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Effects of Boron Deficiency on Geranium Grown under Different Nonphotoinhibitory Light Levels

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Abstract. **Apart from a role in cell wall structure, specific functions for boron (B) in plants are unclear; hence, responses and adaptations to B stress are incompletely understood. We tested hypotheses that net photosynthesis (P_n) decreases with B deficiency before visible foliar symptoms and that higher nonphotoinhibitory light levels enhance soluble carbohydrate status and therefore mitigate B deficiency. Geranium (*Pelargonium* ×*hortorum* L.H. Bailey cv. Nittany Lion Red) plants were grown hydroponically and were then exposed to normal (45 μM) or deficient (0 μM) B at two light levels [100 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (*PAR*)]. Photosynthesis [net CO_2 uptake, carboxylation, and photosystem II (PSII) efficiency] was monitored for 5 days, as were concentrations of B, chlorophyll, soluble sugars, total protein, and several photosynthetic and stress proteins [ribulose 1,5-bisphospate carboxylase oxygenase (rubisco), rubisco activase, oxygen-evolving complex-23 (OEC23), Cu/Zn-superoxide dismutase (SOD), Mn-SOD, and eukaryotic translation initiation factor 5A-2 (eIF5A-2)]. Biomass and sugar concentration were greater in high light, and mass was decreased by B deficiency only in leaves in high light. Boron deficiency decreased [B] in all tissues, especially in new leaves. Carboxylation efficiency and P_n decreased within 1 day of B deficiency in low light, but not until 5 days in high light. Chlorophyll concentration decreased, and Mn-SOD increased transiently, with B deficiency in both light levels, but no other effects of low B were observed. Protection of P_n by higher light was confirmed in a different cultivar (Maverick White) grown at 100, 300, and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*. Thus, in geranium, photosynthesis is affected by B deficiency before effects on leaf growth, and higher light can at least temporarily ameliorate B deficiency, perhaps partly due to enhanced carbohydrate status.**

Boron (B) has long been recognized as an essential micronutrient for higher plants (Warington, 1923), and B stress (deficiency or toxicity) in crops is common and economically important (Shorrocks, 1997). Despite the importance of B in plant nutrition, the specific functions for B in plants (Blevins and Lukaszewski, 1998; Bolaños et al., 2004; Dordas and Brown, 2000), and the specific cellular mechanisms of, and adaptations to, B stress are not well understood (Dordas and Brown, 2005; Kaur et al., 2006; Reid et al., 2004; Roessner et al., 2006; Wimmer et al., 2005). To date, the most widely accepted role of B in plants is that of a structural function in plant cell walls (Brown et al., 2002; Goldbach, 1997; Kobayashi et al., 1996; Matoh, 1997; Power and Woods, 1997). This structural role of B in cell walls is due to its capacity to form diester bridges between adjacent *cis*-hydroxyl–containing molecules, such as mono-, oligo-, and polysaccharides, and diols and hydroxyacids (Power and Woods, 1997). Boron also is involved in plant reproduction, which may or may not be related solely to the structural role of B in cell walls (Blevins and Lukaszewski, 1998; Marschner, 1995).

Boron deficiency affects vegetative and reproductive stages of plant growth. In the vegetative stage, B deficiency leads to the inhibition of growth, the death of growing meristems, and the inhibition of the development of vascular bundles (Goldbach, 1997). During reproduction, B deficiency causes inhibition of, or defects in, flower, seed, and fruit development (Asad et al., 2002; Dell and Huang, 1997; Rerkasem and Jamjod, 1997; Ross et al., 2006). At the cellular level, B deficiency has been implicated in a wide range of processes ranging from cell division and elongation, metabolism of nucleic acids, protein synthesis, metabolism and transport of carbohydrates, synthesis and transport of plant hormones, regulation of plasma membrane-bound ATPase and oxido-reductase activities, to synthesis and metabolism of phenolics (Blevins and Lukaszewski, 1998; Goldbach, 1997; Kouchi, 1977). However, the earliest responses to B stress are debated, largely because of the absence of conclusive evidence regarding the specific roles of B in plant function, apart from the structural function in walls (Bolaños et al., 2004).

For example, it is currently debated as to whether effects of B deficiency (or toxicity) on photosynthesis are primary or secondary, or early or late, effects in leaves (Bolaños et al., 2004; Reid et al., 2004). Comparatively early (defined herein as

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the time between initiation of B stress and initial visible symptoms of B stress in the leaf tissue, unless otherwise defined) decreases in the rate of photosynthetic oxygen evolution and increased sugar levels were observed in B-deficient sunflower (*Helianthus annuus* L.) leaves (El-Shintinawy, 1999; Kastori et al., 1995). In B-deficient cotton (*Gossypium hirsutum* L.) plants, Zhao and Oosterhuis (2003) observed a decrease in net photosynthesis of leaves after 2 weeks, before all other plant responses measured (growth, leaf area, chlorophyll concentration, and membrane leakage). In a proteomic analysis of early protein responses (within 60 h before visible symptoms) to B deficiency and toxicity in *Arabidopsis thaliana* (L.) Heynh. leaves, 13 early B-responsive proteins were identified, including eight chloroplast and four photosynthetic proteins [rubisco activase, 23-kD oxygen-evolving-complex protein of photosystem II (PSII; OEC23), ATPase δ-subunit, and glycolate oxidase] (Chen, 2006). Of these B-responsive proteins, rubisco activase was the earliest detected (within 24 h). Rubisco activase, as the name implies, activates rubisco, and thus is a major determinant of rubisco activation state (Eckardt and Portis, 1997). These B-responsive photosynthetic proteins decreased with B deficiency and toxicity; yet, B stress decreased, or had no effect on, three of four oxidative stress proteins examined, and did not affect total protein concentration. These results indicated early, potentially specific effects of B stress on photosynthetic metabolism, along with specific effects on protein synthesis that were not the result of general damage to cell proteins.

As with cellular responses to B stress, acclimations to B stress are incompletely understood (Brown et al., 1999; Kaur et al., 2006; Wimmer et al., 2005), although with B deficiency, alterations in carbohydrate metabolism may be a key adaptation (Hu et al., 1997). For example, in sunflower, carbohydrate accumulation has been observed as a long-term B-deficiency response (Kastori et al., 1995). Also, transgenically enhanced sorbitol synthesis increases tolerance to B deficiency by increasing B phloem mobility (Brown et al., 1999). Consistent with a link between carbohydrate status and B stress tolerance, our preliminary results indicate that growth of barley (*Hordeum vulgare* L.) plants under elevated CO_2 increases tolerance to low-B conditions (S. Mishra, S. Heckathorn, and J. Frantz, unpublished results).

To investigate the early effects of B deficiency on photosynthesis and how carbohydrate status might affect plant responses to B deficiency, we grew 'Nittany Lion Red' geranium plants under normal B levels and at two levels of light (5.76 and 17.28 $mol \cdot m^{-2} \cdot d^{-1}$), and then transferred some plants to B-deficient conditions (0 μM B). To determine if effects on photosynthesis are an early response to B deficiency, as predicted from the proteomic analysis described above, we monitored various aspects of photosynthesis on a daily basis following B withdrawal, including overall photosynthetic rate, aspects of light and dark reactions, and the B-responsive photosynthetic proteins identified previously. The goal of this research was to determine if net photosynthesis decreases with B deficiency before visible foliar symptoms, and if higher nonphotoinhibitory light levels enhance soluble carbohydrate status and therefore mitigate B deficiency.

