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Effects of irradiance during growth on tolerance of geranium to sub- and supra-optimal boron supply

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Abstract

In our previous study on geranium, we showed that increases in growth irradiance from sub-optimal to near-optimal could delay boron deficiency effects on photosynthesis. In this study, we further investigated the effects of growth irradiance on tolerance to B stress by growing geranium (*Pelargonium* × *hortorum* cv. Maverick White) under sub- to supra-optimal B concentrations (4.5, 45, and 450 μM) and under three irradiances of 100, 300, or 500 μmol m^{-2} s^{-1} PAR for 30 d. In general, at low and medium irradiances, sub- and supra-optimal B availability decreased root and shoot dry masses, but at high irradiance, the B stress was prevented. Net photosynthetic rate decreased by the supra-optimal B concentration at the high irradiance only suggesting B-related photoinhibition. Tissue B content and root specific B uptake only modestly decreased by the low B treatment, but increased greatly by the high B availability, and the higher irradiance decreased the tissue B content and the root B uptake only at the low and medium B supplies. Interestingly, the increases in irradiance decreased the content and uptake of all other nutrients, except Fe uptake. Effects of the B stress on the content of other nutrients were variable, but the B stress often exacerbated decreases in nutrient content with the increasing irradiance which would be especially important under nutrient-limiting conditions. Hence, in this study, the B stress effects on growth were mitigated by the increases in growth irradiance, which offset negative effects on physiology, and the protective effects of irradiance were likely caused by its positive effects on plant carbon/energy status rather than on tissue B content or B uptake.

Additional key words: macronutrients, micronutrients, net photosynthetic rate, *Pelargonium* ×*hortorum*.

Introduction

Boron is an essential micronutrient required for plant growth and development. The best characterized and confirmed role of B in plants is *via* a structural function in plant cell walls (though evidence for a structural role in membranes is strong too), and this structural function likely accounts for the strong effects of B stress (deficiency and toxicity) on root growth and reproduction (Marschner 1995, Kobayashi *et al*. 1996, Goldbach 1997, Matoh 1997, Power and Woods 1997, Blevins and Lukaszewski 1998, Brown *et al*. 2002). B stress can also affect other functions (*e.g.*, metabolism of nucleic acids, proteins, saccharides, phenolics, and photosynthesis), though it is not yet clear if these other effects are direct or indirect (*e.g.*, due to general oxidative damage; Kouchi 1977, Goldbach 1997, Nable *et al*. 1997, Blevins and Lukaszewski 1998, Bañuelos *et al*.1999, Bishnoi *et al*. 2006, Reid 2007, Mishra *et al*. 2009, Mishra *et al*. 2012). Boron stress in agriculture is common, economically-important, and decreases both yield and crop quality (Shorrocks 1997, Brown *et al*. 2002, Roessner *et al*. 2006).

Plant responses to variation in B availability can be affected by irradiance. For example, Cakmak *et al*. (1995) studied B deficiency in *Helianthus annuus* grown under

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Abbreviations: CE - carboxylation efficiency, EDTA - ethylenediaminetetraacetic acid, ICP-OES - inductively coupled plasma optical emission spectroscopy, PAR - photosynthetically active radiation; P_N - net photosynthetic rate.
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low to medium irradiances, and they observed that B deficiency decreases shoot and root growth and increases ion membrane leakage, particularly under higher irradiance. Huang *et al*. (2002) also found that B deficiency effects on leaf growth are the greatest at high irradiance in *H. annuus*. Tanaka (1966) reported increased irradiance (low to medium) increased B deficiency effects on growth but decreased B toxicity in *Lemna paucicostata*. In *Vigna mungo*, shading reduced the effects of low B on leaf elongation (Noppakoonwong *et al*. 1993). Kocábek *et al*. (2009), using *Arabidopsis* mutants with defects in light-signaling pathways, observed stimulation of hypocotyl elongation by elevated B (1 - 3 mM) which increases with increasing irradiance. Notably, excluding effects on membrane ion leakage (Cakmak *et al*. 1995) and stomatal opening (Huang *et al*. 2002), past studies have not examined interactive effects of B and irradiance on responses other than growth, and only one has examined irradiance effects on toxicity (Tanaka 1966). Interestingly, B × irradiance effects on growth are sometimes (Noppakoonwong *et al*. 1993, Cakmak *et al*. 1995, Huang *et al*. 2002) and sometimes not (Tanaka 1966, Mishra *et al*. 2009) related to effects on tissue B content. Also, two past studies observed that increases in irradiance can impart protection from N stress (Tanaka 1966, Mishra *et al*. 2009).

