oxidative stress and antioxidant activity were determined at 30 d. Concentrations of 400 and 800 μM B reduced shoot and root growth in both cultivars, and 800 μM B decreased Ψ_{leaf} in Legacy and significantly increased it in Brigitta. Boron applied at 400 μM resulted in the highest B accumulation in leaves of Brigitta (5-fold) and Legacy (2-fold), whereas Brigitta roots showed up to 60% increased B accumulation in comparison with 50 μM B, respectively. High B caused more severe oxidative stress in leaves of Legacy than B deficiency. The antioxidant activity was increased from 50 to 400 μM B in both cultivars. Boron added at 400 and 800 μM impaired physiological and biochemical performance probably due to toxicity, demonstrating that highbush blueberry in a genotype-dependent manner has relatively low B requirement.

Keywords: Antioxidant activity, boron nutrition, highbush blueberry, oxidative stress

1. Introduction

Boron (B) is an essential micronutrient that has a key function in plant structure and is involved in a range of physiological and biochemical processes (Blevins and Lukaszewski, 1998; Herrera-Rodríguez *et al.*, 2010; Landi *et al.*, 2012; Yadegari, 2016). The critical levels between low and high B in plant tissues are narrow, but the B requirement range is highly variable among species and genotypes, with an optimum for one cultivar being either insufficient or toxic for other species or cultivars (Landi *et al.*, 2012). There is little information regarding deficiency and excess of B in fruit plants grown in acidic soils, such as high-bush blueberry (*Vaccinium corymbosum* L.). Wójcik (2005) reported optimal leaf B concentration in high-bush blueberry cultivars Bluecrop and Jersey were below 15 and 37 mg kg⁻¹ DW, respectively, classifying blueberry as a fruit species with controversial B requirements. In contrast, Caruso and Ramsdell, (1995) observed symptoms of B deficiency (including decreased leaf size and shoot growth) in highbush blueberry plants with leaves containing less than 20 mg kg⁻¹ DW. In White Smith gooseberry (*Ribes grossularia* L.) grown in soil with low B availability, Wójcik and Filipczak (2015) associated soil B fertilization (1 kg ha⁻¹) with greater B concentration in leaves (>40 mg kg⁻¹ DW) compared with control plants without B fertilization that had ~20 mg B kg⁻¹ DW in leaves; higher leaf B concentration resulted in increased shoot length and improved production parameters.

Boron deficiency has been related to disorders in cell wall structure, reduced growth rate (particularly of shoots) (Camacho-Cristóbal *et al.*, 2008; Herrera-Rodríguez *et al.*, 2010; Miwa and Fujiwara, 2010), imbalances in nutrient status (Camacho-Cristóbal *et al.*, 2008; Landi *et al.*, 2012), disturbed water relations (Wimmer and Eichert 2013) and changes in antioxidant activity (Tewari *et al.*, 2010; Dahajipour *et al.*,

2011). On the other hand, B toxicity is related to diminished plant growth, increased oxidative stress as evidenced by exacerbated lipid peroxidation, along with increases in activity of antioxidant compounds (Herrera-Rodríguez *et al.*, 2010; Tewari *et al.*, 2010; Gimeno *et al.*, 2012; Kayaa and Ashraf, 2015). Pandey and Archana (2013) reported an accumulation of thiobarbituric acid-reactive substances in both leaves and roots of *Brassica juncea* under deficient (0.033 mg B L⁻¹) and excess boron (3.3 and 33 mg B L⁻¹) supply, suggesting oxidative damage due to excessive production of reactive oxygen species (ROS). Boron toxicity has also been associated with mottled interveinal chlorosis and scorching of leaf margins (Caruso and Ramsdell, 1995).