Materials and Methods

Plant material and B treatments. Geranium plants were grown hydroponically from cuttings until they were rooted in 4.5-L opaque tubs, in complete nutrient solution. The control nutrient solution provided millimolar concentrations of macronutrients of 7.15 N (all nitrate), 0.67 P (as PO_4), 1.67 K, 2.3 Ca, 1.3 Mg, and 1.3 S (as SO_4), plus micromolar concentrations of 71 Fe, 9 Mn, 1.5 Cu, 1.5 Zn, 45 B, 0.1 Mo, 24 Cl, and 0.2 Na using KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, K-EDTA, Fe-DTPA, $MnCl_2$, $CuCl_2$, $ZnCl_2$, H_3BO_3, and Na_2MoO_4. The nutrient solution was changed weekly and was maintained at pH 5.6 with the addition of 1 N HCl or KOH. Plants were grown in a controlled environment chamber at 23 °C day/19 °C night with 70% humidity under a 16-h photoperiod and at two different light conditions (low or high light = 100 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*, respectively, which provided 5.76 and 17.28 $mol \cdot m^{-2} \cdot d^{-1}$ of integrated *PAR*). Geranium is reported to grow well at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* (Fisher and Both, 2004), therefore we used this level and a lower level of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*. These two light levels are above the photosynthetic light-compensation

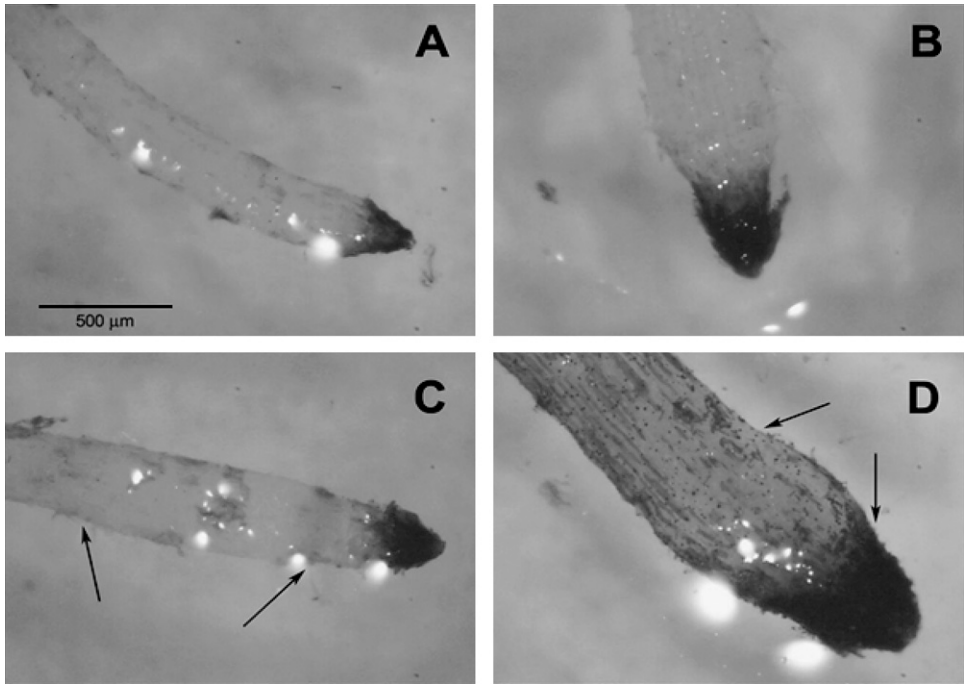

Fig. 1. Effects of acute boron (B) deficiency on root-tip morphology of geranium plants grown under low- or high-light conditions (100 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*). Plants were monitored daily over 6 d of B treatment (control = 45 μM B, -B = 0 μM B). Shown are representative root tips from control (**A–B**) and -B plants (**C–D**) in low (left side) and high (right side) light after 2 d of B-deficiency treatment. Note the characteristic B deficiency-related swelling, indicated by the region between the arrows, at the root tips just above the root cap. In this species, the root cap was always black.

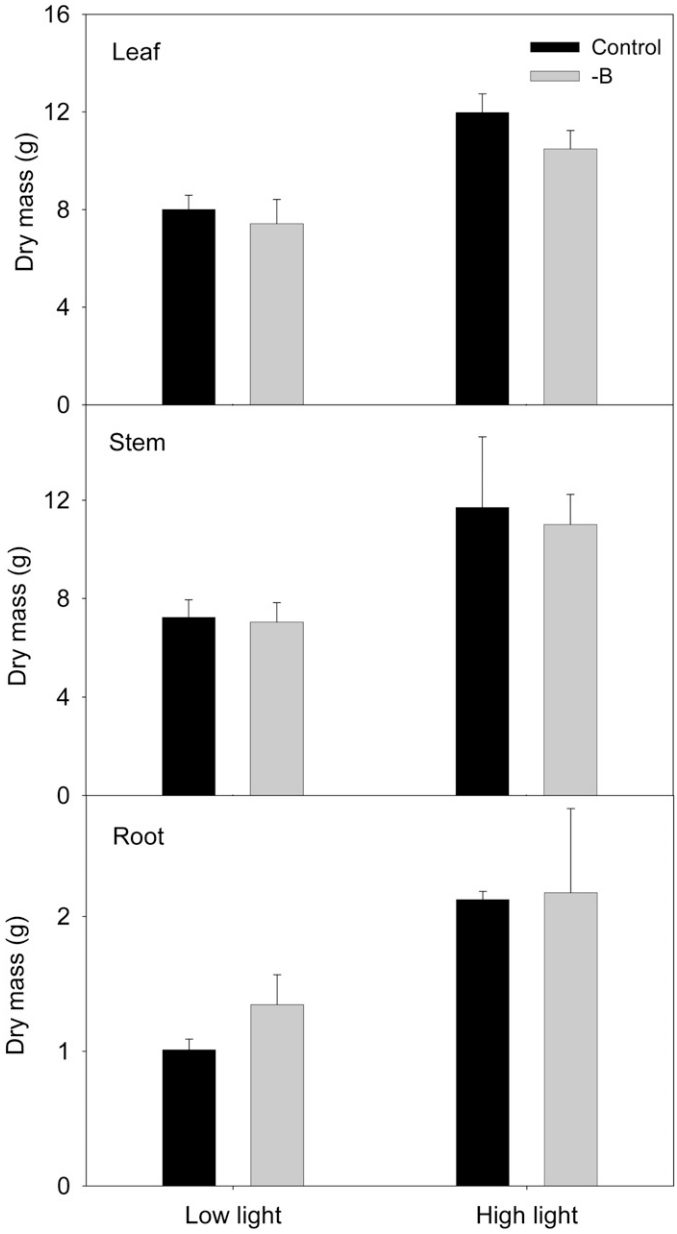

Fig. 2. Effects of acute boron (B) deficiency on biomass of different tissues of geranium plants grown under low- or high-light conditions (100 or 300 μmol·m^{-2}·s^{-1} *PAR*). Plants were harvested after 6 d of B treatment (control = 45 μM B, -B = 0 μM B) and were separated into leaves, stems, and roots. Results are means (n = 3) + SD.

point and are nonphotoinhibitory (see "Results"). Light was measured twice weekly with a line quantum sensor (model LQSV-E; Apogee Instruments, Logan, UT) and intensity was adjusted by installing a neutral-density shade-cloth attached to the lamp barrier of the growth chamber. After full development of shoots and root systems (about 2 weeks), half of the 30 plants were transferred to (otherwise) complete nutrient solution, but containing 0 μM B, while the remaining plants continued to receive complete nutrient solution with 45 μM B (control plants). Plant responses to B withdrawal were monitored for 5 or 6 d, depending on the variable.