Previously, we investigated effects of an increase in growth irradiance on responses to B deficiency (induced by B withdrawal) in geranium grown under limiting non-photoinhibitory irradiance (Mishra *et al*. 2009). We observed that a higher irradiance (300 *vs*. 100 μmol m^{-2} s^{-1} of photosynthetically active radiation, PAR) induces decreases in shoot mass under low B that were not observed under low irradiance. However, the higher irradiance delays B deficiency-related decreases in net photosynthetic rate (P_N) and carboxylation efficiency (CE). The protective effect of the higher irradiance on P_N occurs despite decreases in the concentrations of B, K, and Mg in plant tissue with B deficiency and may have been related to a higher sugar content in leaves of high irradiance grown plants. To investigate the interactive effects of B and irradiance further, in the present study, we grew geranium under both sub- and supra-optimal B for an extended duration (*vs*. acute B deficiency only in the previous study) and under a broader range of irradiances compared to the previous study (100, 300, 500 *vs*. 100 and 300 μmol m^{-2} s^{-1}).

Materials and methods

Geranium (*Pelargonium* × *hortorum* cv. Maverick White) seeds were sown into foam cubes (*LC1*, *Smithers-Oasis*, Kent, OH, USA) and grown in complete Hoagland's nutrient solution in a greenhouse. Uniform seedlings (of similar height and with 3 to 4 true leaves) were transferred to opaque 4.5 dm^3 plastic tubs filled with the same solution as in Mishra *et al*. (2009). The tubs had opaque lids with evenly-spaced 2-cm-diameter holes through which the seedlings were suspended (by wrapping with thin strips of foam around the root-shoot interface to fill the gap between the stem and lid-hole). After 10 d, the plants were transferred to nutrient solutions containing 4.5, 45, or 450 μM B (in the form of boric acid; 3 replicate tubs per B concentration) and then 9 tubs were kept under different non-photoinhibitory irradiances: low (100), medium (300), and high (500 μmol m^{-2} s^{-1} of PAR) for 30 d. Each tub contained two plants, and these two plants were averaged to generate the value for individual tub, with mean tub values being the experimental replicates. The plants were grown at day/night temperatures of 23/19 °C, 70 % humidity, and a 16-h photoperiod. Nutrient solutions were changed weekly, and the plants were rotated within the chambers every other day. Irradiance was monitored twice weekly with a line quantum sensor (*LQSV-E*, *Apogee Instruments*, Logan, UT, USA); chamber CO_2 concentration (400 μmol mol^{-1}) and temperature were monitored several times a day.

Net photosynthetic rate (P_N) of recently fully-expanded intact leaves, which had developed after the exposure to the experimental treatments, was measured with a portable photosynthesis system with infrared gas analyzer (*LI-6400*, *LI-COR*., Lincoln, NE, USA), equipped with CO_2, irradiance, and temperature controls (for details see Mishra *et al*. 2009). Measurements were made within 1 min after the insertion of leaves into the cuvette to ensure that photosynthetic responses reflected those within the growth chambers. All photosynthetic variables of plants were measured at the same irradiance at which the plants were grown (100, 300, or 500 μmol m^{-2} s^{-1}) and at the 400 μmol mol^{-1} CO_2 concentration.

The entire plants were harvested after 30 d of the treatment and immediately separated into roots, stem, leaves, and flowers, and then the tissues were dried to a constant mass at 70 °C before determining dry mass. Total soluble sugar content in the leaf tissue was estimated by using the phenol-sulfuric acid method of Dubois *et al*. (1956) with a minor modification, as mentioned in Mishra *et al*. (2009). To determine tissue nutrient content, we followed our previously-reported method (Mishra *et al*. 2009). Briefly, all the harvested tissues were rinsed with 0.1 M HCl, rinsed again with distilled water, and then oven dried in a forced-air oven at 55 °C for 72 h. The tissue was ground with a mortar and pestle into a powder and 0.15 g was digested in a microwave digester (*MARS Express II*, *CEM Corp.*, Matthews, NC, USA), using a modified U.S. Environmental Protection Agency (EPA) method (Nelson 1988; HNO_3 digestion at 200 ºC with an additional peroxide digestion step). Nutrients content (P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) were determined with inductively

coupled plasma optical emission spectroscopy (ICP-OES; model *IRIS Intrepid II*, *Thermo Corp*., Waltham, MA, USA). The root specific uptake of each nutrient was determined by dividing the total content per plant of each nutrient by the dry mass of the root.

