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Effects of Boric Acid Concentration and Shading on Growth, Leaf Physiology, and Anatomy of *Guzmania*

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***Abstract. Guzmania lingulata* (L.) Mez. 'Cherry' were grown in coco chips and fertigated with half-strength Hoagland solution containing various concentrations of boric acid. Excessive boron induced changes in growth, relative chlorophyll content, and leaf anatomy were investigated. Plants treated with 5 mg·L^{-1} or higher boric acid concentration had reduced SPAD-502 readings and Fv/Fm values and increased leaf necrosis in the lower leaves. Boron was distributed unevenly within a leaf, with the maximum concentration in the leaf tip. Increased necrotic length and new leaves with necrosis were evident where average whole leaf boron concentration was higher than 170 µg·g^{-1} on dry weight basis. More leaf growth and higher transpiration or stomatal conductance were recorded in plants under 40% (average 676 µmol·m^{-2}·s^{-1} *PPF* at noon) than 76% (average 270 µmol·m^{-2}·s^{-1} *PPF* at noon) shade. Excessive boron was not found to affect epidermal cells or water storage tissue, but caused browning and shriveling of the chlorenchyma cells.**

Boron (B) toxicity is a nutritional disorder that can decrease plant growth and cause leaf necrosis. High concentrations of B may occur naturally in the soil and in the groundwater, or B may be added to the growing medium through irrigation and fertilization (Gupta et al., 1985; Nabel et al., 1997). Amongst a wide range of plant species, the typical visible symptom of B toxicity is leaf burn (chlorotic and/or necrotic patches), often at the margins near the tip of older leaves (Nabel et al., 1997). Before visible toxicity symptoms are expressed in young squash plants (*Cucurbita pepo*), an early effect of developing B toxicity is decreased chlorophyll concentration, followed by reduced growth and decreased photosynthesis (Lovatt and Bates, 1984). Boron accumulation is known to be greatly influenced by transpiration rates (Raven, 1980) and shading to reduce transpiration rate decreased B absorption by kiwifruit plants (*Actinidia deliciosa* var. *deliciosa*) in areas where B toxicity is still an agricultural problem (Sotiropoulos et al., 2004).

Guzmanias have attractive leaves and long-lasting inflorescence, making them popular interior potted foliage plants. Commercial growers consider Guzmanias rather sensitive to excess B and thus use low-boron fertilizers and irrigation water (Griffith, 2002). However, no published reports have been found on critical B concentrations in liquid fertilization for B toxicity in Guzmanias. The objective of the present work was to determine the effects of boric acid concentration and shading on growth and leaf physiology of *Guzmania lingulata* (L.) Mez. 'Cherry'.

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Materials and Methods

Experiment 1. Nonflowering plants of *Guzmania* 'Cherry' with 32 to 33 fully expanded leaves were planted in plastic containers containing 0.9 L of 1 to 2 cm diameter coco-chip, commonly used for commercial production of bromeliad. The experiment was conducted from 26 Oct. 2004 to 15 Apr. 2005 and all plants were grown in a shaded greenhouse at 23 ± 5 °C, with an average noon photosynthetic photon flux (*PPF*) of 580 µmol·m^{-2}·s^{-1}. Plants were each fertigated weekly with 0.5 L of Hoagland's solution (Hoagland and Arnon, 1950) without additional water supply, with all macronutrients and micronutrients, except B, supplied at half strength. Treatments included five boric acid concentrations: 0, 1, 5, 10, and 15 mg·L^{-1}; (equivalent to 0, 0.18, 0.89, 1.77, and 2.66 mg·L^{-1} B, respectively). This experiment was arranged in a completely randomized design. Treatments were replicated six times, with a single plant per replication. During the experimental period, the medium EC and pH were measured using pour-through extracts (Wright, 1986). The medium EC ranged from 0.94 to 1.00 dS·m^{-1} as measured with a conductivity meter (SC-170, Suntex, Taipei, Taiwan), and pH was between 5.5 and 6.2 as measured with a microcomputer pH meter (6171; San Diego, Calif).