To confirm light × B effects on photosynthesis observed in the above experiment and to investigate their general effects, we grew a different geranium cultivar (Maverick White) in a second experiment under chronic B deficiency (compared with acute B withdrawal in the first experiment) and at three different nonphotoinhibitory light levels (100, 300, and 500 μmol·m^{-2}·s^{-1} *PAR*). Seed was sown into foam cubes [15 × 15 × 30 mm each (LC1-type; Smithers-Oasis North America, Kent, OH)] and was irrigated with complete fertilizer solution. After 40 d when seedlings had three to four true leaves, seedlings were transferred to hydroponic containers containing complete nutrient solution (including 45 μM B) and were allowed to acclimate for 10 d to minimize transplant stress. At this time, subsets of plants were transferred to nutrient solutions containing 4.5 μM B (in complete nutrient solution as above), while control plants continued to receive 45 μM B. Net photosynthesis at 400 μmol·mol^{-1} CO_2 and respective growth light levels were measured as below, following an additional 10 and 20 d of growth in the low or normal B solutions. Results were similar at 10 and 20 d, therefore only results from 10 d are shown. Nutrient solutions were changed weekly, and plants were rotated within chambers every alternate day.

Growth and nutrient analysis. To determine the time-course of root responses to B deficiency in Expt. 1, small representative subsamples of roots (one per plant) were harvested and examined visually with a dissecting microscope to estimate the fraction of root tips exhibiting B deficiency-related swelling (Gibson et al., 2007). Entire plants were

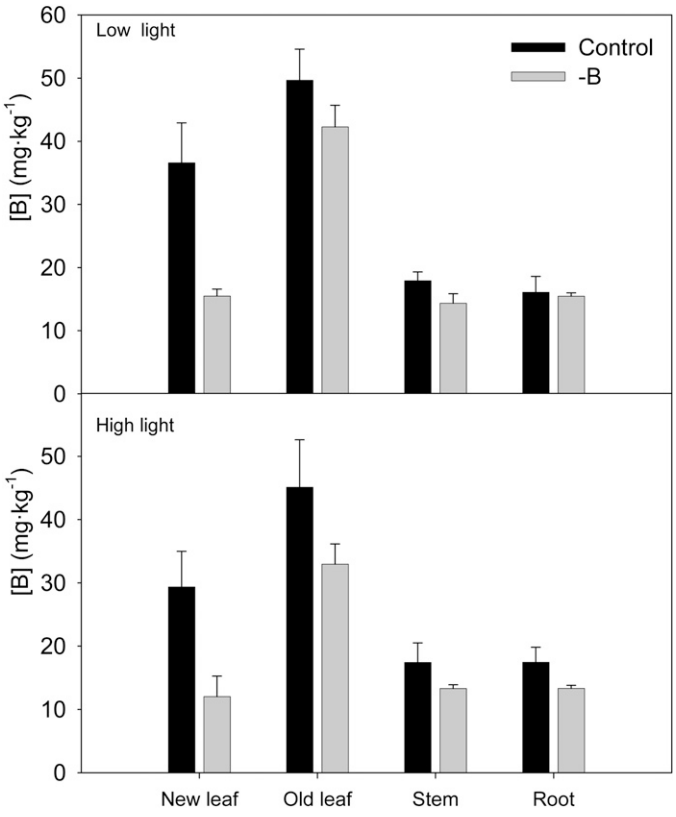

Fig. 3. Effects of acute boron (B) deficiency on the concentration of B in tissues of geranium plants grown under low- or high-light conditions (100 or 300 μmol·m^{-2}·s^{-1} *PAR*). Plants were harvested after 6 d of B treatment (control = 45 μM B, -B = 0 μM B) and were separated into expanding leaves (new leaf), mature leaves (old leaf), stems, and roots. Results are means (n = 3) + SD. The recommended [B] in mature leaf of geranium is 30 to 75 mg·kg^{-1} (Mills and Jones, 1996).

harvested after 6 d, immediately separated into roots, stems, and young (expanding) and mature (fully expanded) leaves, and were then weighed to determine mass (fresh and dry).

To determine tissue nutrient concentration, after harvest tissue was rinsed with 0.1 N HCl, rinsed again with distilled water, and then oven dried in a forced-air oven at 55 °C for 72 h. Tissue was ground by mortar and pestle into a powder and 0.15 g was digested in a microwave digester (MARS Express II; CEM Corp., Matthews, NC) using a modified U.S. Environmental Protection Agency (EPA) method [method 3051, HNO_3 digestion at 200 °C with an additional peroxide digestion step (Nelson, 1988)]. Nutrient concentration, except for N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; model IRIS Intrepid II; Thermo Corp., Waltham, MA). A quality control was run every 10 samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato (*Solanum lycopersicum* L.) standards (NIST reference material 1573; National Institute of Standards and Technology, Gaithersburg, MD) (Sharpless and Gill, 2000) were compared every 20 samples and tomato and spinach (*Spinacia oleracea* L.) standards (NIST reference material 1570a) (Sharpless and Gill, 2000) were compared every 40 samples.

Photosynthesis. Steady-state P_n (net CO_2 exchange) of leaves of intact plants was measured with a portable photosynthesis system with infrared gas analyzer (model 6400; LI-COR, Lincoln, NE) equipped with a 250-mm^3 leaf chamber and CO_2, light and temperature control. P_n at ambient CO_2 (400 $\mu mol \cdot mol^{-1}$) and photosynthetic CO_2 carboxylation efficiency were obtained from analysis of so-called "A-C_i curves" [i.e., response curves of net assimilation rate versus leaf internal CO_2 concentration, where $A = P_n$ (Farquhar and Sharkey, 1982)]. Complete A-C_i curves were obtained for a single leaf of intact plants, and three to four replicate plants were measured per treatment combination each day. Measurements were made at a light level of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* and a cuvette temperature of 20 °C. The measurements were initiated at 400 $\mu mol \cdot mol^{-1}$ CO_2. Once steady-state photosynthesis and stomatal conductance (g_S) was reached (about 15–20 min), the ambient CO_2 concentration was gradually lowered to 50 $\mu mol \cdot mol^{-1}$ (steps of 300, 200, 150, 100, and 50 $\mu mol \cdot mol^{-1}$), and was then increased back to 400 $\mu mol \cdot mol^{-1}$, and was finally increased stepwise from 400 up to 1000 $\mu mol \cdot mol^{-1}$ (steps of 600, 800, and 1000 $\mu mol \cdot mol^{-1}$). Net photosynthesis values were plotted against the respective internal leaf CO_2 concentrations (C_i) to produce an A-C_i response curve. The initial linear slope of an A-C_i curve represents carboxylation efficiency (CE), and is proportional to rubisco activity in C_3 plants, which is dependent on rubisco concentration and activation state (Farquhar and Sharkey, 1982). Consistent with expectations that B deficiency will effect expanding leaves more than already-expanded leaves, due to limited B mobility, preliminary measurements of most-recently expanded and nearly expanded leaves indicated that B deficiency affected photosynthesis in the latter before the former; thus, all subsequent and reported photosynthetic measurements were made on nearly expanded leaves.

To examine treatment effects on PSII function, maximum PSII efficiency (F_v/F_m) in dark-adapted (45 min at growth temperatures) leaves (e.g., Krause and Weis, 1991) was monitored by analysis of chlorophyll fluorescence using a pulse-amplitude-modulated (PAM) fluorometer (model PAM 101/103; Walz, Effeltrich, Germany). Measurements were made on intact plants on individual leaves that were dark-adapted for 45 min. Minimum basal fluorescence (F_o) was measured initially at <0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*, followed by maximum fluorescence (F_m) after a 1.0-s pulse of saturating white light (>5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*) and $F_v/F_m = (F_m - F_o)/F_m$.

Carbohydrate and chlorophyll concentration. Total soluble carbohydrate concentration in leaf tissue was estimated by using the phenol-sulfuric acid method of Dubois et al. (1956) with minor modification. Leaf tissue (50 mg dry weight) was ground in liquid N_2 and was then mixed with 2 mL of 1 M phosphate buffer (pH 7.2) and reground. The homogenate was

Table 1. Effect of B deficiency on the tissue nutrient concentration ($g \cdot kg^{-1}$ for K, Mg, P, S, and Ca; $mg \cdot kg^{-1}$ for Cu, Mn, and Zn) in new leaves of geranium plants grown at low and high light (100 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*). Plants were harvested after 6 d of B treatment. Results from statistical analysis (*P* values) of effects of B, light, and their interactions are shown. Mean values (n = 3) and their SD are shown.