Results were analyzed statistically by the two-way (irradiance × B) analysis of variance (*ANOVA*) using the *JMP 5.0* software (*SAS Corp*, Cary, NC, USA). Differences among the specific treatment groups were determined using Fisher's LSD test following significant *ANOVA* results. Treatment effects were considered significant if $P \leq 0.05$.

Results

In general, the low B availability decreased shoot and root masses at the low and medium irradiances, but at the high irradiance, the low B availability did not decrease shoot or root mass (Fig. 1). The high B availability did not affect the shoot mass, but decreased the root mass at the medium irradiance. The negative effects of high B on the root mass were absent at the high irradiance. Neither B nor irradiance had any significant effect on a root:shoot ratio, though the supra-optimal B concentration tended to decrease it (data not shown).

As expected, net photosynthesis (P_N) increased with irradiance, but the low B availability did not affect P_N (Fig. 1). The high B availability decreased P_N only at the high irradiance; hence, this B concentration was toxic and

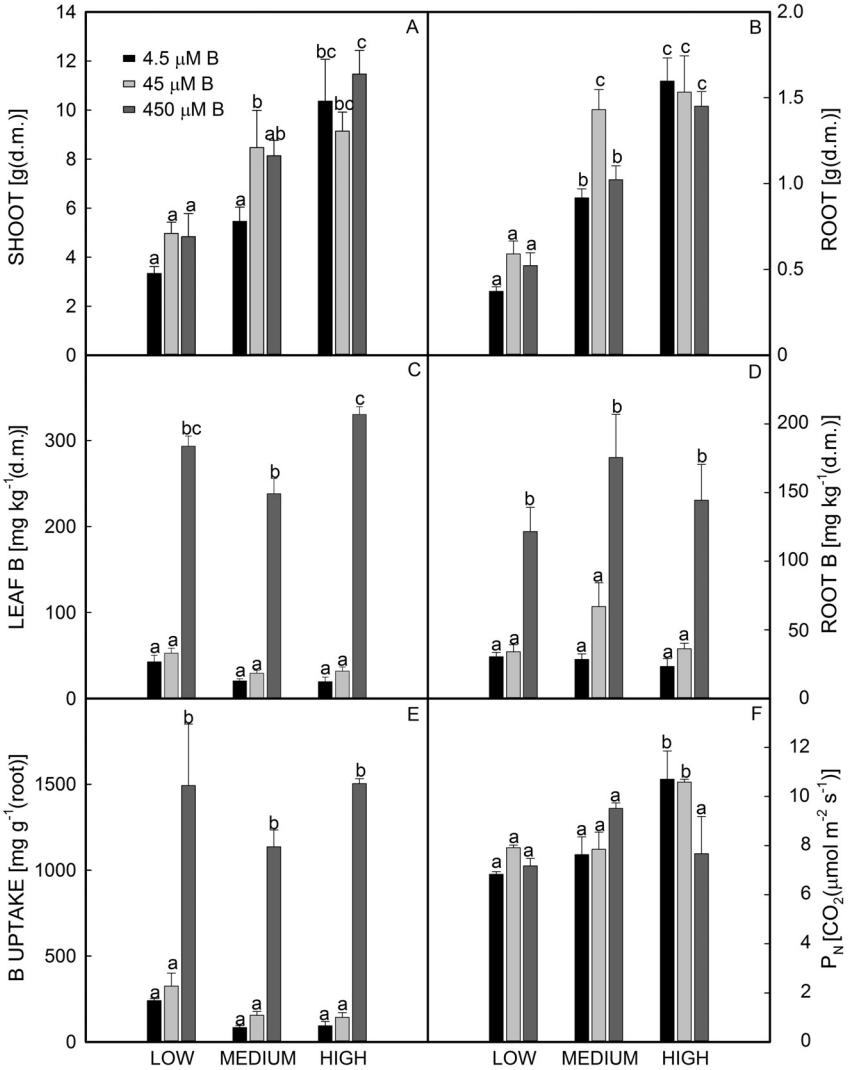

Fig. 1. Effects of B concentration (4.5, 45, or 450 μM) in the nutrient solution on shoot biomass (*A*), root biomass (*B*), boron content (dry mass basis) in leaves (*C*) and roots (*D*), on the uptake of B (the total plant B content per root dry mass unit, *E*), and on net photosynthetic rate (*F*) of geranium plants grown under low, medium, or high irradiances (100, 300, or 500 μmol m^{-2} s^{-1} PAR). Means ± SE, $n = 3$. Among all treatment combinations, different letters above the *bars* indicate significant differences ($P < 0.05$).

induced photoinhibition. As in Mishra *et al*. (2009), the content of soluble sugar in leaves increased with irradiance, but B had little effect on the sugar content (not shown). The chlorophyll content was not affected by low B, but high B increased chlorophyll at the low irradiance and decreased it at the high irradiance (data not shown).