Boron-enriched soils are much rarer than B-deficient soils and concentrated particularly in the areas with the marine evaporates and marine argillaceous sediments (Nable *et al.*, 1997). Plant B deficiency frequently occurs in acidic soils in temperate climates, which exhibit high precipitations and high leaching losses (Shorrocks, 1997). To remediate low B concentration, both soil and foliar applications are useful strategies to correct B levels; however, these practices could lead easily to over-fertilization (Wójcik, 2005). In plants such as flax (*Linum ussitatissimum* L.) grown in acidified hydroponic culture (pH 4.5), B treatments were deficient, normal and excessive at 4.5, 45 and 450 µM, respectively (Dahajipour *et al.*, 2011). Moalemi *et al.* (2012) considered B concentrations of 1, 10 and 1000 µM as control, deficient and excessive, respectively, for marshmallow (*Althaea officinalis* L.) and reported that B deficiency and excess B led to increased concentration of phenolics and H₂O₂. Other authors reported normal B concentrations at 20 µM in navel orange plants (*Citrus sinensis*) (Liu *et al.*, 2014), and 50 µM in laurel (*Viburnum tinus*)

(Bañón *et al.*, 2012). However, the question about which B supply could be deficient or toxic for highbush blueberry grown in acidic conditions remain unresolved.

In Chile, highbush blueberry is an important commercial crop, cultivated on 15,560 ha with a total fruit production of 91,000 t during the season 2014-15 (Chilean Blueberry Committee, 2015). This fruit has received great interest due to its richness in antioxidants compared to other fruits, offering potential benefits for human health (Ehlenfeldt and Prior, 2001; Meriño-Gergichevich *et al.*, 2015). In southern Chile, where acid Andisols ($\text{pH} \leq 5.5$) cover 2,600,000 ha and represent the largest part of the arable area (Besoain, 1985), highbush blueberry is planted on 2,000 ha (Chilean Blueberry Committee, 2015); in these soils availability of many nutrients including B and phytotoxic aluminum (Al) can be a restrictive factor for highbush blueberry growth and yield. Traditionally, agricultural practices recommend B fertilization of blueberries to ensure greater fruit set and yield, as well as better quality parameters of marketable berries (Eichholz *et al.*, 2011); however, an overestimation in B doses could lead to toxicity. In contrast to numerous studies on B deficiency in fruit plants (Liu *et al.*, 2014), toxicity is poorly characterized for commercial fruit crops grown in acid soils, such gooseberry (Wójcik and Filipczak 2015) and highbush blueberry (Wójcik, 2005). Even it is usually assumed that critical ranges related to B deficiency, sufficiency and toxicity are narrow, those ranges vary among species and even among varieties of the same species (Herrera-Rodríguez *et al.*, 2010; Landi *et al.*, 2012).

The objective of this study was to determine the effects of different supplies of B on physiological and

2. Material and Methods

2.1. Plant material and experimental conditions

The study was carried out in a greenhouse at the Universidad de La Frontera, Temuco, La Araucanía Region, Chile. Highbush blueberry cultivars Brigitta and Legacy (one-year-old plants provided by Berries San Luis, Lautaro, Chile), frequently cultivated in southern Chile, were grown in a mixture of peat moss with coconut fiber and vermiculite (Sociedad Rathgeb y Cía. Ltda, www.jardisen.cl) at pH 4.5 under greenhouse conditions (air temperature 20-25 °C, 16/8h light/dark period, air humidity 80% and photosynthetic photon flux density of 300 mmol photons $\text{m}^{-2} \text{s}^{-1}$). Plants were irrigated daily with adequate volume of Hoagland nutrient solution: $\text{Ca}(\text{NO}_3)_2$ 2 mM, KNO_3 3 mM, MgSO_4 1 mM, KH_2PO_4 0.1 mM, MnSO_4 2 μM , NH_4NO_3 1 mM, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 0.07 μM , ZnSO_4 1.6 μM , CuSO_4 0.4 μM , and Fe-EDTA 20 μM , containing either 0, 50, 200, 400 or 800 μM of B, equivalent to 0, 3.10, 12, 25 and 50 $\text{mg L}^{-1} \text{H}_3\text{BO}_3$, respectively. The study considered the following measurements: plant biomass, leaf and root nutrient concentration, water potential, oxidative stress, and radical scavenging activity (RSA). After 30 days, plant leaves and roots were harvested, one-half was used for chemical (inorganic) and physiological analyses and another half was stored at -80 °C for biochemical analyses.