		Tissue nutrient concn		*P* value		
Nutrient	B supply (μM)	Low light	High light	B	Light	B × light
P	45	6.16 ± 0.46	6.26 ± 0.58	0.032*	0.849	0.679
	0	7.47 ± 0.48	7.20 ± 1.21			
K	45	34.9 ± 3.63	29.5 ± 0.96	0.013*	0.019*	0.400
	0	29.1 ± 1.66	26.2 ± 2.66			
Ca	45	13.1 ± 2.40	12.2 ± 2.53	0.167	0.778	0.675
	0	10.7 ± 0.84	10.8 ± 2.51			
Mg	45	4.26 ± 0.53	3.87 ± 0.52	0.072	0.154	0.991
	0	3.75 ± 0.19	3.37 ± 0.38			
S	45	2.47 ± 0.09	2.22 ± 0.07	0.160	0.002*	0.177
	0	2.71 ± 0.1	2.22 ± 0.23			
Cu	45	10.5 ± 0.2	7.00 ± 0.7	0.310	0.473	0.337
	0	11.3 ± 1.8	8.50 ± 0.98			
Mn	45	471.3 ± 83.8	305.2 ± 15.5	0.110	0.003*	0.024*
	0	344.5 ± 23.0	278.1 ± 38.3			
Zn	45	25.3 ± 5.3	15.7 ± 1.4	0.06	0.244	0.066
	0	24.5 ± 3.7	26.7 ± 5.8			

*Indicates significant differences of treatment effect at $P \leq 0.05$.

centrifuged at 21,000 g_n and 1 mL of supernatant was then taken and mixed with 1 mL of aqueous phenol. Concentrated sulfuric acid (5 mL) was added, and absorbance at 470 nm was determined after 20 min. Glucose was used for generating a standard curve. Chlorophyll concentration (per fresh weight) in leaves was estimated spectrophotometrically after extraction in dimethyl sulfoxide (DMSO) using the equations of Barnes et al. (1992). Leaf samples were incubated at 65 °C for 1 h.

Protein quantification. Proteins were extracted from leaf tissues (400 mg fresh weight) by grinding in liquid N_2 in a mortar and pestle and then in an extraction buffer containing 0.15 M Tris-HCl (pH 8.0), 1% SDS (w/v), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM dithiothreitol (DTT), 10% sucrose (w/v), 10 μM leupeptin, 1 mM benzamidine, 10 mM ascorbic acid, 2% polyvinylpolypyrrolidine (PVPP) (w/v), 1 mM EDTA, 10% glycerol (v/v), and 0.05% bromophenol blue. Samples were centrifuged at 21,000 g_n for 10 min at 4 °C to remove insoluble debris, and the supernatant was used for further analysis. The total protein concentration of each sample was determined in triplicate by the Coomassie dye-binding method of Ghosh et al. (1988) using bovine serum albumin as a standard. The colorimetric density of protein in sample spots on filter paper discs was determined using a desktop scanner and densitometry analysis, using National Institutes of Health imaging software (Scion; National Institutes of Health, Bethesda, MD).

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 12% acrylamide, 16 × 20-cm gels (50 μg of total protein per lane) (e.g., Heckathorn et al., 2002). Following electrophoresis, gels were stained for total proteins using Coomassie blue R-250 or were transferred to nitrocellulose membranes by electroblotting. The membranes were then probed with protein-specific antibodies and secondary antibodies conjugated to alkaline phosphatase. Colorimetric detection of secondary antibodies was carried out by treating membranes with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolylphosphate (BCIP). Antiserum to Cu/Zn-SOD and Mn-SOD were purchased commercially (Stressgen Bioreagents, Victoria, BC, Canada), while antiserum to eIF5A-2, rubisco activase, and OEC23 were kindly provided by Drs. T.W. Wang, A. Portis, and E. Camm/A. Eastman, respectively. Concentration of the rubisco large subunit (about 52 kD) was determined by densitometric analysis of Coomassie-stained gels. Densitometric analysis of proteins on gels or blots was performed as above. Cu/Zn-SOD and Mn-SOD are key antioxidant enzymes localized to chloroplasts/cytosol and mitochondria, respectively, and increases in levels of these enzymes would be indicative of increases in cellular oxidative stress (Bowler et al., 1992). The eukaryotic translation initiation factor 5A-2 (eIF5A-2) is thought to regulate translation of specific proteins and is often upregulated by stress and may help regulate apoptosis (Thompson et al., 2004). Thus, increases in eIF5A-2 and decreases in total cell protein would be indicative of stress effects on cell protein synthesis.

Statistics. Each day, three to five (depending on variable) replicate plants were randomly selected from a larger set (about 12 for each treatment combination) for measurement. Results were analyzed statistically by two-way (light × B) or three-way (light × B × day) analysis of variance (ANOVA) using JMP 5.0 software (SAS Institute, Cary, NC). Treatment effects were considered significant if $P \leq 0.05$ and marginally significant if $P \leq 0.10$. Before analysis, results of individual proteins were normalized to day 0 mean values within each B × light treatment combination. Analyses were also conducted for all normalized results following arcsin-square root transformation (in the event of non-normal distributions); these analyses yielded similar results to those with untransformed data, therefore we present only the latter here (means and errors).