After six months, relative chlorophyll content (SPAD-502 reading) of the most recently fully expanded leaves (leaves five to eight from the apex) from all plants in each treatment was measured *in situ* with a chlorophyll meter (SPAD-502; Minolta Camera Co., Tokyo, Japan). Recently fully developed leaves from all six plants in each treatment were sampled to measure necrotic length and necrotic leaf area. The necrotic length was measured from the leaf tip to the border between the brown and green leaf tissues. The necrotic area of each leaf was cut and measured with a leaf area meter (LI-3000; LI-COR, Lincoln, Nebr.). These leaves were divided at every 5 cm length from leaf tip to obtain SPAD-502 readings and the maximal efficiency of photosystem II (PS II) photochemistry (Fv/Fm) values, respectively. Chlorophyll fluorescence was measured at 25 °C with a modulated light MINI-PAM portable fluorometer (Walz, Germany) after the leaves had been dark-adapted for 30 min.

The recently fully developed leaves from all six plants in each treatment were sampled and separated into top, middle and basal leaf blades. Two sections from the same portion of every two plants were combined. These samples were dried, ground to pass a 200-mesh screen, and analyzed for B concentration using the Azomethine-H method (Wolf, 1971). Regression analysis was used to describe the relationship between solution boric acid concentration and leaf B concentration, and between SPAD-502 reading and Fv/Fm values.

Experiment 2. In total, 48 uniform plants, with 14 to 15 expanded leaves, of nonflowering *Guzmania* 'Cherry' which planted in plastic containers containing 0.9 L of 1- to 2-cm-diameter coco chips were grown in a greenhouse from October 2004 to April 2005 at 20 ± 3 under natural day length (11.5 to 13.5 h). Plants received either an average of 676 or 270 µmol·m^{-2}·s^{-1} *PPF* at noon, which accounted for 40% and 76% shade, respectively. The plants were fertigated the same way as described in Expt. 1. The four boric acid treatments, 0, 1, 3, and 5 mg·L^{-1}, which represent 0, 0.18, 0.53, and 0.89 mg·L^{-1} B, respectively, were arranged in a completely randomized block design within each shade level, replicated six times. Shading to reduce *PPF* was achieved by erecting one layer of a polypropylene black shade netting of different light transmissions, on supporting frames suspended above and around the plants. The irradiance for each treatment was uniform and measured at the plant canopy by using a quantum sensor (type QS2; Delta-T Devices Ltd, Cambridge, U.K.).

At the beginning of the experiment, the uppermost visible leaf of each plant was tagged for determing the number of new leaves that unfolded during the experiment. Numbers of new leaves and new leaves with necrosis were recorded in each treatment after six months of growth. The most recently fully developed

Table 1. Effect of boric acid concentration on SPAD-502 reading, necrotic length, and necrotic leaf area of *Guzmania* 'Cherry' (Expt. 1).

H_3BO_3 (mg·L^{-1})	SPAD-502 reading	Necrotic length (cm)	Necrotic leaf area (cm^2)
0	56.9	0.4	37.3
1	54.3	2.5	72.0
5	44.8	10.3	402.4
10	25.0	15.9	599.8
15	18.6	23.5	793.1
Regression	L**	L**	L**

**Significant at $P < 0.01$; linear (L).

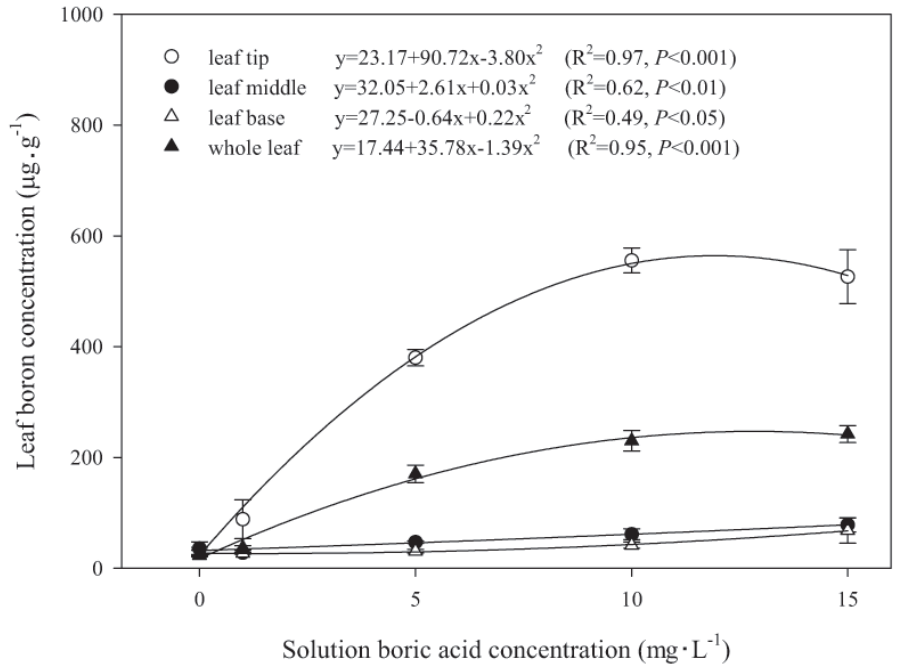

Fig. 1. Relationships between solution boric acid concentration and boron concentration in various leaf positions in *Guzmania* 'Cherry' (Expt. 1). Bars indicate standard error of the mean.