2.2. Plant biomass

At harvest, leaves and root plants were dried by placing them at 70 °C in a forced-air oven for 48 h. To determine mean relative growth rate (MRGR), the procedure of Hoffmann and Poorter (2002) was applied as follows:

$$\text{MRGR} = \frac{(\ln W_2) - (\ln W_1)}{t_2 - t_1}$$

where W1 and W2 represent the dry weight at time 0 d (t1) and 30 d (t2) respectively.

2.3. Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured using a pressure chamber PMS (model 1000, Instrument Co., Corvallis, OR, USA), following the recommendations by Hsiao (1990). Measurements were made at midday, between 13:30 and 15:30 h, on shaded leaves enclosed in plastic bags laminated with aluminum foil for at least 1 hour (h).

2.4. Chemical analyses

Nutrient concentrations were determined in the leaves and roots according to the method described by Sadzawka *et al.* (2007), using a simultaneous multi-element atomic absorption spectrophotometer (Perkin-Elmer 3110, Wellesley, USA).

2.5. Lipid peroxidation

The level of lipid peroxidation (LP) was expressed as malondialdehyde (MDA) concentration (nmol MDA g⁻¹ FW) and determined as 2-thiobarbituric acid reactive-substances (TBARS), according to the protocol modified by Du and Bramlage, (1992). Thus, the absorbance was spectrophotometrically measured at 532, 600 and 440 nm in order to correct the interference caused by TBARS–sugar complexes.

2.6. Radical scavenging activity

Fresh leaves and root samples were snap-frozen in liquid nitrogen, powdered in a mortar, homogenized

in 1 mL of 80% (v/v) methanol, and centrifuged at 10,000 rpm for 5 min (at 4 °C). The supernatant was collected and stored at -80 °C until analysis. Radical scavenging activity (RSA) of roots and leaves was assayed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging method as described by Chinnici *et al.* (2004). Absorbance was measured at 515 nm using Trolox as standard.

2.7. Experimental design and statistical analyses

The experiment was arranged in a split-plot design with two cultivars x five B treatment in four replicates each. All data were tested by Kolmogorov-Smirnov test for homogeneity of variances. A two-way analysis of variance (ANOVA) and Tukey test were used to determine significance of differences between the means with a significance level at $P \leq 0.05$. All statistical analyses were performed with Sigma Stat 3.1 (SPSS® Inc., Chicago, IL, USA).

3.1. Plant biomass and water potential

After 30 days, both main effects (B treatment and cultivar) significantly influenced MRGR (mg DW day⁻¹), but the interaction was not significant (Table 1). Significantly decreased MRGR was found in Brigitta and Legacy, with a reduction up to 75% and 90% respectively, under high B (800 µM) compared to 50 and 200 µM B ($P \leq 0.05$; Table 2). In Brigitta, a significant decrease (75%) in MRGR for deficient B (0 µM) was found in comparison to 50 µM, maximal MRGR values, up to 8.8 in Brigitta and 10.8 mg DW day⁻¹ for Legacy were observed in plants grown at 50 µM B (Table 2; $P \leq 0.05$).

No significant interactions between B and cultivar were found for dry weight (DW) production of root and leaves. Compared to high B (800 µM), the other treatments showed increased leaf DW production in

Brigitta ($P \leq 0.05$; Table 2), eg. up to 20% and 24% at 0 and 50 μM B, respectively (Table 2). In Legacy, leaf DW was reduced by 56% at 800 μM compared to 50 μM B ($P \leq 0.05$). Cultivars differed significantly with respect to root DW (Table 1), whereby Brigitta for all treatments showed on average 85% greater root DW production than Legacy (Table 2). The highest root DW in Brigitta was noted in the 50 μM

B treatment (Table 2; $P \leq 0.05$). Regarding the root/shoot ratio, the interaction B x cultivar was non-significant and the cultivar was the main factor that influenced this parameter (Table 1). The lowest root/shoot ratio in Brigitta was observed at 0 μM B ($P \leq 0.05$), whereas in Legacy significant increases were registered at 400 and 800 μM B compared with the 50 μM B (Table 2).

Table 1. ANOVA of the mean relative growth rate (MRGR), root:shoot ratio, leaf water potential (Ψ_{leaf}), dry matter (DM), mineral status, and oxidative stress assessed by lipid peroxidation (LP) and radical scavenging activity (RSA).