Notably, the low B concentration in the nutrient solution decreased the B content in leaf and root tissues only slightly (excluding roots at the medium irradiance, wherein the decrease was larger) (Fig. 1). In contrast, the high B concentration in the nutrient solution increased the B content in leaves and roots dramatically. The B content tended to decrease with increasing irradiance at the low and medium B concentrations, but not at the high B availability. The root-specific B uptake exhibited similar B and irradiance responses as tissue B content: small decreases with low B, large increases with high B, small decreases with increasing irradiance at low and medium B, and no effect of irradiance at high B (Fig. 1).

In contrast to B, growth irradiance had strong negative effects on the content of other minerals in leaf tissue (Figs. 2 and 3, $P < 0.02$ for all nutrients). On the other

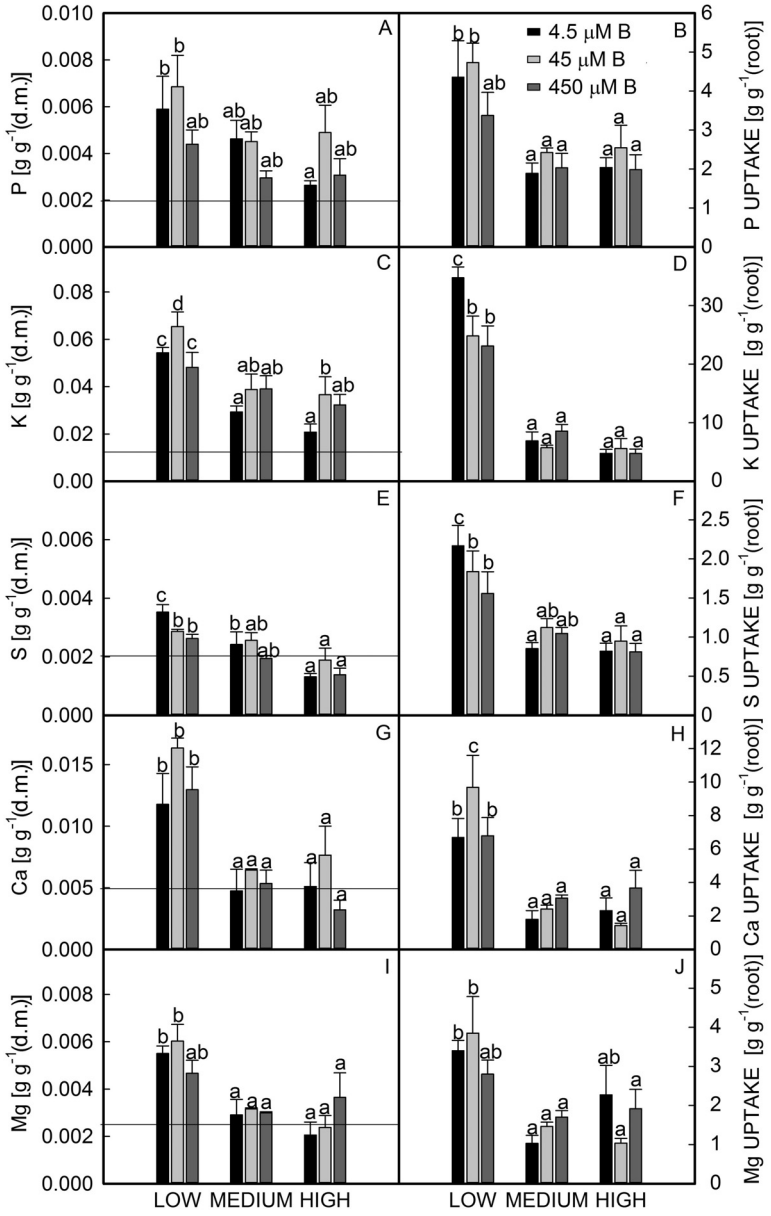

Fig. 2. Effects of B concentration (4.5, 45, or 450 μM) in the nutrient solution on the content of macronutrients in leaf tissue (*A*, *C*, *E*, *G*, *I*) and on the specific uptake of each mineral (the total content of mineral in plant per root dry mass unit; *B*, *D*, *F*, *H*, *J*) of geranium plants grown under low, medium, or high irradiances (100, 300 or 500 μmol m^{-2} s^{-1} PAR). Means ± SE, $n = 3$. Among all treatment combinations, different letters above the *bars* indicate significant differences ($P < 0.05$). Nutrient content corresponding deficiency (Taiz and Zeiger 2002) is indicated by a *horizontal line*.

