

Foliar Application of Zinc and Boron Improves Walnut Vegetative and Reproductive Growth

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SUMMARY. An experiment was conducted in a persian walnut (*Juglans regia*) orchard in the north of Iran to evaluate the effects of zinc (Zn) as zinc sulfate and/or boron (B) as boric acid in foliar spray with different concentrations and combinations. Three B and three Zn concentrations (0, 174, and 348 mg·L⁻¹ for B and 0, 1050, and 1750 mg·L⁻¹ for Zn) were applied either independently or in combination. Leaf nutrient concentrations, pollen germination, fruit set, leaf chlorophyll index, nut and kernel characteristics, vegetative growth, nut weight, and nut yield were measured to assess the effects of treatments. The results showed that all B and Zn applications and combinations had a significant effect on all traits except nut and kernel diameter, shell percent, husk thickness, and pistillate flower abscission (PFA). Pollen germination, fruit set, vegetative growth, nut weight, kernel percent, nut and kernel length, and chlorophyll index were highest when B and Zn were applied simultaneously at 174 and 1050 mg·L⁻¹ concentrations, respectively.

Persian walnut is sensitive to B and Zn deficiency (Ramos, 1997), especially in sandy soils with low organic matter and also in calcareous soils (Storey, 2007). These conditions are predominant in many walnut orchards around the world, particularly in Iran (Momeni, 2003; Ziaecian and Malakouti, 2001). Boron and Zn deficiencies are more probable early in the season because the translocation of elements from the root to the aboveground portion may not be adequate before leaf expansion (Nielsen et al., 2004). Zinc and B have a critical effect on flowering and fruit set and for this reason spring foliar application of these elements are frequently recommended in walnut orchards. In soils with a strong Zn fixation capacity, spring foliar fertilization has several advantages

including low application rate, uniform distribution of fertilizer materials, efficacy before leaf development, and quick response (Umer et al., 1999).

Severe B deficiency symptoms in walnut trees consist of long, leafless shoots, mostly in the tops of trees, and flattened and twisted shoots at the tips. These shoots die during the following winter. Moderate B deficiency in walnut results in reduced yields in otherwise healthy looking trees (Ramos, 1997). Boron plays an important role in pollen germination and pollen tube growth (Storey, 2007) and foliar sprays of B increase pollen germination in a number of tree species including almond [*Prunus amygdalus* (Nyomora et al., 1997)],

pear [*Pyrus communis* (Lee et al., 2009)], and meyer spruce [*Picea meyeri* (Wang et al., 2003)] and fruit set in almond, sweet cherry (*Prunus avium*), hazelnut (*Corylus avellana*), and apple (*Malus domestica*) (Nyomora et al., 1997; Shrestha et al., 1987; Silva et al., 2003; Usenik and Stampar, 2002; Wojcik and Treder, 2006).

Zinc is a cofactor of over 300 enzymes and proteins and has an early and specific effect on cell division, nucleic acid metabolism, and protein synthesis (Marschner, 1986). In many deciduous species, Zn deficiency can have a marked effect on pollen production, pollen physiology, floral anatomy, and yield (Usenik and Stampar, 2002; Ute and Clemens, 2005). Zinc deficiency in walnut is visually expressed as small leaves and nuts, delayed opening of vegetative and flower buds, leaf chlorosis between the lateral veins, wavy leaves with upward folded leaf margins and terminal dieback (Ramos, 1997). Foliar applications of Zn have been successfully used to promote tree vigor, fruit set, and yield in apple (Wojcik, 2007) and 'Washington Navel' orange (*Citrus sinensis*) (Hafez and El-Metwally, 2007). Brown et al. (1995a) stated that applications of foliar Zn to mature leaves were ineffective and did not provide significant Zn to new leaves developed after spray application or in the subsequent spring. The best time for Zn foliar application was found to be immediately after pistillate flower senescence and for the 2 weeks subsequently (Brown et al., 1995a). Both Zn and B applications have been observed to have a positive effect on chlorophyll contents in B- and Zn-deficient plants