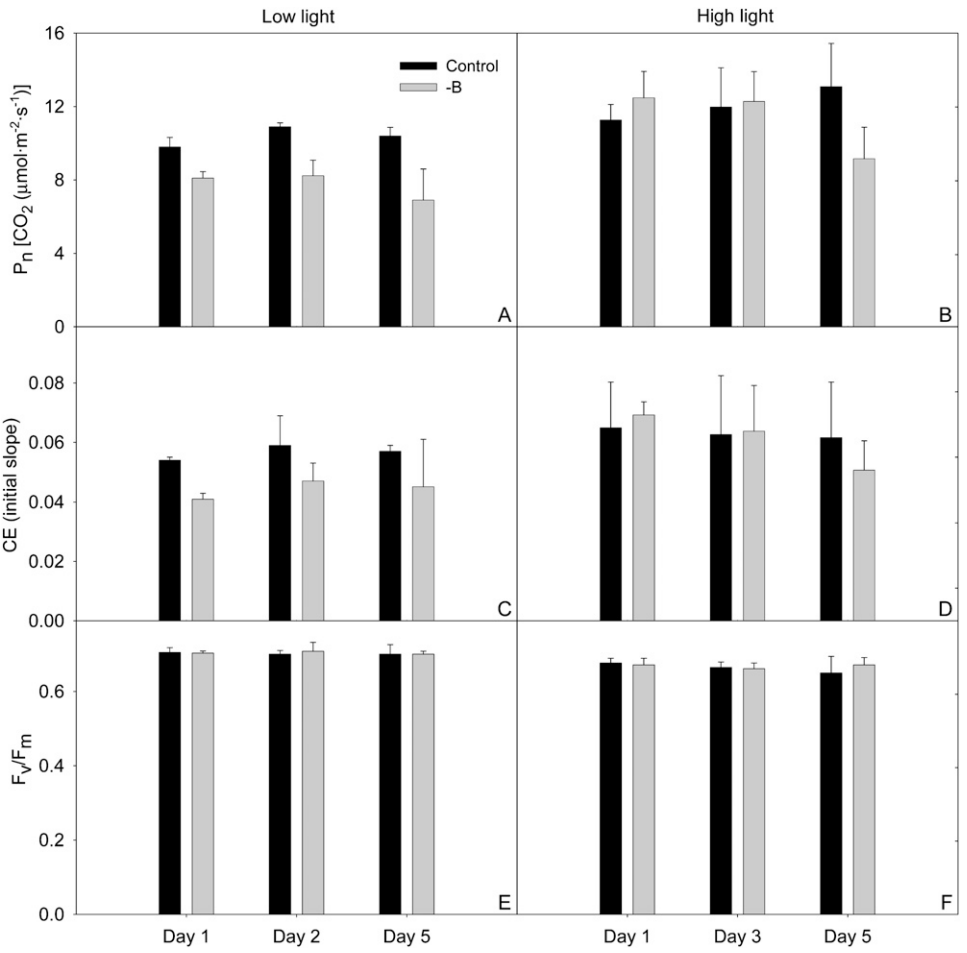

Fig. 4. Effects of acute boron (B) deficiency on net photosynthesis (P_n) (**A–B**), carboxylation efficiency (CE) (**C–D**), and PSII efficiency (F_v/F_m) (**E–F**) in geranium plants grown under low- or high-light conditions (100 or 300 μmol·m^{-2}·s^{-1} *PAR*). Plants were measured repeatedly over 6 d of B treatment (control = 45 μM B, -B = 0 μM B). P_n was measured at ambient CO_2 concentration (400 μmol·mol^{-1}); CE is the initial linear slope of the response of P_n to leaf internal (CO_2); F_v/F_m is the ratio of variable-to-maximum chlorophyll fluorescence in dark-adapted leaves (arbitrary units). Results are means (n = 3) + SD.

Table 2. Results from statistical analysis (*P* values from ANOVA) of treatment effects of B, light, and measurement day, and their interactions on various response variables. Geranium plants were grown under acute B deficiency (0 μM) under two light levels (100 or 300 μmol·m^{-2}·s^{-1} *PAR*) for 6 d. Values are averages of leaves from three replicates.

	Treatment effects						
Response variable[z]	B	Light	Day	B × light	B × day	Light × day	B × light × day
P_n	0.0007*	<0.0001*	0.2191	0.0497*	0.0106*	0.9294	0.2279
CE	0.0180*	0.0002*	0.6723	0.2632	0.2720	0.3043	0.4525
F_v/F_m	0.6077	0.0004*	0.5618	0.8272	0.6725	0.7833	0.5698
Rubisco	0.2311	0.0026*	0.9294	0.3003	0.9742	0.9767	0.8396
Rubisco activase	0.3585	<0.0001*	0.9216	0.5078	0.8531	0.9277	0.9093
OEC23	0.9919	0.0134*	0.4904	0.5040	0.6765	0.8091	0.8915
Chlorophyll	0.0010*	<0.0001*	0.9701	0.4381	0.9808	0.5309	0.7665
Soluble sugars	0.9695	<0.0001*	0.0397*	0.7349	0.8041	0.8735	0.9724
Total protein	0.9682	0.2433	0.0078*	0.5149	0.7822	0.1296	0.6347
eIF5A-2	0.3866	<0.0001*	0.4828	0.5051	0.0356*	0.7687	0.9569
Cu/Zn-SOD	0.4446	0.3580	0.2067	0.1115	0.4254	0.8605	0.7773
Mn-SOD	0.0240*	0.0365*	0.0977	0.7673	0.0234*	0.5958	0.478

*Indicates significant differences of treatment effect at $P \leq 0.05$.
[z]P_n = net photosynthesis, CE = carboxylation efficiency, F_v/F_m = maximum PSII efficiency, OEC23 = 23-kD oxygen-evolving-complex protein of PSII, eIF5A-2 = eukaryotic translation initiation factor 5A-2, SOD = superoxide dismutase.

Results

Symptoms of acute B deficiency were observed rapidly in roots, apparent as slight swellings at the root tip just above the root cap (Fig. 1). This swelling was observed on a small proportion of roots in B-deficient plants within 1 day of B withdrawal in low-light plants, and the proportion of roots exhibiting this swelling increased with each successive day of B deficiency. After 5 d, plant mass was greater in high versus low light for all tissues ($P \leq 0.05$; two-way ANOVA within each tissue), but mass was decreased by acute B deficiency only in leaves in high light (Fig. 2; $P \leq 0.05$ only for high-light leaves).

As expected, acute B deficiency generally decreased the concentration of B in most tissues at both light levels (Fig. 3). Decreases in B concentration with B deficiency were most pronounced for new, expanding leaves, with smaller decreases in older mature leaves, stems, and roots. Boron concentration was lower in leaves of high-light plants, indicating that the increased growth rate of plants in high light was not matched by B uptake rate; nevertheless, the proportional decreases in B concentration in leaves and stem with B deficiency were similar in low- and high-light plants.

Acute B deficiency also affected the concentration of some other nutrients in plants (Table 1). In new expanding leaves, B deficiency increased the concentration of P (low and high light) and Zn (high light only; marginally significant), whereas K, Mg, and Mn decreased in concentration with B deficiency (marginally significant for Mg, low light only for Mn). The light level also affected the nutrient concentration of leaves, wherein high light decreased K, Mn, and S concentration. In stems, only Ca concentration (22320.66 and 20670.69 g·kg^{-1} in low and high light, respectively, under B deficiency) was changed (increased) significantly ($P = 0.003$) by B deficiency. Boron deficiency did not affect the concentration of any nutrients in roots (not shown).

P_n and CE were decreased significantly by acute B deficiency and were increased by higher light (Fig. 4; Table 2). Decreases in P_n and CE were observed within the first day of B withdrawal in plants grown under low-light condition (Fig. 4, A and C); however, in plants growing under high light, decreases in P_n and CE were not observed until day 5 (Fig. 4, B and D). In

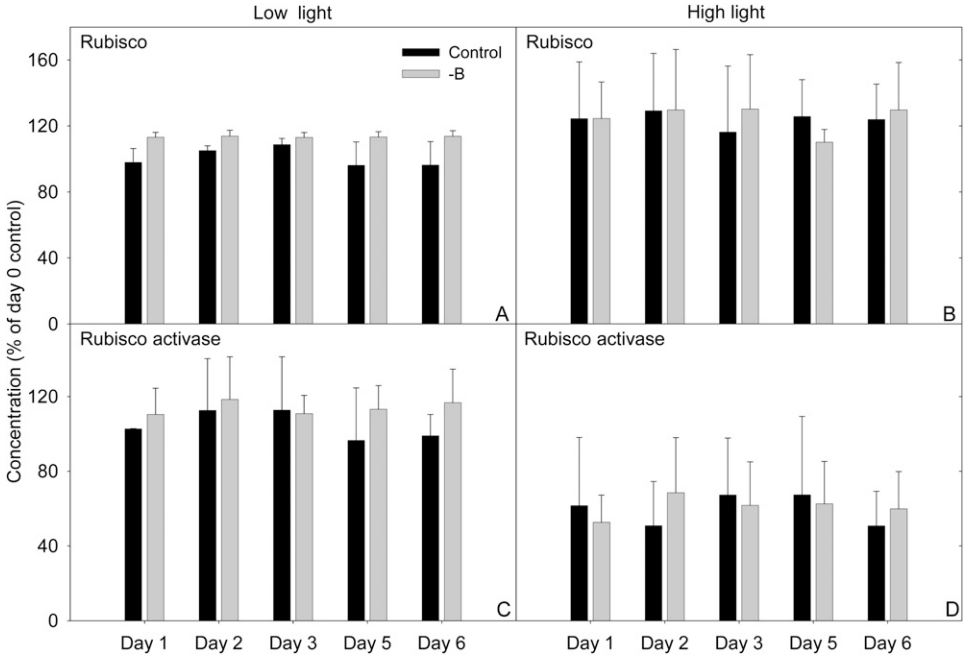

Fig. 5. Effects of acute boron (B) deficiency on concentration of rubisco (**A–B**) and rubisco activase (**C–D**) in leaves of geranium plants grown under low- or high-light conditions (100 or 300 μmol·m^{-2}·s^{-1} *PAR*). Plants were measured repeatedly over 6 d of B treatment (control = 45 μM B, -B = 0 μM B). Results are means (n = 3) + SD. Values are normalized to day 0 means within each light × boron treatment combination.

contrast, acute B deficiency did not affect F_v/F_m (Fig. 4, E and F; Table 2), and although F_v/F_m decreased with higher light, this decrease was biologically small; hence, PSII was not damaged by B deficiency.