leaf (leaf 6 from the apex) from all six plants in each treatment was sampled to measure leaf width and necrotic length. Leaf transpiration rate and stomatal conductance were measured on the middle section of the acropetal sixth leaf with a steady-state porometer (LI-1600; LI-COR). The tip portion of the sixth 1eaf was taken from plants treated with 0, 1, or 5 mg·L^{-1} boric acid concentration under 40% or 76% shade for anatomical observation. All shoots were then dried, ground, and analyzed for B concentration. All data were subjected to analysis of variance using the general linear models procedure. The difference between the shading levels was compared by *t* test.

Results

In Expt. 1, the SPAD-502 reading decreased but necrotic length and necrotic leaf area increased as the concentration of boric acid increased from 0 to 15 mg·L^{-1} (Table 1). Whole-leaf B concentration increased quadratically with increasing boric acid concentration (Fig. 1). Marked boron toxicity symptom expressed as large necrotic areas was observed in plants at 5 mg·L^{-1} and higher boric acid concentrations, containing over 170 μg·g^{-1} B in the whole leaf (Table 1, Fig. 1). Leaf tip contained 520 to 550 μg·g^{-1} B while the middle and basal leaf blade contained 40 to 80 μg·g^{-1} B in plants treated with 10 or 15 mg·L^{-1} boric acid (Fig. 1). Boron accumulation in the leaf tip was evident.

In plants treated with 0 or 1 mg·L^{-1} boric acid, all leaf segments showed Fv/Fm values at around 0.83 and SPAD-502 reading maintained between 50 and 60 (Fig. 2). In plants treated with 5 mg·L^{-1} or higher boric acid concentrations, the Fv/Fm value and SPAD-502 reading decreased toward the leaf tip, while increasing boric acid level caused more leaf segments with declined Fv/Fm value and SPAD-502 reading. A linearly relationship existed between Fv/Fm value and SPAD-502 reading measured on different leaf segments in each treatment (Fig. 3).

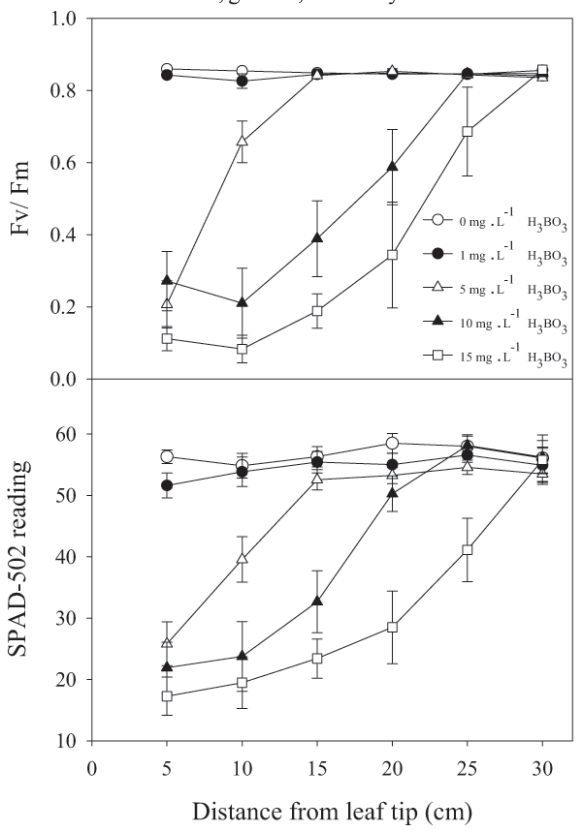

Fig. 2. Effect of nutrient solution boric acid concentration on Fv/Fm and SPAD-502 reading of various portions of *Guzmania* 'Cherry' leaves (Expt. 1). Bars indicate standard error of the mean.