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Units

| To convert U.S. to SI, multiply by | U.S. unit | SI unit | To convert SI to U.S., multiply by |
|------------------------------------|-----------------------|----------------------------------|------------------------------------|
| 0.4047 | acre(s) | ha | 2.4711 |
| 0.3048 | ft | m | 3.2808 |
| 0.0283 | ft ³ | m ³ | 35.3147 |
| 0.0700 | ft ³ /acre | m ³ ·ha ⁻¹ | 14.2913 |
| 3.7854 | gal | L | 0.2642 |
| 9.3540 | gal/acre | L·ha ⁻¹ | 0.1069 |
| 2.54 | inch(es) | cm | 0.3937 |
| 25.4 | inch(es) | mm | 0.0394 |
| 0.4536 | lb | kg | 2.2046 |
| 1.1209 | lb/acre | kg·ha ⁻¹ | 0.8922 |
| 1 | mmho/cm | dS·m ⁻¹ | 1 |
| 28.3495 | oz | g | 0.0353 |
| 1 | ppm | mg·kg ⁻¹ | 1 |
| 1 | ppm | mg·L ⁻¹ | 1 |
| (°F - 32) ÷ 1.8 | °F | °C | (1.8 × °C) + 32 |

(Kaya and Higgs, 2002; Zheng et al., 1989). Foliar B and Zn have both been observed to increase vegetative growth in mango (*Mangifera indica*) when trees were sprayed with 800 mg·L⁻¹ Zn (Rajput et al., 1976).

Several studies have emphasized the critical role that concentration of Zn and B in the spray solution has on efficacy of the application. Nyomora et al. (1997) showed that foliar application of 245 and 490 mg·L⁻¹ of sodium octaborate (Solubor; U.S. Borax, Greenwood Village, CO) in almond resulted in greater fruit set and yield than either the application of 0 or 735 mg·L⁻¹. Solar and Stampar (2001) reported that yield of hazelnut trees was highest in the treatment with 2000 mg·kg⁻¹ B + 2000 mg·L⁻¹ Zn and lowest in the treatment with 1000 mg·L⁻¹ B + 1000 mg·L⁻¹ Zn. Silva et al. (2003) stated that the foliar application of 600 and 900 mg·L⁻¹ of B increased nut yield of hazelnut more than 300 mg·L⁻¹ treatment.

The possible synergistic effect of combined Zn and B applications has been examined in several species. In gladiolus (*Gladiolus grandiflorus*), height and number of effective leaves per plant were progressively increased by the addition of B and Zn up to 2 and 3 kg·ha⁻¹, respectively, in contrast with the application of each individually. A greater or lower concentration of either element resulted in lower productivity than the combined spray (Memon et al., 2009). Moreover, foliar application of B and Zn, simultaneously in almond trees, increased fruit set by 38%, whereas when B or Zn was applied alone, fruit set was 27.7% and 22.2%, respectively (Sotomayor et al., 2000). Combined foliar application of Zn and B on sweet cherry increased fruit set and yield to a greater extent than application of either element alone (Usenik and Stampar, 2002).

Despite many studies on the response of deficient fruit trees to Zn and B foliar application, there is little knowledge directly relevant to walnut trees. Because of the high cost of labor and application machinery, it is currently a standard practice to combine foliar materials into a single spray. The main objectives of this study were to determine the best concentration and combination of Zn and B for application to persian walnut.

Material and methods

The experiment was conducted from 2007 to 2009 in a 400-ha commercial persian walnut orchard located in Shahmirzad, Semnan Province, Iran (lat. 52°21'N, long. 35°41'W). The height of trees was 3 m and crown size was 11.5 m³. The orchard was planted on strongly sloping land (8% grade), and the soil texture was silty loam with pH 7.9, containing about 60% calcium carbonate and less than 0.8% organic matter (Table 1). Walnut trees require a soil of fairly neutral pH between 6.5 and 7.2. In soils with pH of 7.0 or higher, Zn and some other micronutrients are held very tightly by the soil and are unavailable for plant uptake. The effect of pH on B at these pH ranges is not well characterized but would generally be expected to be minimal. The plant materials consisted of 10-year-old Z₆₃ domestic walnut seedling of promising genotype spaced 15 × 15 m². Trees were drip irrigated using one drip line for each row, with seven emitters per tree, each with a flow rate of 4 L·h⁻¹. Irrigation was applied from early April until mid December. The volume of irrigation water was about 4000 m³·ha⁻¹, and trees were irrigated with 16 m³ of water weekly. The experiment was a completely block randomized 3 × 3 factorial design with two factors, three B concentrations (0, 174, and 348 mg·L⁻¹ for B₀, B₁, and B₂, respectively) and three Zn concentrations (0, 1050, and 1750 mg·L⁻¹ for Zn₀, Zn₁, and Zn₂, respectively) in four replications (blocks). Boron was applied as boric acid (17.4% B) and Zn was applied separately as Zn sulphate monohydrate (35% Zn) manufactured by Golzar (Tehran, Iran). In B₀Zn₀, trees received no foliar-applied Zn or B. Foliar sprays were performed by means of a handgun sprayer (34 L·min⁻¹) using 1000 L·ha⁻¹. There were 10 trees in each block, and data from 10 trees were averaged and treated as a single replicate, so that 360 trees were tested in total. Trees were visually similar in morphological features such as canopy volume and trunk diameter.