	Source of variation					
	Boron (B)		Cultivar (c)		B x c	
Growth parameter						
MRGR (mg DW d^{-1})	3.6*		6.1*		0.9 ns	
Root/shoot ratio	3.1*		225**		0.9 ns	
Ψ_{leaf} (MPa)	10***		128***		36***	
	Leaf	Roots	Leaf	Roots	Leaf	Roots
DW (g plant^{-1})	7.4***	1.3 ns	135***	479***	1.6 ns	0.4 ns
Mineral concentration (mg kg^{-1})						
	Leaf	Roots	Leaf	Roots	Leaf	Roots
B	40***	7.2***	13***	58***	23***	3.8**
Al	0.9 ns	0.5 ns	5.2*	17***	0.6 ns	0.9 ns
Ca	3.9*	4.2**	46***	40***	5.2**	1.5 ns
Mg	1.3 ns	4.5**	6.5**	194***	1.6 ns	5.9***
K	25***	1.2 ns	7.8**	4.7**	51***	3.0*
Na	13***	1.2 ns	33***	39***	8.8***	4.9**
Antioxidant parameter						
Lipid peroxidation ($\text{nmol MDA g}^{-1} \text{FW}$)	0.41 ns	7.65***	0.56 ns	61.98***	6.18***	8.29***
RSA ($\text{TE g}^{-1} \text{FW}$)	0.51 ns	0.74 ns	7.06**	32.52***	4.29**	1.03 ns

Significance designated as ns: not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 2 Mean relative growth rate (MRGR), leaf and root dry weight and leaf water potential (Ψ_{leaf}) in two high-bush blueberry cultivars subjected to different B treatments in substrate after 30 days. Values are means of four replicates $\pm$ S.E. Different uppercase letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) among B rates for the same cultivar.

Parameter	Boron supply (μM)									
	0		50		200		400		800	
	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy
MRGR (mg DW d^{-1})	2.1 $\pm$ 0.30bB	9.4 $\pm$ 1.8aA	8.8 $\pm$ 1.7aA	10.8 $\pm$ 2.1aA	1.5 $\pm$ 0.4bcB	9.2 $\pm$ 2.6aA	1.2 $\pm$ 0.5cB	2.9 $\pm$ 0.2bA	1.1 $\pm$ 0.2cA	0.9 $\pm$ 0.1cA
Leaf DW (g plant^{-1})	8.6 $\pm$ 0.44aA	4.2 $\pm$ 0.1bB	9.0 $\pm$ 0.6aA	6.5 $\pm$ 0.1aB	8.2 $\pm$ 0.8bA	3.8 $\pm$ 0.5bB	8.3 $\pm$ 0.9bA	4.0 $\pm$ 0.2bB	6.8 $\pm$ 0.3cA	2.8 $\pm$ 0.1cB
Root DW (g plant^{-1})	6.0 $\pm$ 0.3bA	0.7 $\pm$ 0.0bB	7.2 $\pm$ 0.5aA	1.2 $\pm$ 0.1aB	6.1 $\pm$ 0.4bA	0.8 $\pm$ 0.1bB	6.6 $\pm$ 0.9bA	0.9 $\pm$ 0.1aB	6.1 $\pm$ 0.3bA	1.0 $\pm$ 0.1aB
Root/shoot	0.7 $\pm$ 0.0bA	0.1 $\pm$ 0.0bB	0.8 $\pm$ 0.0aA	0.1 $\pm$ 0.0bB	0.7 $\pm$ 0.1aA	0.1 $\pm$ 0.0bB	0.8 $\pm$ 0.0aA	0.2 $\pm$ 0.0aB	0.9 $\pm$ 0.0aA	0.4 $\pm$ 0.0aB

The water potential (Ψ_{leaf}) was influenced by the boron x cultivar interaction ($P \leq 0.001$) (Table 1). Cultivars showed similar Ψ_{leaf} at 0 μM B but differed in treatments containing 50 μM B or higher (Figure 1). Boron application improved Ψ_{leaf} in Brigitta from -0.629 to -0.283 MPa at 800 μM ($P \leq 0.001$) and Legacy showed decreased Ψ_{leaf} at 800 μM B compared to 0 μM B (-0.575 and -0.683 MPa, respectively) ($P=0.03$).