In Expt. 2, both leaf transpiration rate and stomatal conductance were higher in plants under 40% than 76% shade (Table 2). Both transpiration rate and stomatal conductance increased as the concentration of boric acid increased from 0 to 5 mg·L^{-1}.

Plants grown under 40% shade produced more leaf number, leaf dry weight and wider leaves than plants under 76% shade (Table 3). Necrotic length and new leaves with necrosis increased with increasing boric acid concentration. There were interactions between shading and boric acid concentration on new leaf number, new leaves with necrosis and shoot boron concentration.

Leaf anatomy reveals that plants grown under 40% shade produced thicker leaves than those under 76% shade (250 to 270 μm vs. 210 to 230 μm). Thickness of adaxial epidermis (10 to 20 μm), water storage tissue (63 to 73 μm), and abaxial epidermis (10 to 20 μm) did not differ significantly between the boric acid concentration treatments. There was no interaction between shading and boric acid concentration on leaf thickness. Observations on the anatomy of leaves (Fig. 4) indicated that, regardless of shade level, chlorenchyma browning was seen in the leaves at 5 mg·L^{-1} boric acid treatment as compared with the green chlorenchyma in the 0 or 1 mg·L^{-1} boric acid-treated leaves.

Discussion

Excessive B reduced chlorophyll concentration, and increased necrosis in lower leaves of *Guzmania* 'Cherry' (Table 1). Similar B toxicity symptoms have been reported for kiwifruit, bean, tomato, and chrysanthemum (Gupta, 1983; Smith and Clark, 1989). For *Guzmania* 'Cherry', B was distributed unevenly within leaf portions, with maximum concentration in leaf tip (Fig. 1), presumably at and near the end of the transpiration stream. This is consistent with previous results that B accumulated on leaf tip and margins (Nable et al., 1997; Sotiropoulos et al., 2002).

Critical values for B toxicity have been established in many crop and tree species such as 125 μg·g^{-1} B for *Phaseolus vulgaris* L., 172 μg·g^{-1} B for tomato, 98 μg·g^{-1} B for *Zea mays* L., 176 μg·g^{-1} B for *Phleum pretense* L., and 100 μg·g^{-1} B for *Actinidia deliciosa* var. *deliciosa* (Gupta, 1993). However, one particular concern with the use of foliar analysis for diagnosing B toxicity is that there is a wide range of values for B toxicity, even for the same species (Nable et al., 1997). Therefore, the present study did not attempt to establish the critical value for B toxicity for *Guzmania.* Based on the present results, leaf necrosis was evident where average whole leaf boron concentration was higher than 170 μg·g^{-1} DW (Fig. 1). In the future, boric acid concentrations below 5 mg·L^{-1} should be studied to further refine the critical value for B toxicity.

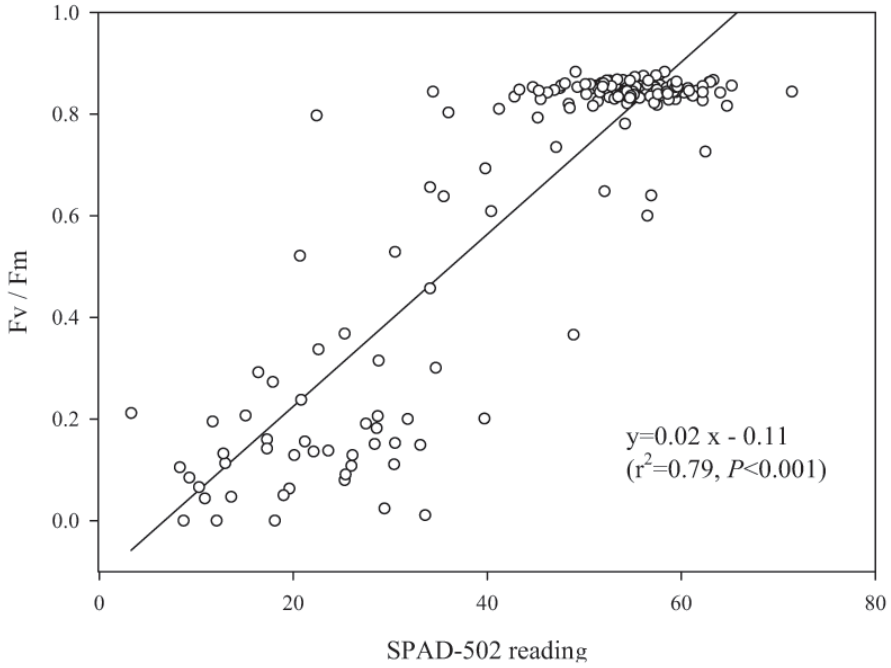

Fig. 3. Relationship between Fv/Fm and SPAD-502 reading of *Guzmania* 'Cherry' leaves at several boric acid concentrations (Expt. 1).