Soil samples were taken from a depth of 0–30 and 30–60 cm from the orchard before treatments were applied in Oct. 2007. Soil characteristics are presented in Table 1. All trees were sprayed three times in each season. The first spray was performed at the beginning of fall, 1 week after

harvest (20 Sept.). These trees did not lose leaves for at least 30 d after harvest. The second spray was applied at the beginning of growth when feathers of the pistillate flowers had turned brown in mid-April, and the third spray was applied at the beginning of May (2 weeks later).

Two representative branches were selected from each tree to monitor the total number of flowers and fruits on selected dates throughout 2008 and 2009 seasons. The branches were selected from east and west sides of the canopies and received about equal hours of direct sunlight. Selected branches from each tree were monitored for pistillate flower formation, and the total number of pistillate flowers was counted on 11 May 2007. On 15 July 2007, the number of fruits was counted to estimate the intermediate fruit set (number of nuts/number of initial flowers × 100). On 12 Sept. 2007, just before harvest, the number of fruits was counted to estimate final fruit set (number of nuts/number of initial flowers × 100).

Pollen was collected during Spring 2009. Staminate inflorescences having some flowers with dehiscent anthers were brought into the laboratory and pollen shed over the next 2 h was collected. Pollen were germinated on a medium containing 20% (w/v) sucrose, 1.0 mol·m⁻³ calcium chloride, 100 g·m⁻³ boric acid, and 0.65% (w/v) agar (Luza and Polito, 1991). After incubation for 24 h, the plates were sampled randomly and germination percentage was determined for at least 500 pollen grains of each tree.

Pistillate flower abscission was determined by the methods of McGranahan et al. (1994). Nitrogen (N), phosphorus (P), potassium (K), sodium (Na), Zn, iron (Fe), copper (Cu), magnesium (Mn), and B in leaf tissues were determined 90 d after flowering. Forty leaflets from middle leaflet pairs of mature leaves on terminal shoots were collected from the mid-third portion of current season growth. Leaf samples were washed with distilled water, dried at 65 °C for 48 h, ground to pass 1-mm sieve, stored in plastic bags before analyses, and analyzed.

Nitrogen was measured according to Kjeldahl's method (Ostrowska et al., 1991). Phosphorus was determined colorimetrically using the vanado-molybdophosphoric method (Ostrowska et al.,

Table 1. Soil characteristics of the studied persian walnut orchard located in Shahmirzad, Iran (lat. 52°21'N, long. 35°41'W), before Zn and B treatments were applied in Oct. 2007.

| Texture | Clay (%) | Silt (%) | Sand (%) | N (mg·kg ⁻¹) ^z | P (mg·kg ⁻¹) ^z | K (mg·kg ⁻¹) ^z | Fe (mg·kg ⁻¹) ^z | Mn (mg·kg ⁻¹) ^z | Cu (mg·kg ⁻¹) ^z | Zn (mg·kg ⁻¹) ^z | B (mg·kg ⁻¹) ^z | OM (%) | pH | EC (dS·m ⁻¹) ^y | Saturation point (%) | Depth (cm) ^x |
|------------|----------|----------|----------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|--|--|---------------------------------------|--------|------|---------------------------------------|----------------------|-------------------------|
| Silty loam | 18 | 59 | 23 | 1.8 | 22 | 200 | 5.97 | 10.64 | 1.46 | 0.90 | 0.31 | 0.97 | 8.27 | 0.62 | 21 | 0-30 |
| | 22 | 16 | 63 | 1.5 | 18 | 210 | 10.00 | 8.00 | 1.00 | 0.80 | 0.42 | 0.83 | 8.09 | 0.54 | 36 | 30-60 |

OM, organic matter; EC, electrical conductivity
^x 1 mg·kg⁻¹ = 1.0 ppm; available N in the soil was determined by alkaline permanganate method, OM by Walkley and Black method, available P by sodium bicarbonate or Olsen method, and available K by neutral normal ammonium acetate method; extractable Fe, Mg, Cu, and Zn from soils were determined by the diethylene triamine pentaacetic acid method available B soluble in hot water was measured by the Azometine-H method in spectrophotometer at 420 nm.
^y 1 dS·m⁻¹ = 1.0 mmho/cm.
^z 1 cm = 0.3937 inch.