hand, the effects of B availability on the content of other nutrients were variable and when affected by B, effects were only modest. However, both B deficiency and B toxicity often tended to (*i.e.*, non-significantly) exacerbated decreases in nutrients content caused by the increases in irradiance (*e.g.*, P, K, S, Ca, Cu, and Fe). In several instances, the high irradiance and/or B stress (low or high B) resulted in the nutrient content in leaves corresponding to nutrient deficiency symptoms (*i.e.*, Ca, Fe, and Mn). Similar results were observed for roots, wherein the increased growth irradiance also decreased the nutrients content, but B and B × irradiance effects were weaker and non-significant (excluding Ca) (data not shown). The increases in irradiance also decreased the root specific uptake of non-B nutrients, excluding Fe (Figs. 2 and 3). Boron effects on the nutrient uptake per g of root were mostly absent, excluding P, K, S, and Ca at the low irradiance and Fe at the medium irradiance. Hence, unlike the nutrient content, the combination of the B stress and high irradiance did not cause additive negative effects on the nutrient uptake per root dry mass.

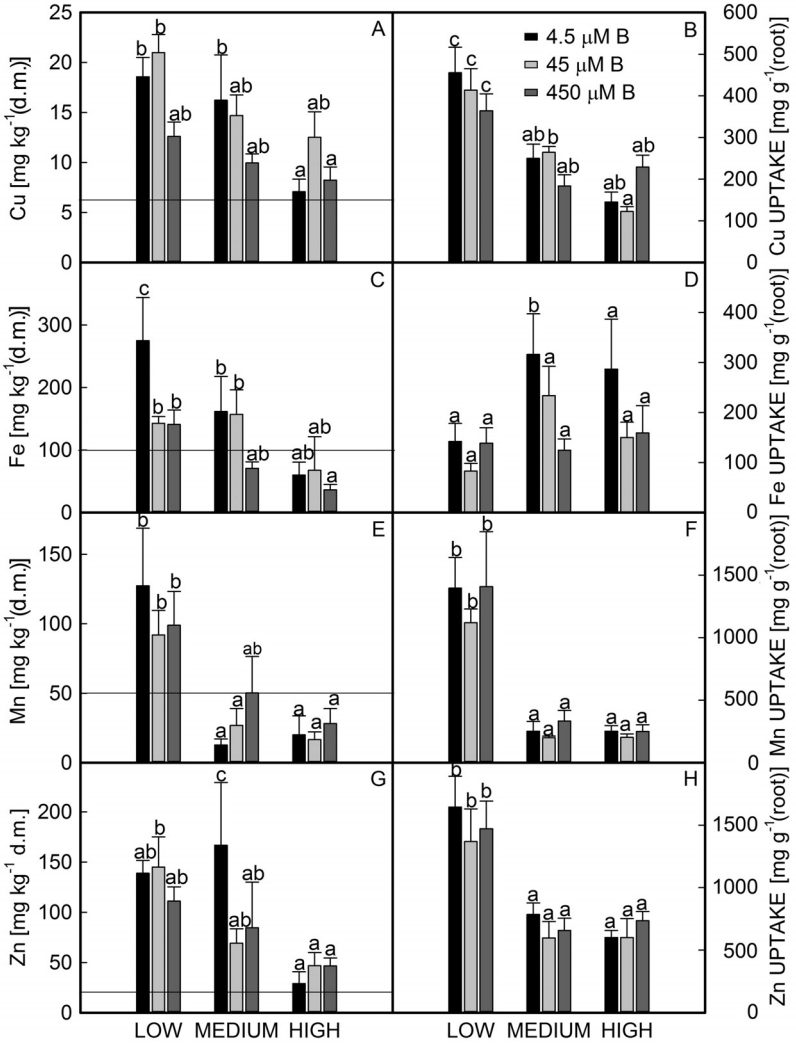

Fig. 3. Effects of B concentration (4.5, 45, or 450 μM) in the nutrient solution on the content of micronutrients in leaf tissue (*A*, *C*, *E*, *G*) and on the specific uptake of each mineral (the total content of mineral in plant per root dry mass unit; *B*, *D*, *F*, *H*) of geranium plants grown under low, medium, or high irradiance (100, 300 or 500 μmol m^{-2} s^{-1} PAR). Results are means ± SE, $n = 3$. Among all treatment combinations, different letters above the *bars* indicate significant differences ($P < 0.05$). Nutrient content corresponding deficiency (Taiz and Zeiger 2002) is indicated by a *horizontal line*.