Table 2. Effects of shading and boric acid concentration on transpiration rates and stomatal conductance of *Guzmania* 'Cherry' (Expt. 2).

Treatment	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)
Shading (%)		
40	5.9	230.5
76	2.9	135.8
Significance (*t* test)	***	***
Boric acid concentration ($mg \cdot L^{-1}$)		
0	3.5	162.2
1	3.4	139.3
3	4.5	186.5
5	6.1	244.7
Significance	$L^{*}Q^{*}$	$L^{*}Q^{**}$
Significant interactions ($P < 0.05$)	NS	NS

NS, *,**,*** Nonsignificant or Significant at $P < 0.05$, 0.01, or 0.001, respectively; linear = L, quadratic = Q.

Table 3. Effects of shading and boric acid concentration on leaf growth and shoot boron concentration of *Guzmania* 'Cherry' (Expt. 2).

Treatment	New leaf no.	Leaf dry wt (g)	Leaf width (cm)	Necrotic length (cm)	New leaves with necrosis (no.)	Shoot B concn ($\mu g \cdot g^{-1}$)
Shading (S)(%)						
40	11.0	0.27	3.2	1.0	1.6	71.7
76	8.5	0.20	2.5	1.3	1.6	96.6
Significance (*t* test)	***	***	***	NS	NS	NS
Boric acid concentration (B) ($mg \cdot L^{-1}$)						
0	10.4	0.21	2.7	0.0	0.0	46.3
1	9.4	0.26	2.8	0.0	0.0	65.3
3	9.6	0.22	2.9	1.2	2.1	82.2
5	9.6	0.25	2.8	3.6	4.3	101.5
Significance	NS	NS	NS	$L^{***}Q^{***}$	$L^{***}Q^{***}$	$L^{***}Q^{***}$
Significant interaction ($P < 0.05$)	S × B	NS	NS	NS	S × B	S × B

NS,***Nonsignificant or significant at $P < 0.001$, respectively; linear = L, quadratic = Q.

Excessive B affected relative chlorophyll concentration and PS II in *Guzmania* 'Cherry', as shown by reduced SPAD-502 readings and Fv/Fm values (Fig. 2). The SPAD readings were often used to represent greenness and relative chlorophyll content of leaves. The Fv/Fm represents the maximum efficiency of PS II in transforming light energy to chemical energy. In our current study, leaf turned brown and SPAD readings decreased in plants treated with high B concentrations (Figs. 2 and 4). The decrease in Fv/Fm values accompanied by lower SPAD readings might be due to the degradation of chlorophyll and the failure to maintain efficient of PS II system. Under the conditions constructed in Experiment 1 (Table 1, Fig. 2), 5 $mg \cdot L^{-1}$ boric acid resulted in SPAD-502 reading around 45. This appears to be the start of PS II impairment, where the Fv/Fm values were lower than 0.80 ± 0.05, corresponding to highly efficient use of the excitation energy in photochemical processes (Bjorkman and Demmig, 1987).

The transpiration rate seems to be the most important factor governing B absorption (Hu and Brown, 1997). Partial shading decreased transpiration and shoot B concentration in kiwifruit, thereby reducing B toxicity (Sotiropoulos et al., 2004). In the present study, Guzmanias under 40% shading had higher transpiration and stomatal conductance than plants under 76% shade. Transpiration and stomatal conductance increased with increasing boric acid concentration (Table 2). However, highest shoot B concentration and necrotic leaf number were found in plants under 76% shade at 5 $mg \cdot L^{-1}$ boric acid (data not shown). Since plants grown under 76% shade had reduced new leaf number, less leaf dry weight, and narrower leaf width (Table 3), the B in leaf tip or shoot was probably not diluted by growth as much as in plants under 40% shade in the 5 $mg \cdot L^{-1}$ boric acid treatment. Most commercial bromeliads have been recommended to be grown under 50% to 70% of natural irradiance (Griffith, 2002), while the present results suggest that Guzmanias should be produced under a brighter condition to promote growth.