Figure 1. Changes in leaf water potential (Ψ_{leaf}) in two highbush blueberry cultivars subjected to different B treatments in substrate after 30 days. Values represent the average of four replicates $\pm$ SE. Different uppercase letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters indicate significant differences ($P \leq 0.05$) among treatments for the same cultivar.

3.2. Mineral concentration in leaves and roots

The main factors B and cultivar as well as their interaction significantly influenced B concentration (mg kg^{-1} DW) in leaves and roots of blueberry plants (Table 1). Non-significant differences were observed in leaves of both cultivars under treatments 0 and 50 μM B; however, at 200 μM Legacy accumulated 98% more B than Brigitta ($P \leq 0.05$). With higher B concentration 400 and 800 μM , was Brigitta the cultivar that showed the greatest B in leaves (Table 3) with maximal value of 240 mg B kg^{-1} DW (ranges from 196 to 296 mg B kg^{-1} DW) and 114 mg B kg^{-1} DW (ranges from 73 to 138 mg B kg^{-1} DW) respectively ($P \leq 0.05$; Table 3). In roots, B x cultivar interaction was significant for Brigitta where B in roots were gradually increased under treatments 200 μM (44%) and 400 μM (60%), in comparison to B 50 μM ($P \leq 0.05$), whereas in Legacy no changes in B accumulation were observed (Table 3).

Table 3. Mineral concentrations in leaves and roots of highbush blueberry cultivars subjected to different B treatments in substrate after 30 days. Values are means of four analytical replicates $\pm$ S.E. Different uppercase letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters indicate significant differences ($P \leq 0.05$) among treatments for the same cultivar.

