

Research article

Effect of boron nutrition on American ginseng in field and in nutrient cultures

John T.A. Proctor*, Barry J. Shelp

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

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ABSTRACT

Field and nutrient cultures of American ginseng (*Panax quinquefolius* L.) were used to establish foliar symptoms related to boron (B) concentration in leaves and soils, and to evaluate radish as a time-saving model system for B nutrition. Application of excess B, 8 kg/ha versus the recommended 1.5 kg/ha, to field plantings of 2-, 3-, and 4-yr-old American ginseng plants just prior to crop emergence caused, within 4 wk after crop emergence, leaf symptoms of chlorosis followed by necrosis starting at the tips and progressing along the margins. The B concentration in leaves of 2–4-yr-old plants receiving 1.5 kg/ha B was 30 µg/g dry mass compared to 460 µg/g dry mass where 8 kg/ha B was applied. Similarly, B concentration in soils receiving the lower B concentration was 1.8 µg/g dry mass and 2.2–2.8 µg/g dry mass where the higher B concentration was applied. Application of 8 kg/ha B reduced the dry yield of 3rd-yr roots by 20% from 2745 kg/ha to 2196 kg/ha and 4th-yr roots by 26% from 4130 kg/ha to 3071 kg/ha. Ginseng seedlings and radish were grown under greenhouse conditions in nutrient culture with four B concentrations ranging from 0 mg/L to 10 mg/L. At 5 mg/L and 10 mg/L ginseng and radish developed typical leaf B toxicity symptoms similar to those described above for field-grown plants. Increasing B in the nutrient solution from 0.5 mg/L to 10 mg/L decreased, in a linear fashion, the root and leaf dry mass of ginseng, but not radish. Given the many similarities of ginseng and radish to B utilization, radish might be used as a time-saving model system for the study of B, and other micronutrients, in the slow-growing perennial ginseng.

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1. Introduction

American ginseng (*Panax quinquefolius* L.) is a minor crop in North America and there is little research information to assist growers of the crop [1,2]. Even data for mineral nutrition of the crop are sparse. Stoltz [3] described various foliar deficiency symptoms for ginseng grown in nutrient solutions. He reported that root fresh mass gain, the most important economic yield component, was most reduced by the omission of calcium, phosphorus, or magnesium from the nutrient solution. He did not study boron (B) nutrition. Khwaja and Roy [4] have given nutrient ranges in ginseng based on extensive sampling of growers' fields. Minimum and maximum B concentrations in leaves of 2–4-yr-old plants were: 5 µg/g, deficient; 5–15 µg/g, low; 16–50 µg/g, sufficient; 51–100 µg/g, high; and >100 µg/g, excessive.

Konsler and Shelton [5] and Konsler et al [6] described the effect of lime and phosphorus on the growth, nutrient status, and ginsenoside content of the ginseng root.

Ginseng production in Ontario, Canada, the major center for American ginseng culture, is on sandy and sandy-loam soil with low organic matter content, along the north shore of Lake Erie [7]. In general, these soils are low in B for production of many crops [8,9]. Previously, we reported that the rusty root of ginseng and associated internal browning of roots grown in the above-mentioned soils may be linked to B deficiency [10].

B is required by plants only in small amounts, therefore, over-application to crops can occur easily. Oliver [11] recommended that to maintain adequate soil levels of B for ginseng cultivation, 1–2 kg/ha should be applied when soil tests show ≤ 0.5 µg/mL. B is taken up through the plant roots as boric acid and transported with the transpiration flow. In most plants, B is highly immobile [12], being restricted to the transpiration stream. Accumulation of B can occur at the end of the transpiration stream in the leaves [13]. Manifestation of B toxicity shows as damage to tissues where it accumulates. Although B toxicity is crop-specific, it generally leads to chlorosis and necrosis starting at the edges of mature leaves [12,13].

* Corresponding author. Department of Plant Agriculture, University of Guelph, Guelph, Ontario N1G 2W1, Canada.
E-mail address: jprocto@uoguelph.ca (J.T.A. Proctor).

This development of necrotic areas can reduce leaf photosynthetic potential, cause a reduction of photosynthetic supply to the developing root system, the economic part of the ginseng plant, and restrict activity in the meristematic tissues. It is unclear why B is toxic to plants, or why some plants can tolerate B and evade toxicity [13]. Reid et al [14] concluded that, at high B concentrations, many cellular processes are retarded and these are often made worse in light by photooxidative stress.