The relative concentration (per unit total cell protein) of rubisco was greater in high- versus low-light-grown plants, consistent with higher P_n and CE (the reverse was true for rubisco activase), but rubisco and rubisco activase concentration was unaffected significantly by acute B deficiency (Fig. 5; Table 2). The lack of significant B effects on rubisco and rubisco activase concentration indicate that B deficiency-related decreases in CE, a leaf-level measure of rubisco activity, must reflect decreases in rubisco activation state.

As with F_v/F_m, acute B deficiency did not affect the concentration of OEC23 (per unit total cell protein), while high light increased OEC23 compared with low light. (Fig. 6, A and B; Table 2). In contrast, acute B deficiency and high light decreased total chlorophyll concentration (per unit mass), with B deficiency decreases especially pronounced in high light (Fig. 6, C and D; Table 2). However, there were no significant differences in ratio of Chl *a*-to-*b*, indicating that the decreases in total chlorophyll reflected a decrease in the concentration of PSII per gram of leaf tissue rather than a change in the ratio of PSII reaction centers (rich in chl *a*)-to-PSII light-harvesting complexes (rich in chl *b*) (Taiz and Zeiger, 2002).

As expected, growth of plants in high light increased the concentration of soluble sugars in leaves compared with plants in low light, especially in newly expanding leaves; the reverse was true in roots (Fig. 7, A and B; Table 2). However, acute B deficiency had no effect on the sugar concentration of tissues.

There was no significant effect of acute B deficiency or light level on total protein concentration of leaves (Fig. 8, A and B; Table 2), although B deficiency decreased total protein on days 5 and 6 in high-light plants (*t* test, $P \leq 0.05$). Acute B deficiency also had no significant effect on the concentration of eIF5A-2, although there was a significant B × day interaction, with higher levels of eIF5A-2 with B deficiency in the 1 to 2 d immediately following B withdrawal, and lower levels on the last days before harvest (days 5–6); also, plants grown in low light had significantly higher levels of eIF5A-2 than high-light plants (Fig. 8, C and D; Table 2).

Neither acute B deficiency nor light level significantly affected the concentration of Cu/Zn-SOD in leaves (Fig. 9, A and B; Table 2). In contrast, both B deficiency and light level affected Mn-SOD, with B deficiency increasing Mn-SOD concentration on the days just after B withdrawal, but with no B effect thereafter, and with higher Mn-SOD levels in low- versus high-light plants (Fig. 9, C and D; Table 2).

As with acute B deficiency (induced by complete B withdrawal), chronic B deficiency (caused by growth at low levels of B) exhibited interactive effects with growth light level on P_n (Fig. 10). Chronic B deficiency increased P_n per unit leaf area in plants at 300 and especially at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* (medium and high light, respectively), but at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR* (low light), P_n did not differ between control and B deficient.

Discussion

In this study, we tested the hypotheses, suggested by previous studies, that photosynthesis is affected by B deficiency before visible symptom development in leaves (Chen, 2006; Kastori et al., 1995; El-Shintinawy, 1999; Zhao and Oosterhuis, 2003), and that enhanced plant carbohydrate status could help alleviate the effects of B deficiency and delay symptom expression on plants (e.g., Brown et al., 1999; Kastori et al., 1995). We found that changes in photosynthesis were indeed an early response to B deficiency in geranium, decreasing within 24 h of B withdrawal (in low light-grown plants), and photosynthesis was affected before aboveground plant growth (but simultaneous with effects on root-tip morphology).

Negative effects of B deficiency on photosynthesis were observed for overall (net) photosynthetic rate, and for specific aspects of light and dark (Calvin cycle) reactions of photosynthesis; namely, CE and total chlorophyll concentration. Decreases in net photosynthesis and carboxylation efficiency occurred sooner following B withdrawal in low light-grown plants (within 1 d) than in plants grown at higher light (not observed until day 5), although the B concentration of leaves (and other tissues) was lower with B withdrawal (and in controls) in high- versus low-light plants. In contrast, decreases in chlorophyll concentration occurred within 1 day of B withdrawal in low- and high-light plants. Boron deficiency

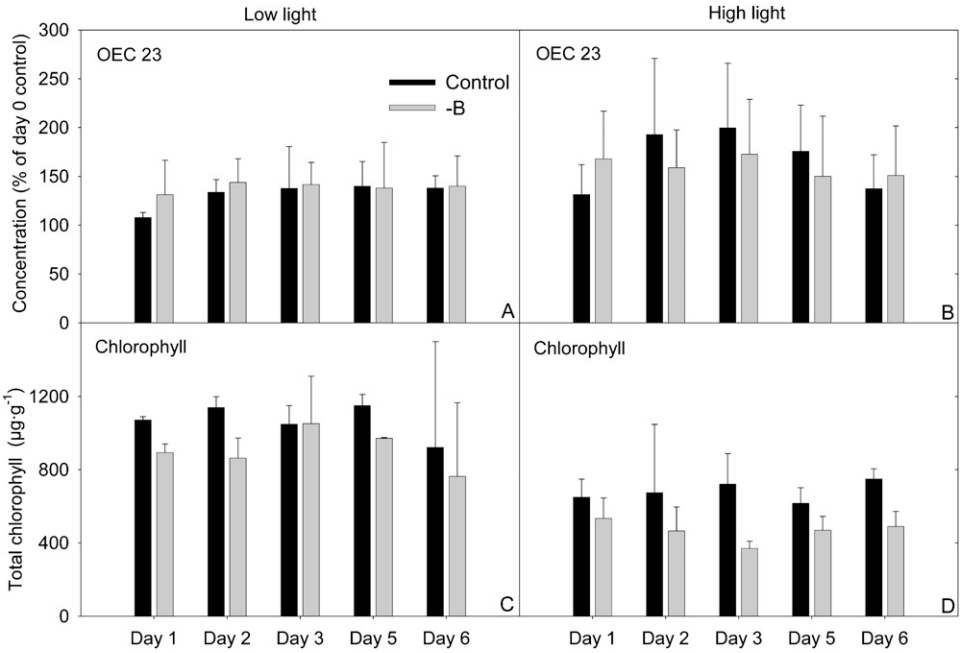

Fig. 6. Effects of acute boron (B) deficiency on concentration of the 23-kD oxygen-evolving-complex protein of PSII (OEC23) (**A–B**) and total chlorophyll (per unit fresh weight) (**C–D**) in leaves of geranium plants grown under low- or high-light conditions (100 or 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PAR*). Plants were measured repeatedly over 6 d of B treatment (control = 45 µM B, -B = 0 µM B). Results are means (n = 3) + SD. Values for OEC23 are relativized to day 0 means within each light × boron treatment combination.