Mineral status	Boron supply (μM)									
	0		50		200		400		800	
	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy	Brigitta	Legacy
Leaf										
B (mg kg^{-1})	47 $\pm$ 1.0cA	55 $\pm$ 3.8cA	48 $\pm$ 3.4cA	49 $\pm$ 2.7cA	52 $\pm$ 2.7cB	103 $\pm$ 21.4aA	240 $\pm$ 20.9aB	96 $\pm$ 5.5aA	114 $\pm$ 14.3bB	70 $\pm$ 1.7bA
Al (mg kg^{-1})	204 $\pm$ 13aA	176 $\pm$ 5.2aA	211 $\pm$ 10aA	191 $\pm$ 18aA	189 $\pm$ 14aA	153 $\pm$ 6.7aA	175 $\pm$ 25aA	180 $\pm$ 24aA	209 $\pm$ 13aA	165 $\pm$ 23aA
Ca (g kg^{-1})	7.30 $\pm$ 0.14aA	7.16 $\pm$ 0.21bA	7.42 $\pm$ 0.22aA	8.23 $\pm$ 0.31abA	7.23 $\pm$ 0.23aB	9.46 $\pm$ 0.52aA	7.06 $\pm$ 0.28aB	9.12 $\pm$ 0.56aA	6.49 $\pm$ 0.21aB	8.44 $\pm$ 0.34abA
Mg (g kg^{-1})	1.77 $\pm$ 0.03aB	2.02 $\pm$ 0.04aA	1.87 $\pm$ 0.03aA	1.74 $\pm$ 0.07aA	1.95 $\pm$ 0.08aA	2.05 $\pm$ 0.18aA	1.83 $\pm$ 0.06aB	2.16 $\pm$ 0.14aA	1.83 $\pm$ 0.06aB	2.13 $\pm$ 0.17aA
K (g kg^{-1})	13.30 $\pm$ 0.45bA	12.26 $\pm$ 0.79cA	12.94 $\pm$ 0.68bB	16.64 $\pm$ 0.67bA	12.72 $\pm$ 0.44bB	25.61 $\pm$ 0.60aA	16.66 $\pm$ 1.30aA	9.96 $\pm$ 0.36cB	14.41 $\pm$ 0.94bA	12.10 $\pm$ 0.79cB
Na (g kg^{-1})	0.58 $\pm$ 0.03aB	0.90 $\pm$ 0.10bA	0.55 $\pm$ 0.02aA	0.67 $\pm$ 0.05cA	0.57 $\pm$ 0.01aB	1.16 $\pm$ 0.03aA	0.52 $\pm$ 0.04aA	572 $\pm$ 0.09cA	5.03 $\pm$ 0.04aA	5.28 $\pm$ 0.06cA
Roots										
B (mg kg^{-1})	21 $\pm$ 0.8bA	14 $\pm$ 0.5aB	25 $\pm$ 1.9bA	15 $\pm$ 1.9aB	36 $\pm$ 7.2aA	16 $\pm$ 0.9aB	40 $\pm$ 1.8aA	18 $\pm$ 0.2aB	22 $\pm$ 2.4bA	16 $\pm$ 1.3aA
Al (mg kg^{-1})	3163 $\pm$ 340aA	1675 $\pm$ 156aB	2618 $\pm$ 492aA	1635 $\pm$ 274aA	2765 $\pm$ 532aA	1831 $\pm$ 209aA	2462 $\pm$ 266bA	1400 $\pm$ 336aB	2176 $\pm$ 377bA	2011 $\pm$ 387aA
Ca (g kg^{-1})	5.12 $\pm$ 0.44bA	4.24 $\pm$ 0.13bA	6.77 $\pm$ 0.26abA	4.39 $\pm$ 0.55bB	8.17 $\pm$ 0.33aA	5.11 $\pm$ 0.70aB	6.56 $\pm$ 0.14abA	4.64 $\pm$ 0.47bB	6.18 $\pm$ 0.76bA	4.82 $\pm$ 0.47bA
Mg (g kg^{-1})	1.73 $\pm$ 0.16bA	1.30 $\pm$ 0.11aB	2.37 $\pm$ 0.04aA	1.31 $\pm$ 0.09aB	2.61 $\pm$ 0.15aA	1.29 $\pm$ 0.08aB	2.36 $\pm$ 0.02aA	1.18 $\pm$ 0.06aB	2.09 $\pm$ 0.16bA	1.40 $\pm$ 0.06aB
K (g kg^{-1})	2.45 $\pm$ 0.08aA	2.40 $\pm$ 0.40abA	2.50 $\pm$ 0.11aA	2.30 $\pm$ 0.22abA	2.83 $\pm$ 0.16aA	2.00 $\pm$ 0.13bB	2.96 $\pm$ 0.12aA	2349 $\pm$ 132abB	2545 $\pm$ 142aA	2916 $\pm$ 201aA
Na (g kg^{-1})	0.50 $\pm$ 0.02bA	0.36 $\pm$ 0.04aB	0.41 $\pm$ 0.01bA	0.44 $\pm$ 0.01aA	0.57 $\pm$ 0.04aA	0.36 $\pm$ 0.02aB	0.634 $\pm$ 0.03aA	359 $\pm$ 20aB	496 $\pm$ 35bA	380 $\pm$ 68aB

There were significant B $\times$ cultivar interactions for Ca, K and Na concentrations in leaves, whereas in roots significant interactive effects of B $\times$ cultivar influenced Mg, Na and K accumulation (Table 1). Boron treatments did not affect Al in leaves of cultivars, whereas in roots of Brigitta the accumulation of this cation was decreased at 400 μM B (Table 3). Contrary, Ca concentration in leaves was significantly increased in Legacy from 200 μM B in comparison to 0 μM B treatment (Table 3).

3.3. Lipid peroxidation and radical scavenging activity

After 30 days, lipid peroxidation in leaves and roots showed a significant B $\times$ cultivar interaction (Table 1). Leaves of Brigitta showed a maximal decrease in

lipid peroxidation at 200 μM B in comparison to the 0 μM B (64%) and 800 μM (55%) treatments in lipid peroxidation at 200 μM B in comparison to the 0 μM B (64%) and 800 μM (55%) treatments ($P \leq 0.05$; Figure 2), whereas Legacy showed a significant increased lipid peroxidation in leaves from 200 to 800 μM B. Legacy exhibited an increased lipid peroxidation in leaves (45%) treated with 400 and 800 μM B, in comparison to the treatments 0 and 50 μM B ($P \leq 0.05$). In roots of Brigitta a higher lipid peroxidation than in Legacy was found in all treatments ($P \leq 0.05$), except in roots at 50 μM B (Figure 2). In Brigitta lipid peroxidation was reduced up to 70% in roots subjected to 50 μM B compared to the other treatments ($P \leq 0.05$). However, roots of Legacy were not affected by B treatment (Figure 2).