Ginseng is a perennial plant requiring about 4 yr from seeding to root harvest, therefore, we examined the possibility of using radish as a time-saving model system in our B nutritional studies. Radish requires 3–6 wk from seeding to root harvest and B deficiency induces root splitting and brown heart disorder [15], similar to brown heart in ginseng [10]. Also, B toxicity in radish reduces root growth [16,17].

Lack of definitive data on B nutrition of American ginseng, the supposed deleterious effects on the leaves, roots, and meristematic regions, and an application of a high concentration of B to commercial ginseng plantings prompted this investigation. The objectives of this work with ginseng were to describe, in field plantings and nutrient cultures, the foliar symptoms associated with the application of different rates of B; to establish that foliar symptoms were due to B; to obtain general information about B nutrition, particularly toxicity; and to evaluate radish as a model system for the B nutrition of ginseng.

2. Materials and methods

2.1. Field experiments

For the field experiments, seedlings were established at a seeding rate of 112 kg/ha (about 215 seeds/m² or 46.5 cm² space per seedling) and grown following standard cultural methods for American ginseng [18]. Seeds were planted on raised soil beds and covered with 5–10 cm of straw mulch. Woven black polypropylene shade was placed 2 m above the beds to reduce solar radiation to an optimal 20–30% of full sunlight. Standard commercial practices for pest control were followed [18]. Field experiments were carried out with 2-, 3-, and 4-yr-old plants using 1-m² plots having guards also of 1 m². Treatments of B were 1.5 kg/ha (control) and 8 kg/ha. They were replicated four times in a randomized complete block design with four blocks. The broadcast soil-applied commercial fertilizer was applied prior to crop emergence and was based on superphosphate, potassium chloride, ammonium sulfate, magnesium sulfate, and zinc sulfate (N 9.0%, P 7.0%, K 7.4%, Ca 8.5%, S 9.8%, Mg 8%, and Zn 0.9%). Sodium borate (14% B) was added to the blended mixture to produce final B rates of 1.5 kg/ha and 8 kg/ha.

2.2. Greenhouse experiments with ginseng and radish

These pot experiments were carried out in a greenhouse without supplemental lighting at the University of Guelph, Guelph, Ontario; latitude 43° 32' N, longitude 80° 15' W. Ginseng mature stratified seeds were purchased from a local Ontario grower in October. These seeds were mixed with moistened mortar sand (1 seed/3 sand, v/v) and put in plastic containers that were held in a controlled-environment room (4 ± 1 °C, 50 ± 5% relative humidity) until the experiments were started in January. For the radish (*Raphanus sativus* L. cv. Cherry Belle), experimental seeds were purchased from a commercial seed house.

For the pot experiments with the two plant species, 10 seeds were planted equidistant within each wide (21 cm diameter) and

Table 1

Effect of broadcast application of B at two rates on the soil elemental composition of test fields containing different age ginseng plants¹⁾

B rate, plant age	Soil pH	OM (%)	Macroelement (ppm)				Microelement (µg/g dry mass)		
			P	K	Ca	Mg	Mn	Zn	B
1.5 kg/ha									
3 yr	5.4	2.1a*	93.2a	138.7ab	456.5	51.5ab	52.5	30.5a	1.8a
8 kg/ha									
2 yr	5.5	2.0b	63.0c	159.7a	470.7	46.0b	47.2	41.0b	2.7bc
3 yr	5.4	1.5b	79.0b	116.5b	473.0	49.7ab	60.5	47.0b	2.8c
4 yr	5.4	2.1a	83.2b	149.2a	482.7	57.7a	62.2	47.0b	2.2ab

* Mean separation in columns by different letters by Duncan's multiple range test, $p < 0.05$.

OM, organic matter.

¹⁾ The soil was sampled 2 mo after the fertilizers were applied.

deep (21 cm) pot. Seed germination averaged 60%. Seeding depths of 40 mm for ginseng and 20 mm for radish were used. The germination and growing medium for all seedlings was vermiculite. The pots were filled to within 3 cm of the top with the vermiculite.

Light transmission of the greenhouse was measured with a quantum, or line quantum, sensor (LI-COR, Lincoln, NE, USA). For the ginseng greenhouse experiments, 30% of the incident light at the top of the seedlings was established by suspending different thicknesses of knitted black polypropylene shade cloth above the pots. Radish plants were grown under ambient light. For each experiment, repeated at least twice, there was a minimum of four pots per treatment in a completely randomized design.