Adaxial epidermis and abaxial epidermis were not found any effects by B toxicity (Fig. 4). Similar results were obtained for peach (Kamali and Childers, 1966) and kiwifruit (Sotiropoulos et al., 2004). Bromeliads have a unique water storage tissue to overcome drought stress. For example, in *Tillandsia ionantha*, the cross-sectional area of the water storage tissue decreased rapidly and was completely exhausted after 50 d of drought treatment, while the cross-sectional area of the chlorenchyma had declined by only 12% (Nowak and Martin, 1997). Excessive B did not affect the water storage tissue in *Guzmania* leaves (Fig. 4), suggesting that leaf necrosis was probably not associated with drought stress. The effects of B toxicity on leaf structure are similar in different plant species and limited to the mesophyll cells (Papadakis et al., 2004). while B toxicity in *Guzmania* appeared to be limited to chlorenchyma cells (Fig. 4). The browning and shrunken chlorochyma cells could result in decreases of SPAD-502 reading in *Guzmania* treated with excessive B. In conclusion, boron toxicity in *Guzmania* may affect growth and photosynthesis by decreasing chlorophyll content and PSII efficiency and increasing necrotic leaf area.

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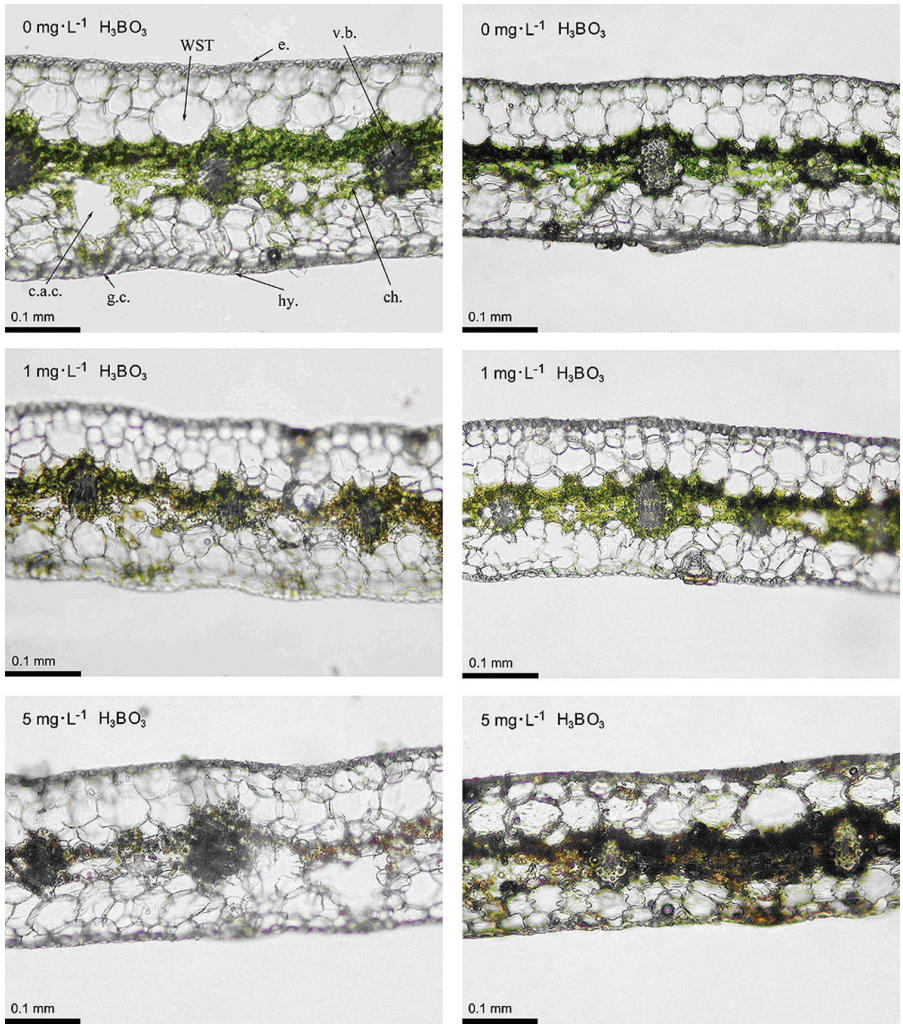

Fig. 4. Effects of shading and boric acid concentration on the anatomy of *Guzmania* 'Cherry' leaves (Expt. 2). Abbreviations: c.a.c = central aerating canal; ch. = chlorenchyma; ad. e. = adaxial epidermis; g.c. = guard cell; ab. e. = abaxial epidermis; v.b. = vascular bundle; WST = water storage tissue.

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