Figure 2. Lipid peroxidation (LP) measured as malondialdehyde content in leaves and roots of two highbush blueberry cultivars subjected to different B treatments in substrate after 30 days. Values represent the mean of four replicates $\pm$ S.E. Different uppercase letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters indicate significant differences ($P \leq 0.05$) among treatments for the same cultivar.

There was the significant interaction influencing radical scavenging activity (RSA) in leaves (Table 1). The RSA in leaves increased by 8-11% from 50 to 400 μM B in Brigitta (Figure 3; $P \leq 0.05$). The B treatment at 200 and 400 μM significantly increased RSA in leaves of Legacy compared to the 0 μM B treatment. In roots, the

interaction B x cultivar was not significant ($P \leq 0.05$). Increased B additions improved RSA in Brigitta roots; moreover, Brigitta exhibited higher RSA in roots than Legacy in all treatments (Figure 3; $P \leq 0.05$).

Figure 3. Radical scavenging activity in leaves and roots of two highbush blueberry cultivars subjected to different B treatments in substrate after 30 days. Values represent the mean of four replicates $\pm$ S.E. Different uppercase letters indicate significant differences ($P \leq 0.05$) between cultivars for the same treatment. Different lowercase letters indicate significant differences ($P \leq 0.05$) among treatments for the same cultivar.

4. Discussion

In our studied highbush blueberry plants, the cultivars achieved best MRGR at 50 μM , whereas significant growth depression occurred at high B supply, resulting that excessive B application decreased plant growth more severely than low B. Similar findings were reported by Nasim *et al.*, (2015) for barley (*Hordeum vulgare* L.) cultivar Clipper, with excess B supply (3 mg kg^{-1} soil) resulting in a significant decrease in plant dry matter. In the present study, cultivar Brigitta had higher leaf and root dry weight than Legacy at high B rates, such B tolerance has been reported for vegetative tissues in fruit crops such as (*Malus domestica* Borkh) (Molassiotis *et al.*, 2006). Tolerance to B excess is associated with enhanced expression and activity of specific transmembrane B exporters (BORs) and nodulin-26-like intrinsic proteins (NIPs) capable of excluding B at the root-cell plasma membranes (Miwa and Fuyiwara, 2010; Landi *et al.*, 2012).

Decreased DM production at high B was more accentuated in shoots than roots, particularly in Legacy, where the root/shoot ratio was higher at 400 and 800 μM B than 0 and 50 μM B. These findings can be explained by strong permeability of the plasma membrane lipid bilayers to H_3BO_3 , followed by transport upward via xylem to the leaf tissues where B may accumulate and cause visual B-toxicity symptoms (Herrera-Rodríguez *et al.*, 2010; Miwa and Fujiwara, 2010; Landi *et al.*, 2012).

According to Wimmer and Eichert (2013), B deficiency affects water relations by altering root uptake, water transport through xylem and impairment transpiration in leaves. In our study both cultivars showed more negative water potential under 0 μM compared with treatment 50 μM B, but there was a tendency of less negative Ψ_{leaf} in Brigitta with an increase in B rates ($r=-0.51$), whereas in Legacy plants B excess (i.e. 800 μM) was associated with a more negative Ψ_{leaf}

($r=0.60$) in comparison to B at 0 μM . Similar results were reported by Gimeno *et al.* (2012) in lemon tree (*Citrus limon* Burm. F. cv. Verna) grown under B at 0.25, 2, 4.5 and 7 mg L^{-1} . Authors found that in plants subjected to high B (i.e 4.5 and 7 mg L^{-1}) the water potential in leaf was decreased to more negatives values. These findings are in agreement with our experiment, which suggests that B may cause a reduction of osmotic potential regulated by compounds such as soluble sugars. However, two cultivars showed different water relation performance which would be related to a decrease in root transmembrane aquaporins in cultivar Legacy, due to physiological, biochemical and anatomical differences, aspects should be considered in the future research.