1991). Potassium was determined according to the method of Evenhuis and De Waard (1980). Zinc, Fe, and Mn concentrations were determined by atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT). Boron was measured with the curcumin spectrophotometric method (Lieten, 2002).

The initial nutrient status of trees was 2.38% N, 1.60% K, 0.17% P, 1.2% Ca, 89.8 mg·kg⁻¹ Mn, 118.30 mg·kg⁻¹ Fe, 12 mg·kg⁻¹ Zn, and 55 mg·kg⁻¹ B. All leaf nutrient values are above existing standards, with the exception of Zn and B (Ramos, 1997). However, it should be noted that the experimental basis for definition of B and Zn standards is not known.

Chlorophyll measurements were collected with a hand-held dual-wavelength chlorophyll meter (SPAD 502; Minolta, Tokyo, Japan). On each tree, four leaves were sampled from four shoots located at midheight of the canopy in the four cardinal directions (16 values per tree) on 15 July. The mean of these 16 readings was used for all treatment comparisons.

A sample of 30 nuts was collected from each tree at harvest time (23 Sept. 2008 and 10 Sept. 2009) for the determination of nut weight (grams), nut length (centimeters), nut diameter (centimeters), and length/diameter ratio. Husks and shells were removed manually. Husk thickness was measured by a vernier caliper. Kernel length, kernel diameter, kernel weight, and shell and kernel percent were also determined.

The data were subjected to analysis of variance using SAS (version 9; SAS Institute, Cary, NC), and the differences were compared by using the Duncan's multiple range test at $P \leq 0.05$.

Results

INFLUENCE OF FOLIAR B APPLICATIONS. The main effect of B was significant as applications of B at 174 mg·L⁻¹ (B1) markedly increased yield and quality characteristics (Table 2). Furthermore, the 174 mg·L⁻¹ B treatment resulted in higher nut yield compared with other B treatments. For all variables measured, the application of B at 348 mg·L⁻¹, although superior to 0 mg·L⁻¹ B applications, was less effective than 174 mg·L⁻¹ B application. Boron applied at 174 mg·L⁻¹ also increased pollen germination. There was a significant

positive effect of B treatments on chlorophyll, with highest values recorded at 174 mg·L⁻¹ B application. Vegetative growth, assessed as current shoot growth, was influenced by B applications in 2009 and 2008, but on increasing B concentration, vegetative growth decreased compared with B1. Boron at 174 mg·L⁻¹ was more effective than at 348 mg·L⁻¹ B (Table 2). Boron applications increased tissue N, P, K, Zn, Fe, and B (Table 3).

INFLUENCE OF FOLIAR ZN APPLICATIONS. The main effect of Zn was significant as application of Zn alone at 1050 mg·L⁻¹ resulted in an increase in yield, nut length, and higher kernel percent compared to 0 or 1750 mg·L⁻¹ Zn (Table 2), but in other parameters, Zn1 was more effective than Zn0 and Zn2, and there was no difference between Zn0 and Zn2 (Table 3).

INTERACTIVE EFFECTS. For many of the parameters measured, the combination of Zn and B resulted in a greater response than either element supplied individually and was significant (Table 2). The combined applications of B and Zn at 174 and 1050 mg·L⁻¹, respectively, increased walnut yield by 400% when compared with unsprayed controls (Table 2). While combined Zn and B sprays had a positive effect on fruit set in both years, the interaction between B and Zn differed between years. In 2008, the highest fruit set was obtained from 174 and 1050 mg·L⁻¹ concentration of B and Zn, respectively, while in 2009, the highest fruit set was obtained with 174 mg·L⁻¹ B and 1050 mg·L⁻¹ Zn and 174 mg·L⁻¹ B and 1750 mg·L⁻¹ Zn (Table 2). Foliar Zn applications were effective only in promoting pollen germination when applied at 1050 mg·L⁻¹ in combination with 174 mg·L⁻¹ B. Chlorophyll index was the highest at 1050 and 174 mg·L⁻¹ Zn and B, respectively (Table 2). In 2008, 174 mg·L⁻¹ B + 1050 mg·L⁻¹ Zn and 174 mg·L⁻¹ B + 1750 mg·L⁻¹ Zn and, in 2009, 174 mg·L⁻¹ B + 1050 mg·L⁻¹ of Zn resulted in the longest new shoot length (Table 2). Leaf analysis showed that the highest concentration of N was achieved with application of 174 mg·L⁻¹ B + 1750 mg·L⁻¹ Zn, while the lowest N concentration was obtained in the control treatment when neither treatment was applied (Table 3).