Plants were managed and fertilized as described previously [15]. Every 3rd day plants were fertilized with 1 L full-strength Hoagland's solution as described by Knott et al [19]. The standard (control) solution was prepared with distilled water and contained 0.5 mg/L B and 200 mg/L calcium. Four B treatments were used: 0 mg/L, 0.5 mg/L, 5 mg/L, and 10 mg/L.

2.3. Soil and plant sampling

In the field experiments, soil samples were taken 2 mo after fertilizer application (Table 1) [20]. At the end of the growing season, the 2-yr-old plantings were discarded because leaf damage was extensive and root growth was reduced to the point that predicted yield at harvest would not generate a profit. At the end of the growing season, all roots in the 1-m² areas of each of 3- and 4-yr-old plantings were dug by hand. The harvested roots were washed free of soil, dried to constant weight at 38 °C, and weighed. These yields were then converted to kg/ha.

In the pot experiments, at the end of the growing season of 70 d for radish and 100 d for ginseng, plants were assessed for foliar symptoms and then harvested. The roots were also assessed visually for deficiency or toxicity symptoms of root color and surface texture and cracking, and given a rating of 0 for no symptoms and 1, 2, and 3 for mild, moderate, and severe, respectively. Each seedling was then separated into leaves and roots and dried to constant weight at 80 °C.

Where appropriate, data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC, USA). Descriptive statistics such as means and standard deviations were calculated. Regression analysis was used to evaluate relationships between ethephon application and plant response in field experiments, and between ethephon application and plant response of both ginseng and radish plants grown in pots in greenhouse experiments.

3. Results and discussion

3.1. Leaf symptoms of boron toxicity

The first sign of B injury observed in the field was leaf-tip yellowing. The soil-applied fertilizer containing the excess B, 8 kg/ha instead of 1.5 kg/ha, was applied to the bare soil in late April. Crop emergence started in early May and was completed by late May [21,22]. During May, transpiration would have increased with canopy growth and the B translocated to the transpiring leaves for accumulation at the leaf tips [12,23]. Gupta and Arsenault [24] also applied B to the soil at 8.8 kg/ha to field-grown tobacco (*Nicotiana tabacum* L.) and found B toxicity symptoms of spotting, browning, and burning of the leaf edges.

In another perennial species like ginseng, grapevine, *Vitis vinifera* L. 'Sugarone', Yermiyahu et al [25] reported that B toxicity symptoms appeared about 1 mo after leaf emergence. Here, leaf-tip yellowing on ginseng leaves spread along the leaf margins and then necrosis progressively developed from the tips and along the margins towards the leaf mid-rib. The leaf tips and margins took on a burned appearance that did not cover the entire leaf or lead to premature leaf senescence. Thus, ginseng is like most plant species in the way it displays leaf toxicity symptoms in response to high levels of B [13].

Flowering, fruit set, and berry growth were unaffected by the B toxicity of the leaves. Unfortunately, B analysis of the berry mesocarp and seed was not carried out to determine if these strong sinks [26] are an alternative site for B accumulation in ginseng as they are in plants with developing sinks such as *Prunus*, *Malus*, and *Pyrus* [13].

3.2. Boron concentrations in the soil and leaves of ginseng

The soils of this ginseng growing area are slightly acidic (pH 5.4–5.5) and contain low organic matter, 1.5–2.1%, (Table 1) [8]. These soils are also low in B [9], with a normal concentration of 1.8 µg/g. Application of a high rate of B (8 kg/ha) raised the average B soil concentration available for the three ages of ginseng to 2.6 µg/g, (range 2.2–2.8 µg/g, Table 1); a 40% increase. Gupta and Arsenault [24] reported soil B levels of 3.0–3.4 µg/g where B had been applied at 8.8 kg/ha. There were no differences among the treatments in calcium and manganese (Table 1). Although there were some differences in phosphorus, potassium, magnesium, and zinc, these were relatively minor and did not show a pattern. Smith and Clark [27] also reported no significant effect of excess B on the soil concentration of mineral elements other than B.