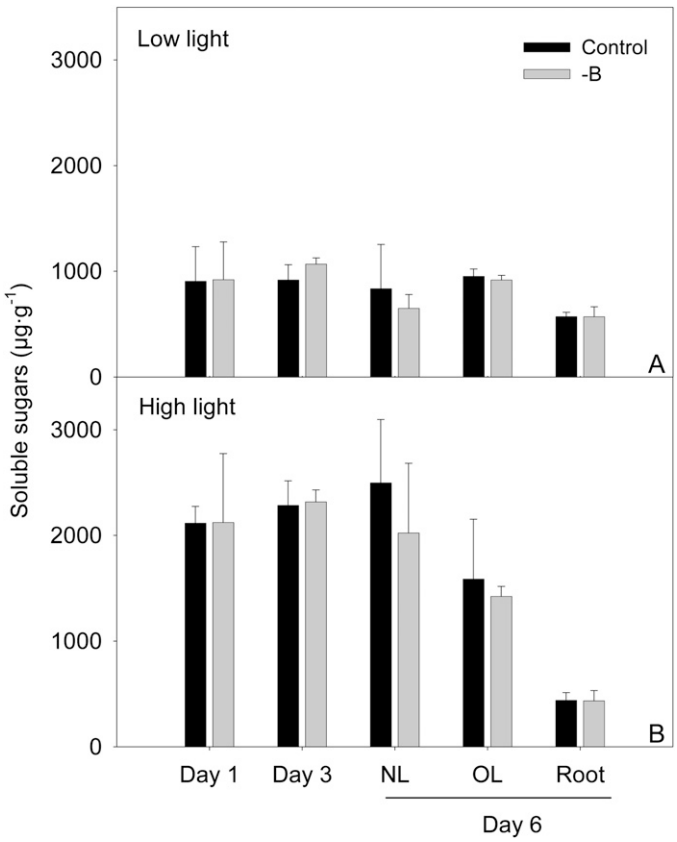

Fig. 7. Effects of acute boron (B) deficiency on concentration of total soluble sugar (per unit dry weight) in leaves of geranium plants grown under low- or high-light conditions (100 or 300 µmol·m^{-2}·s^{-1} *PAR*). Plants were measured after 1, 3, and 6 d of B treatment (control = 45 µM B, -B = 0 µM B). Expanding leaves were measured on days 1 and 3; on day 6, expanding new (NL) and mature older (OL) leaves and roots were measured. Results are means (n = 3) + SD.

did not affect the concentration of total soluble carbohydrates in leaves, but as expected, high-light plants had higher carbohydrate levels than low-light plants; thus, as predicted, enhanced carbohydrate status was correlated with delayed onset of B-deficiency effects on net photosynthesis and CE. Importantly, the interactive effect of growth light level and B on P_n was also observed in a different cultivar grown under chronic low B levels and under a wider range of light levels, wherein B deficiency increased P_n per unit leaf area in higher-light plants (i.e., 300 and 500 µmol·m^{-2}·s^{-1} *PAR*), but not in plants grown at low light levels (100 µmol·m^{-2}·s^{-1} *PAR*). Thus, the benefits of higher nonphotoinhibitory growth light levels to P_n during B deficiency may be common.

Of the other aspects of photosynthesis examined, no effects of B deficiency were observed for concentration of rubisco or rubisco activase (dark-reaction components), or OEC23 concentration, F_v/F_m, or chlorophyll *a*-to-*b* (light-reaction components). The negative effects of B deficiency on CE of leaves must reflect a decrease in rubisco activation state because CE depends on rubisco concentration and activation state (or activity) (Eckardt and Portis, 1997; Farquhar and Sharkey, 1982). Whether B deficiency-related decreases in rubisco activity were due to direct effects on rubisco or to indirect effects on rubisco activase activity are not yet known. The negative effects of B deficiency on total chlorophyll were not associated with negative effects on PSII structure (chlorophyll *a*-to-*b*, OEC concentration per unit total cell protein) or function (F_v/F_m = maximum PSII efficiency), and thus must reflect decreases in the concentration of PSII per unit leaf mass.

Previous studies have also observed early effects of B deficiency on photosynthesis. At the whole-plant level, B deficiency may indirectly affect photosynthesis by decreasing leaf expansion, and thus photosynthetic area, and by altering the leaf constituents (Dell and Huang, 1997; Furlani et al., 2003). In cotton, Zhao and Oosterhuis (2003) demonstrated that B-deficient plants had lower net photosynthesis (per leaf area), but higher total chlorophyll concentration, and decreases in photosynthesis were evident before effects on growth or membrane leakage (all weekly measurements; photosynthesis affected by week 2). Kastori et al. (1995) observed a decrease in photosynthetic oxygen evolution and CO_2 assimilation in 30-d-old sunflower leaves during B deficiency, which was accompanied by an increase in nonstructural carbohydrate concentration (starch + sugar) (versus soluble sugars only in this study). In mustard (*Brassica campestris* L. cv. T42), B deficiency decreased leaf stomatal frequencies and apertures, resulting in a decrease in CO_2 uptake (Sharma and Ramchandra, 1990). In sunflower leaves, Cakmak et al. (1995) observed that increased light intensity enhanced B-deficiency symptoms and decreases in chlorophyll concentration. Notably, not all past studies have observed effects of B deficiency on photosynthesis [e.g., on chlorophyll concentration, stomatal density, and PSII function; Stavrianakou et al. (2006)].

Interestingly, in the present study, B deficiency decreased the concentration of several nutrients in leaves (K, Mg, Mn, and trending so for Ca), but increased the concentration of others (P, Zn, and trending so for S and Cu). The decreases in Mg (a component of chlorophyll) and Mn (a component of the OEC of PSII) are consistent with, and potentially an explanation for, the decreases in the concentration in total chlorophyll and PSII. Effects of B deficiency on the concentration or uptake of other nutrients have been observed previously (Cakmak et al., 1995; Cakmak and Römheld, 1997), although such effects are not universal (Papadakis et al., 2003). For example, B deficiency is associated with substantial and rapid alterations in ion fluxes (Cakmak and Römheld, 1997). Also, B deficiency has been associated with disruption of phenolic metabolism (Blevins and Lukaszewski, 1998; Dordas and Brown, 2005), and it has been suggested that B-related effects on phenolics result in reversible alterations in membrane permeability and ion transport through membranes (Glass, 1973). Light intensity also plays a role in B deficiency-induced K^+ leakage (Cakmak et al., 1995), which might be responsible for the decreases in K^+ concentration in new leaf tissues of geranium in the present study. In the present study, nutrient concentrations in the mature (old) leaves were all above the critical levels reported for geranium (Mills and Jones, 1996), including the recommendations for B. Only in new leaves did the B concentration drop below the reported critical level of 20 mg·kg^{-1}; however, those guidelines are based on mature tissue, which normally has concentrations of B higher than that of new leaves (personal observation).

There is currently no evidence of a direct role for B in photosynthesis (e.g., Blevins and Lukaszewski, 1998), therefore the early negative effects of B deficiency on photosynthesis observed in this study and others may result from indirect pleiotrophic effects, which may be common for B stress (Bolaños et al., 2004), or from indirect general cellular damage [e.g., from reactive oxygen species (ROS)] (Cakmak et al., 1995;