Discussion

As in Mishra *et al*. (2009), increases in growth irradiance mitigated certain aspects of B stress in geranium in the current study. In the previous study, an increase in irradiance from 100 to 300 μmol m^{-2} s^{-1} delays

B-deficiency effects on photosynthesis, but the higher irradiance does not prevent decreases in leaf mass. In the current study, an increase in growth irradiance from 300 to 500 (but not from 100 to 300) μmol m^{-2} s^{-1} prevented decreases in growth caused by both the sub- and supra- optimal B availabilities, but the higher irradiance did not confer protection on P_N and related parameters (the chlorophyll and sugar content). In fact, neither the sub- nor supra-optimal B concentration had any negative effects on P_N or the chlorophyll or sugar content, except for decreases in P_N and the chlorophyll content at the highest irradiance and the highest B concentration indicating that B toxicity caused photoinhibition. In our previous study, we investigated only B deficiency, imposed by complete B withdrawal for 5 d, and only at 100 and 300 μmol m^{-2} s^{-1} PAR, whereas in the current study, we examined both B deficiency and toxicity, imposed continuously at 100, 300, and 500 μmol m^{-2} s^{-1} PAR for 30 d. It is likely that differences in the negative effects of B stress and the protective effects of increased growth irradiance between the two studies are mainly related to differences in B treatments.

Protective effects of increased growth irradiance against B toxicity were also observed in *Lemna pausicostata* (Tanaka 1966), but increased irradiance exacerbated B deficiency in this and past studies (Noppakoonwong *et al*. 1993, Cakmak *et al*. 1995, Huang *et al*. 2002, Mishra *et al*. 2009). In the current study, the protective effects of higher irradiance against B deficiency and toxicity were not associated with irradiance effects on tissue B content, since the B content in leaves and roots decreased with increasing growth irradiance in the low-B plants, which should exacerbate B deficiency, and the B content was not affected by irradiance in the high-B plants. Past studies of B × irradiance interaction have yielded inconsistent results with some finding correlation between irradiance effects and B content (Noppakoonwong *et al*. 1993, Cakmak *et al*. 1995, Huang *et al*. 2002) and some not (Tanaka 1966, Mishra *et al*. 2009). Hence, effects of irradiance on B stress are not likely mediated by the irradiance effect on the B tissue content.

Because we observed a mitigation of both B deficiency and toxicity effects on biomass with increases in growth irradiance level in this study, and increases in growth irradiance levels increased the biomass and P_N in general (*i.e.*, growth irradiance levels were limiting at the low or medium irradiances), we conclude that protective responses of the plants to the B stress were energy or carbon costly and could be constrained by growth under the low irradiance. Though excess irradiance can be phototoxic during B toxicity (Cakmak *et al*. 1995, Reid *et al*. 2004, and this study), we observed increases in leaf soluble sugar with increases in growth irradiance, and an enhanced sugar status can increase tolerance to B stress (Kastori *et al*. 1995, Brown *et al*. 1999, Zhao and Oosterhuis 2003). Hence, although we could not identify the specific reason for protection from the B stress with the increasing irradiance in this study, we can conclude that the increases in growth irradiance increased the energy or carbon status of the plants and this was correlated with plant resistance to the B stress.

Interestingly, we observed that both the sub- and supra-optimal B concentrations sometimes affected the concentration of the other (non-B) mineral nutrients in leaves (especially P, K, Cu, and Fe). Several previous studies, in addition to the current study, examined the effect of B deficiency (five studies, seven species) and toxicity (one study) on the concentration of nutrients in plant tissues (Mozafar 1989, López-Lefebre *et al*. 2002, Davis *et al*. 2003, Eraslan *et al*. 2007, Krug *et al*. 2009, Mishra *et al*. 2009), and it is evident that B deficiency and toxicity can both affect tissue nutrient content. However, these studies indicate no consistent effects of B stress on mineral content. For example, B deficiency can increase, decrease, or have no effect on Ca, Fe, K, P, Mg, and Zn content depending on conditions, species (Krug *et al*. 2009), and even genotypes within a single species (Mozafar 1989). Within a single study, B stress can decrease the content of some nutrients, but increase others, and effects are not different for cations and anions. Hence, though it should come as no surprise that B stress may affect other nutrients, given the importance of B to root growth, it is clear that we do not understand the effects of B stress on the uptake of nutrients aside from B.