The most striking aspect about the distribution of B in ginseng plants grown on soil supplemented with 8 kg/ha B was elevated concentrations in the leaves of each age of plant, compared to the treatment with 1.5 kg/ha B (Table 2). The B concentration in the leaves was increased by about 10–19-fold in response to the treatment, whereas the concentration in the root was decreased by

Table 2
Distribution of B in different age field-grown ginseng plants following broadcast application at two rates

B rate (kg/ha), plant age	Tissue B (µg/g dry mass)		
	Root	Stem	Leaves
1.5 ¹⁾ , 2–4 yr	35 ± 2 ²⁾	30 ± 2	30 ± 3
8, 2 yr	23 ± 2	39 ± 3	580 ± 50
8, 3 yr	21 ± 1	25 ± 2	340 ± 26
8, 4 yr	21 ± 1	26 ± 2	337 ± 41

¹⁾ Plants receiving 1.5 kg/ha B were pooled for presentation.

²⁾ Data represent the mean ± standard deviation (n = 4).

about 40%, and that in the stem was unaffected. From ginseng field survey work, Khwaja and Roy [4] considered >100 µg/g B in leaves as excessive. In another perennial plant, kiwifruit, *Actinidia deliciosa* var. *deliciosa*, Smith and Clark [27] reported that symptoms of B toxicity in leaves were associated with B levels in excess of 100 µg/g dry mass. Gupta and Arsenault [24] found that B toxicity symptoms in tobacco were associated with B levels of 113–119 µg/g. Nable et al [13], in a review of B leaf analysis in relation to toxicity, noted that B concentrations >300 µg/g generally indicate the presence of B toxicity.

There was a good relationship ($R^2 = 0.38$, $p < 0.01$) between B levels in the top 15 cm of soil and B levels in leaves of 2-, 3- and 4-yr-old ginseng (Fig. 1). Also, plants growing in soil containing >1.8 µg/g B showed toxicity symptoms in the leaves that had B in excess of 200 µg/g (Fig. 1). For each increase of 1 µg/g B in the soil, the leaf B increased by 236 µg/g (Fig. 1). Smith and Clark [27], working with the woody perennial, kiwifruit, also growing in field soil, reported an increase of 117.5 µg/g B in the leaves for each increase of 1 µg/g B in the soil. Previously, Yermiyahu et al [25] irrigated grapevines growing in perlite in pots with four concentrations of B and found that B accumulated in leaves linearly, as found here for ginseng. The rate of B accumulation for the grapevines varied from 22.9 mmol/kg per mM in March to 515 mmol/kg per mM in September. These uptake patterns of B are similar to those reported for many other plant species [12].

3.3. Yield reduction

Although B toxicity symptoms in these ginseng leaves were readily visible, and diagnostic (see above), visible symptoms in roots did not develop as has been reported for other plants [13]. However, the spring broadcast application of 8 kg/ha B to the soil reduced the root yield of 3- and 4-yr-old ginseng by 20% and 26%, respectively (Table 3). An explanation for this result is not known but chlorotic and necrotic damage to the leaves early in the growing season may have reduced the photosynthetic area and activity of the leaves, leading to reduced photoassimilate partitioning to the roots. It is unlikely that root growth was directly affected, because work with tomato roots concluded that B toxicity does not cause major oxidative or membrane damage and that lignification is not a factor in reducing root growth [28]. Previous research demonstrated that B application of 8 kg/ha reduced tuber yield of potato (*Solanum tuberosum* L. cv. Sebago) by 15% [29] and tobacco yield by

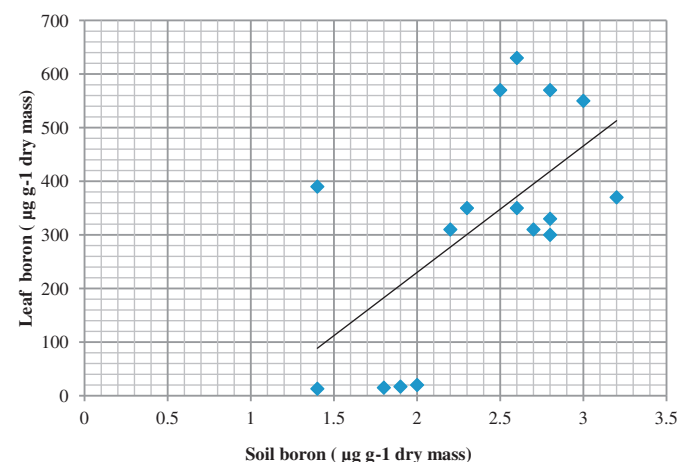


Fig. 1. Relationship between the concentration of B in 2-, 3- and 4-yr-old ginseng leaves and the B in the topsoil (0–15-cm depth). The equation for the regression line is Y (leaf B) = 235.8 X (soil B) – 241.5, $R^2 = 0.38$, and $p < 0.01$.