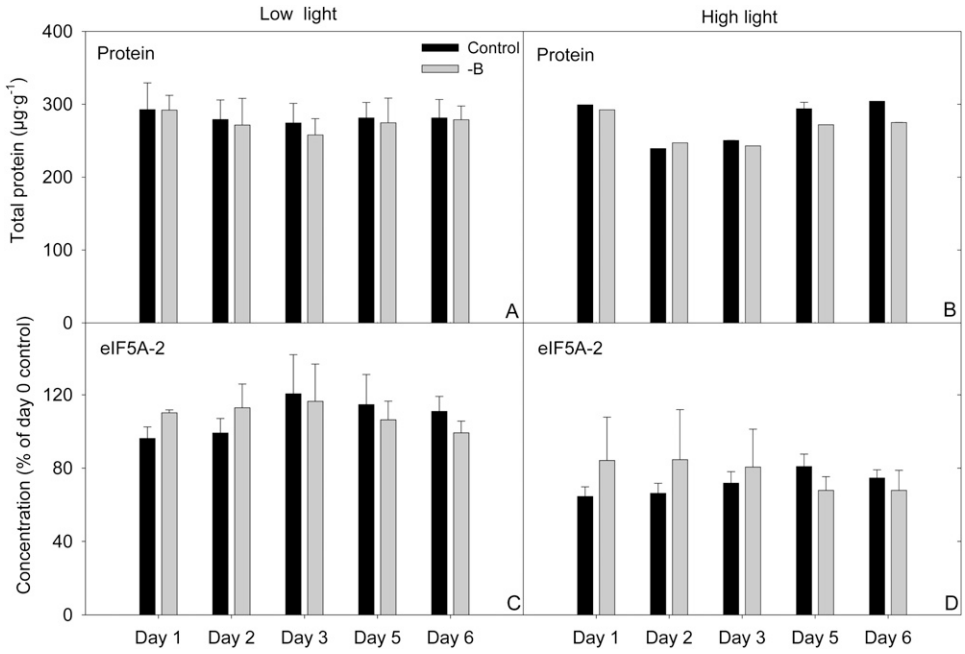

Fig. 8. Effects of acute boron (B) deficiency on concentration of total protein (per unit fresh weight) (**A–B**) and translation initiation factor eIF5A-2 (**C–D**) in leaves of geranium plants grown under low- or high-light conditions (100 or 300 µmol·m^{-2}·s^{-1} *PAR*). Plants were measured repeatedly over 6 d of B treatment (control = 45 µM B, -B = 0 µM B). Results are means (n = 3) + SD. Values for eIF5A-2 are normalized to day 0 means within each light × boron treatment combination.

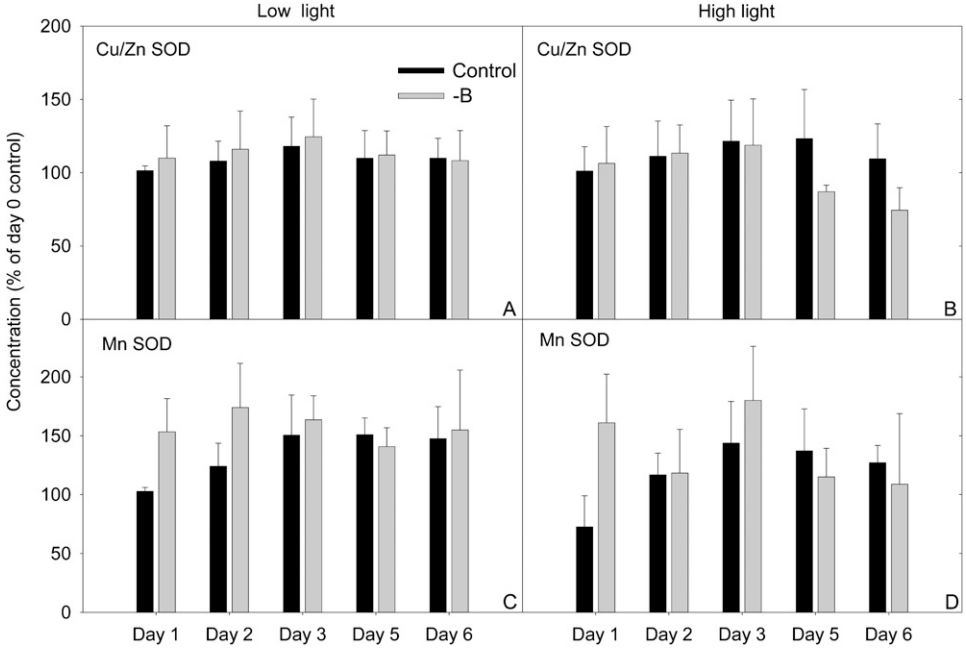

Fig. 9. Effects of acute boron (B) deficiency on concentration of Cu/Zn-SOD (**A–B**) and Mn-SOD (**C–D**) in leaves of geranium plants grown under low- or high-light conditions (100 or 300 µmol·m^{-2}·s^{-1} *PAR*). Plants were measured repeatedly over 6 d of B treatment (control = 45 µM B, -B = 0 µM B). Results are means (n = 3) + SD. Values are normalized to day 0 means within each light × boron treatment combination.

Dordas and Brown, 2005; El-Shintinawy, 1999). There was little evidence for the latter possibility in the present study. For example, while we observed a transient increase in Mn-SOD levels with B deficiency (first 1–2 d following B withdrawal), we saw no such increase in Cu/Zn-SOD, and increases in Mn-SOD were observed for low- and high-light plants, yet decreases in net photosythesis and CE were observed at this time only in the low-light plants. Mn-SOD is localized to the mitochondria, therefore increased levels of this protein suggest an increase in oxidative stress in mitochondria, but Cu/Zn-SOD is localized in the cytosol and chloroplasts, and the lack of B deficiency-related increases in Cu/Zn-SOD suggest a lack of increase in oxidative stress in chloroplasts during B deficiency in this study (Bowler et al., 1992; Mittler, 2002)—a conclusion that is consistent with the absence of B-deficiency effects on soluble carbohydrates and PSII because PSII is susceptible to oxidative damage (Mittler, 2002).

As with Mn-SOD, we also observed a small transient increase in eIF5A-2 levels with B deficiency (i.e., in the first 2 d following B withdrawal), but the decreases were observed in low- and high-light plants and so were not correlated with decreases in photosynthesis and CE. Furthermore, no effects of B deficiency were observed on total protein (or rubisco, rubisco activase, or OEC23) concentration, suggesting that B-related damage to proteins was not of a general nature, but was restricted to specific proteins. Consistent with this notion, although eIF5A-2 is known to be upregulated during many stresses and is involved in determination of cell growth and senescence by regulating mRNA turnover and protein synthesis, it appears that eIF5A-2 is involved only in regulation of specific mRNAs and not global protein expression (Chou et al., 2004, Thompson et al., 2004, Wang et al., 2001). Regardless of the cause, be it direct or indirect, net photosynthesis, CE, and chlorophyll concentration were early targets of B deficiency in geranium in this study.

There is a belief that high light intensities increase B sensitivity due to increased B requirement from growth and secondary metabolites (Cakmak et al., 1995; MacInnes and Albert, 1969; Marschner, 1995; Tanaka, 1966). In this study, symptoms were delayed by higher levels of nonsaturating, nonphotoinhibitory light. However, all indications are that if the stress persisted beyond the time used herein,

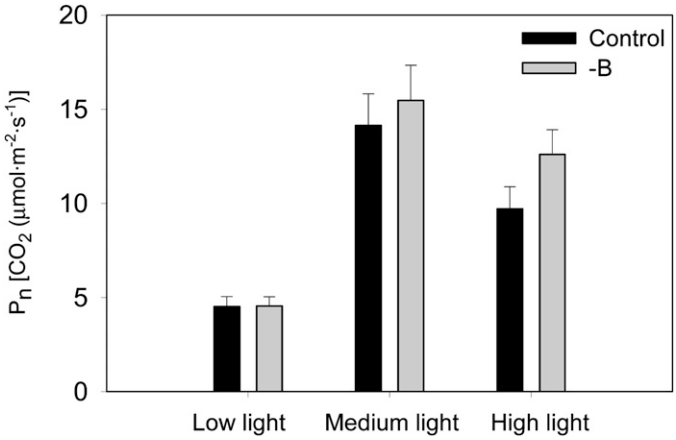

Fig. 10. Effects of chronic boron (B) deficiency on net photosynthesis (P_n) in geranium plants grown under low-, medium-, or high-light conditions (100, 300, or 500 μmol·m^{-2}·s^{-1} *PAR*). P_n was measured in recently expanded leaves at ambient CO_2 concentration (400 μmol·mol^{-1}) and under growth light levels. Results are means (n = 3) + SD.

the symptom severity may have been greater in higher light. It is important to note that the highest light level used in this study was only one-third of daily maximums commonly encountered in the field in most growing areas in the United States during the summer months. This study indicates that increased light can protect P_n and growth during the initial phase of B stress, perhaps due in part to increased carbohydrates. Importantly, specific B effects differed between acute and chronic B stress, therefore conclusions from acute stress in the laboratory may not be relevant to chronic stress occurring in potted or field-grown plants.

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