Notably, the increases in growth irradiance caused a general, and often pronounced, decrease in tissue nutrient content, sometimes to levels corresponding nutrient deficiency (*i.e*., Fe, Mn, and Mg). Decreases in nutrient content with increasing irradiance indicate that the plant nutrient uptake was not keeping pace with increases in plant biomass production under the higher irradiance, and this was consistent with decreases in nutrient uptake per root dry mass with increasing irradiance (excluding Fe). Other studies have also noted decreases in tissue nutrient content or nutrient uptake rates with increases in irradiance, though in some species or situations, nutrient content and uptake rate can increase with irradiance (*e.g.*, Baligar *et al*. 2006, Taylor *et al*. 2008, and references therein). Importantly, the B stress (both the sub- and supra-optimal concentrations) often exacerbated the general decrease in the nutrients content with increasing growth irradiance, such that the combination of the high irradiance and B stress resulted in the nutrient content approaching deficiency (*e.g.*, Ca, Cu, and P). The B stress had little negative effect on the nutrient uptake per root dry mass at the high irradiance and did not decrease the root mass at the high irradiance, so the reason for the decrease in nutrient content with the B stress in the high-irradiance plants is unclear. The deleterious effect of B stress on nutrient content at high irradiance would likely be especially important for plants growing in nutrient-limited soil or in soil where the acquisition of nutrients require significant root growth (since B stress strongly limits root growth).

References

Baligar, V.C., Fageria, N.K., Paiva, A.Q., Silveira, A., Pomella, A.W.V., Machado, R.C.R.: Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. - J. Plant Nutr. **29**: 1959-1974, 2006.

Bañuelos, G.S., Ajwa, H.A., Caceres, L., Dyer, D.: Germination responses and boron accumulation in germplasm from Chile and the United States grown with boron-enriched water. - Ecotox. Environ. Safety **43**: 62-67, 1999.

Bishnoi, S.K., Kumar, B., Rani, C., Datta, K.S., Kumarip, P., Sheoran, I.S., Angrish, R.: Changes in protein profile of pigeonpea genotypes in response to NaCl and boron stress. - Biol. Plant. **50**: 135-137, 2006.

Blevins, D.G., Lukaszewski, K.M.: Boron in plant structure and function. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 481-500, 1998.

Brown, P.H., Bellaloui, N., Hu, H., Dandekar, A.: Transgenically enhanced sorbitol synthesis facilitates phloem boron transport and increases tolerance of tobacco to boron deficiency. - Plant Physiol. **119**: 17-20, 1999.

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer H., Dannel, F., Römheld V.: Boron in plant biology. - Plant Biol. **4**: 205-223, 2002.

Cakmak, I., Kurz, H., Marschner, H.: Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. - Physiol. Plant. **95**: 11-18, 1995.

Davis J.M., Sanders, D.C., Nelson, P.V., Lengnick, L., Sperry, W.J.: Boron improves growth, yield, quality, and nutrient content of tomato. - J. amer. Soc. hort. Sci. **128**: 441-446, 2003.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. - Anal. Chem. **28**: 350-356, 1956.

Eraslan, F., Inal, A., Gunes, A., Alpaslan, M.: Boron toxicity alters nitrate reductase activity, proline accumulation, membrane permeability, and mineral constituents of tomato and pepper plant. - J. Plant Nutr. **30**: 981-994, 2007.

Goldbach, H.E.: A critical review on current hypothesis concerning the role of boron in higher plants: suggestions for further research and methodological requirements. - J. Trace Microprobe Tech. **15**: 51-91, 1997.

Huang, L., Gherardi, M., Bell, R., Dell, B.: High light intensity increases external boron (B) requirements for leaf growth of sunflower (*Helianthus annus* L. cv. Hysun 25) in B-buffered solution culture. - In: Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier, M., Bell, R.W. (ed.): Boron in Plant and Animal Nutrition. Pp. 213-225. Kluwer Academic Press, New York 2002.