Table 3

Effect of broadcast application of B at two rates on yield of dried root of different age field-grown ginseng plants

B rate (kg/ha), plant age (y)	Root yield (kg/ha)
1.5, 3	2,745 ± 178a ¹⁾
8, 3	2,196 ± 101b
1.5, 4	4,130 ± 216a
8, 4	3,071 ± 48b

¹⁾ Data represent the mean ± standard deviation ($n = 4$); means within columns followed by a different letter are significantly different by Student's *t* test, $p < 0.05$.

19% [24]. By contrast, other research on grapevines did not find yield reductions from high B applications and suggested that this may be due to early crop ripening and harvesting [30]. However, grapevines have an indeterminate growth habit and new growth may compensate for damaged leaf tissue. Ginseng has a determinate growth habit producing one set of leaves at the beginning of the season [22], and therefore lacks the ability to compensate for loss of leaf photosynthetic area caused by application of high B rates.

3.4. Greenhouse studies – ginseng and radish

3.4.1. Ginseng

Ginseng seedlings receiving 0 mg/L or 0.5 mg/L B nutrient solution appeared normal with green leaves, whereas those receiving 5 mg/L or 10 mg L B developed typical leaf symptoms of marginal leaflet yellowing and necrosis similar to those described above for plants growing in the field that had received 8 kg/ha B. There were no visual signs of B toxicity on these ginseng roots (Table 4).

The leaf B concentration of ginseng seedlings receiving no applied B was about 50% lower than the leaf B concentration of plants receiving 0.5 mg/L B (Table 4). By contrast, root B concentration was only about 20% lower, although the resulting concentration of 20 µg/g would typically be considered as inadequate [19,23]. These findings have implications for the fertilization of ginseng seedlings because they may be B deficient but not display any leaf symptoms.

As the concentration of B was increased in the nutrient solution from 0.5 mg/L to 10 mg/L, there were seven- and onefold increases in leaf and root B concentrations, respectively (Table 4). This was accompanied by a linear decline in the dry masses of ginseng leaves and roots (Table 5, $R^2 = 0.49–0.52$, $p < 0.01$). The rate of loss of dry mass was 2.10 mg, 1.17 mg, and 3.89 mg for each increase of 1 mg/L B of nutrient solution for roots, leaves, and total mass, respectively. For roots, the loss in dry mass was 25% at 10 mg/L B. These results are similar to those for the 3- and 4-yr-old field-grown roots where yield was reduced by 20% and 26%, respectively, by the application of 8 kg/ha B (see above).

Table 4

Distribution of B in greenhouse-grown ginseng seedlings and radish plants supplied with four B rates in nutrient solution

B rate (mg/L)	Tissue B (µg/g dry matter) ¹⁾			
	Ginseng		Radish	
	Leaves	Roots	Leaves	Roots
0	27 ± 1 ²⁾ (none)	20 ± 2 (none)	62 ± 7 (none)	27 ± 3 (deficient)
0.5	50 ± 1 (none)	25 ± 2 (none)	213 ± 14 (none)	40 ± 1 (none)
5.0	270 ± 4 (toxic)	31 ± 2 (none)	375 ± 18 (toxic)	54 ± 2 (none)
10.0	419 ± 17 (toxic)	41 ± 2 (none)	503 ± 63 (toxic)	68 ± 3 (none)

¹⁾ Visual response symptoms to B of none, deficient, or toxic are noted in parentheses.

²⁾ Data represent the mean ± standard deviation ($n = 8$).

Table 5

Dry mass accumulation of greenhouse-grown ginseng seedlings and radish plants supplied with four B rates in nutrient solution

B rate (mg/L)	Dry mass (mg)					
	Ginseng			Radish		
	Shoot	Root	Total	Shoot	Root	Total
0	47 ± 9 ¹⁾	50 ± 14	97 ± 21	28 ± 6	79 ± 17	107 ± 14
0.5	61 ± 12	66 ± 15	127 ± 26	38 ± 8	91 ± 23	129 ± 31
5	55 ± 6	59 ± 2	111 ± 3	36 ± 4	75 ± 11	111 ± 14
10	44 ± 1	49 ± 2	93 ± 3	36 ± 5	82 ± 12	118 ± 17
Regression analysis (0.5-, 5-, 10-mg/L)	L	L	L	NS	NS	NS

L, effect linear at $p < 0.01$; NS, not significant.