In our study, plants had foliar B concentration reached over 70 mg B kg^{-1} DW in plants grown from 200 μM B (Table 3), while plants under B deficiency (0 μM), as well as those plants treated with 50 μM B showed foliar B ranging from 47 to 55 mg B kg^{-1} DW (Table 3). These concentrations were reported by Wójcik (2005) as normal ranges (from 15 to 55 mg kg^{-1} DW) for B fertilized highbush blueberry plants cv. Bluecrop, whereas Sanderson *et al.* (2008) found leaf B concentration of 32 mg kg^{-1} for lowbush blueberries (*Vaccinium angustifolium* Ait.). Several studies have reported molecular and physiological mechanisms of B tolerance in plants and highlighted the role of root aquaporins involved in B transport to shoots, regulated by different genes in different cereal species. In the present study, higher concentration of B in leaves than roots suggested strongly B transport out of roots via plasma membrane diffusion and protein transporters (Herrera-Rodríguez *et al.*, 2010). Similarly, Nasim *et al.*, (2015) attributed transported-mediated B exclusion by the tolerant cultivar of barley under excessive B (and that means against the concentration gradient in the soil solution surrounding roots).

In the study presented here, the interaction (B x cultivar) was significant regarding leaf concentration of Ca and K (Table 1). Boron and Ca metabolism and their localization are interrelated in several functions in plants, such as being a constituent of the cell wall and acting synergistically in the transport and accumulation of sugars in fruits (Herrera-Rodríguez *et al.*, 2010). Leaf concentration of Ca increased in Legacy, but was not affected in Brigitta, similarly to Simón *et al.* (2013), who reported unaltered Ca concentration in leaves of *Jatropha curcas* L. seedlings under increasing B treatments. Singh *et al.* (2012) and Simón *et al.* (2013) reported similar findings in relation to an increased foliar K by B application in carrot (*Daucus carota*) and *Jatropha curcas* respectively.

In our study, the high concentration of B in the substrate was related to a decreased growth and DW yield in plants. The literature reported that B toxicity was associated with oxidative stress by its accumulation in leaf cell walls causing imbalances in cytoplasmic metabolism (Molassiotis *et al.*, 2006; Herrera-Rodríguez *et al.*, 2010; Landi *et al.*, 2012). According to Herrera-Rodríguez *et al.* (2010) excessive B in tissues decreased plant capacity to avoid the photooxidative damage arising from the accumulation of ROS that trigger lipid peroxidation. Our study demonstrated that lipid peroxidation was increased at both low and high B in leaf and root tissues of Brigitta, whereas leaves of Legacy were more sensitive to high B ($r=0.54$) than lower B rates, as also reported by Kayaa and Ashraf (2015) in B stressed tomato (*Lycopersicon esculentum* Mill.) plants. However, Tewari *et al.* (2010) reported that lipid peroxidation was not influenced significantly in leaves of mulberry (*Morus alba* L.) grown at three B rates.

Boron acts as a regulatory factor of the antioxidant protective system in plants (Dahajipour *et al.*, 2011; Landi *et al.*, 2012; Singh *et al.*, 2012). In the present study, medium and high B rates (50, 200 and 400 μM)

had a greater influence on RSA in leaves than low B, as also reported for marshmallow by Moalemi *et al.* (2012). However, there was no significant difference in RSA in leaves at 0 and 800 μM B (Figure 3), indicating that B deficiency, as well as B excess, can decrease enzymatic and non-enzymatic antioxidant capacity (Tewari *et al.*, 2010; Singh *et al.*, 2102).

5. Conclusions

In conclusion, the B addition at 50 μM and 200 μM increased highbush blueberry root and shoot biomass and growth rate by up to 75%, particularly in cultivar Brigitta, concomitantly with improved leaf water potential. Cultivar Brigitta had higher B accumulation in root and leaves than Legacy when exposed to B excess. Both B deficiency (0 μM) and B excess (400 and 800 μM) caused oxidative stress in leaves and roots to a greater extent in Brigitta than Legacy. Finally, the study provides evidence that an adequate B level in highbush blueberry plays an important role in plant growth and physiology; however, the responses were dependent on cultivar and B rate. Hence, the addition of B to remediate its deficiency should be managed carefully to avoid toxicity and consider the cultivar effects.

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