Kastori, R., Plesničar, M., Panković, D., Sakač, Z.: Photosynthesis, chlorophyll fluorescence and soluble carbohydrates in sunflower leaves as affected by boron deficiency. - J. Plant Nutr. **18**: 1751-1763, 1995.

Kouchi, H.: Rapid cessation of mitosis and elongation of root tip cells of *Vicia faba* by boron deficiency. - Soil Sci. Plant Nutr. **23**: 113-118, 1977.

Kobayashi, M., Matoh, T., Azuma, J.: Two chains of rhamnogalacturonan-II are cross-linked by borate-diol ester bonds in higher plant cell walls. - Plant Physiol. **110**: 1017-1020, 1996.

Kocábek, T., Svoboda, Z., Al-Zwi, A.M., Rolfe, S.A., Fellner, M.: Boron-regulated hypocotyl elongation is affected in *Arabidopsis* mutants with defects in light signaling pathways. - Environ. exp. Bot. **67**: 101-111, 2009.

Krug, B.A., Whipker, B.E., Frantz, J., McCall, I.: Characterization of calcium and boron deficiency and the effects of temporal disruption of calcium and boron supply on pansy, petunia, and gerbera plugs. - Hort. Sci. **44**: 1566-1572, 2009.

López-Lefebre, L.R., Rivero, R.M., García, P.C., Sánchez, E., Ruiz, J.M., Romero, L.: Boron effect on mineral nutrients of tobacco. - J. Plant Nutr. **25**: 509-522, 2002.

Marschner, H.: Mineral Nutrition of Higher Plants. - Academy Press, London 1995.

Matoh, T.: Boron in plant cell walls. - Plant Soil **193**: 59-70, 1997.

Mishra, S., Heckathorn, S., Frantz, J.: Elevated CO_2 affects plant responses to variation in boron availability. - Plant Soil **350**: 117-130, 2012.

Mishra, S., Heckathorn, S., Frantz, J., Gray, J., Yu, F.: Effects of boron deficiency on geranium grown under different nonphotoinhibitory light levels. - J. amer. Soc. hort. Sci. **134**: 183-193, 2009.

Mozafar, A.: Boron effect on mineral nutrients of maize. - Agron. J. **81**: 285-290, 1989.

Nable, R.O., Bañuelos, G.S., Paull, J.G.: Boron toxicity. - Plant Soil **193**: 181-198, 1997.

Nelson, M.R.: Index to EPA Methods. EPA circular 901/3-88-01. - U.S. Environmental Protection Agency, Washington 1988.

Noppakoonwong, R.N., Bell, R.W., Dell, B., Loneragan, J.F.: An effect of shade on the boron requirement for leaf blade elongation in black gram (*Vigna mungo* L. Hepper). - Plant Soil **155**: 317-320, 1993.

Power, P.P., Woods, W.G.: The chemistry of boron and its speciation in plants. - Plant Soil **193**: 1-13, 1997.

Reid, R.J., Hayes, J.E., Post, A., Stangoulis, J.C.R., Graham, R.D.: A critical analysis of the causes of boron toxicity in plants. - Plant Cell Environ. **25**: 1405-1414, 2004.

Reid, R.: Update on boron toxicity and tolerance in plants. - In: Xu, F., Goldbach, H.E., Brown, P.H., Bell, R.W., Fujiwara, T., Hunt, C.D., Goldberg, S., Shi, L. (ed.): Advances in Plant and Animal Boron Nutrition. Pp. 83-90. Springer, Dordrecht 2007.

Roessner, U., Patterson, J.H., Forbes, M.G., Fincher, G.B., Langridge, P., Bacic, A.: An investigation of boron toxicity in barley using metabolomics. - Plant Physiol. **142**: 1087-1101, 2006.

Shorrocks, V.M.: The occurrence and correction of boron deficiency. - Plant Soil **193**: 121-148, 1997.

Taiz, L., Zeiger, E.: Plant Physiology. - Sinauer Associates, Massachusetts 2002.

Tanaka, H.: Response of *Lemna paucicostata* to boron as affected by light intensity. - Plant Soil **25**: 425-434, 1966.

Taylor, M.D., Nelson, P.V., Frantz, J.M.: Substrate acidification by geranium: light effects and phosphorus uptake. - J. amer. Soc. hort. Sci. **133**: 515-520, 2008.

Zhao, D., Oosterhuis, D.M.: Cotton growth and physiological responses to boron deficiency. - J. Plant Nutr. **26**: 855-867, 2003.