¹⁾ Data represent the mean ± standard deviation ($n = 8$).

3.4.2. Radish

The leaves of the radish plants not receiving supplementary B did not show any B leaf injury symptoms, which agrees with reports by Francois [17] and Shelp et al [15]. The roots of these plants were sometimes misshapen with rough, dull skin and had a moderate to severe cracking and were considered to be B deficient (Table 4). Plants that received 5 mg/L and 10 mg B showed leaf marginal chlorosis and necrosis but no root damage. The leaf damage was similar to that reported by Kelly et al [16], who noted some marginal leaf chlorosis on plants receiving 5 mg/L B. Generally, visible symptoms of B toxicity do not appear in roots, because B concentrations in the roots remain relatively low compared to those in leaves [13] (Table 4).

In the absence of B, the top dry mass was reduced by 26% but the total radish plant dry mass was reduced by only 17% (Table 5). However, this was a trend only, because there was considerable biological variability in the data. As the B concentration in the applied solution was increased from 0.5 mg/L to 10 mg L, the total dry masses appeared to be reduced, although this result was not significant, even using regression analysis; probably because of the considerable variability. Previous research reported a reduced root mass of 1.4% and top weight of 2.0% with radish for each increase of 10 mg/L B in the soil solution [17].

There was a strong linear relationship (Table 6, $R^2 = 0.87–0.98$, $p < 0.001$) between the concentration of B in the applied nutrient solution and the concentration of B in the leaves and roots of both ginseng seedlings and radish plants. These results are similar to those of Yermiyahu et al [25] working with grapevine leaves growing in perlite in pots and irrigated with B solutions. They reported R^2 values of 0.85–0.99. In earlier work, Yermiyahu et al [30] reported R^2 values of 0.90–0.98 for B accumulation in grapevine roots.

3.5. Ginseng root regrowth study

None of the leaves of plants growing in vermiculite displayed B toxicity symptoms. Also, flowering and fruit set were normal. These leaves did not display B toxicity symptoms, therefore, it is suggested that relatively low, nontoxic concentrations of B accumulated in the roots during the previous growing season. Normal

Table 6

Relationship between tissue and nutrient solution B in greenhouse-grown ginseng seedlings and radish

Equation	R^2
(1) Ginseng leaf B = 37.3 + 39.8 solution B	0.98*
(2) Radish leaf B = 39.0 + 137.1 solution B	0.87*
(3) Ginseng root B = 21.8 + 1.9 solution B	0.91*
(4) Radish root B = 32.9 + 3.7 solution B	0.91*

* $p < 0.001$.

development of the leaves, flowers, fruit set, and berries occurred in plants growing in soil with 1.8–2.4 $\mu\text{g/L}$ B suggesting that the B levels carried over in the soil were not phytotoxic. Nable et al [13] suggested that many plant species can tolerate soil B levels in excess of 5 $\mu\text{g/g}$. In summary, this root regrowth study suggests that high levels of applied B are rapidly translocated to the transpiring ginseng leaves, which are then lost during fall senescence. The B concentrations in the persisting roots and soil were not high enough to be phytotoxic in the next plant growing cycle.

In conclusion, the Lake Erie counties of Southern Ontario, Canada, where these field studies were carried out, are the major area of *P. quinquefolius* production in the world [7]. The soils of this area are of lacustrine origin and are sandy to sandy-loam with low organic matter content (Table 1), and [8]. Management of micronutrients, such as B, in these soils requires precision as there is a narrow margin between adequate and toxic concentrations. These studies emphasize this point.

B accumulation in ginseng leaves correlated with B toxicity symptoms, which included chlorosis and necrosis starting at the leaf margins. B levels in ginseng leaves were linearly related to soil B levels. B accumulation patterns and levels in greenhouse-grown ginseng and radish were similar to those found in the field. High levels of B reduced ginseng root yield in both field and greenhouse experiments. In the context of these results, it is suggested that B concentrations should not exceed 100 $\mu\text{g/g}$ in ginseng leaves or 2 $\mu\text{g/g}$ dry mass in the topsoil.

The greenhouse studies with ginseng and radish complemented and confirmed the findings in the field studies. Radish responded similarly in many instances to B deficiency and toxicity in ginseng, therefore, it may serve as a time-saving model system for the study of B, and other micronutrients, in the perennial plant, ginseng.

Conflicts of interest

All authors have no conflicts of interest to declare.

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