All yield and quality parameters except nut and kernel diameter, shell

Table 2. Mean kernel weight, mean nut weight, mean kernel percent, mean kernel length, mean nut length, mean nut length, fruit set, pollen germination, chlorophyll index, and vegetative growth (current season growth) in 10-year-old persian walnut that received foliar applications with Zn as zinc sulfate and B as boric acid in spring and fall for two growing season (2007–2009).

| Treatments ^a | Kernel length (cm) ^w | | Kernel percent (%) ^y | | Nut length (cm) ^w | | Nut wt (g) ^x | | Kernel wt (g) | | Nut yield (kg/tree) ^x | | Pollen germination (%) | | Fruit set (%) ^y | | Chlorophyll index ^z | | Vegetative growth (cm) | | |
|-------------------------|---------------------------------|---------------------|---------------------------------|--------|------------------------------|----------|-------------------------|---------|---------------|---------|----------------------------------|---------|------------------------|------|----------------------------|---------|--------------------------------|--------|------------------------|----------|--------|
| | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | |
| B0 | Zn0 | 2.28 e ^t | 2.29 f | 46.7 d | 47.8 c | 3.60 d | 3.59 d | 61.5 e | 61.7 e | 32.0 d | 31.5 d | 11.3 g | 11.0 f | — | 60 c | 50.0 f | 50.7 e | 29 e | 31 e | 39.0 e | 38.7 d |
| B0 | Zn1 | 2.64 c | 2.55 de | 53.2 c | 54.5 b | 3.70 cd | 3.71 cd | 64.8 d | 65.0 d | 35.4 cd | 37.4 c | 15.9 f | 15.5 e | — | 65 c | 64.0 d | 64.3 bc | 33 d | 34 d | 55.7 bc | 55.7 c |
| B0 | Zn2 | 2.68 c | 2.68 cd | 57.8 b | 55.2 b | 3.62 d | 3.61 d | 66.7 d | 66.9 cd | 37.0 bc | 36.7 c | 19.3 d | 18.6 d | — | 66 c | 66.0 cd | 66.5 bc | 37 bc | 37 bc | 57.2 bc | 56.6 c |
| B1 | Zn0 | 2.85 b | 2.88 b | 56.2 b | 55.8 b | 3.69 cd | 3.72 cd | 68.6 cd | 68.7 cd | 38.0 bc | 38.4 bc | 37.9 b | 37.5 b | — | 78 b | 71.0 ab | 67.8 b | 39 b | 40 b | 50.7 d | 49.8 c |
| B1 | Zn1 | 3.00 a | 3.21 a | 61.2 a | 60.6 a | 4.23 a | 4.27 a | 86.2 a | 86.3 a | 51.2 a | 51.0 a | 46.1 a | 45.4 a | — | 89 a | 74.0 a | 73.7 a | 45 a | 47 a | 78.4 a | 78.5 a |
| B1 | Zn2 | 2.74 d | 2.47 e | 56.6 b | 55.5 b | 3.91 b | 3.91 b | 73.7 b | 73.8 b | 40.6 b | 40.7 b | 17.4 ef | 16.8 c | — | 65 c | 69.0 b | 75.1 a | 39 b | 40 b | 72.8 a | 67.2 b |
| B2 | Zn0 | 2.65 c | 2.68 cd | 55.8 b | 55.6 b | 3.62 d | 3.59 d | 64.7 de | 64.6 de | 35.5 cd | 35.7 c | 24.1 c | 23.5 c | — | 65 c | 59.0 c | 51.9 d | 36 bcd | 37 bcd | 47.2 d | 48.7 c |
| B2 | Zn1 | 2.74 bc | 2.77 bc | 57.1 b | 55.4 b | 3.83 bc | 3.83 bc | 64.5 de | 64.8 de | 35.2 cd | 35.3 c | 20.6 d | 19.7 d | — | 67 c | 65.0 d | 65.1 bc | 39 bc | 40 bc | 60.1 b | 57.3 c |
| B2 | Zn2 | 2.7 bc | 2.72 bcd | 57.4 b | 54.7 b | 3.71 bcd | 3.76 bcd | 72.0 bc | 69.6 c | 37.0 bc | 37.0 bc | 18.9 de | 18.8 d | — | 65 c | 62.0 cd | 62.9 cd | 36 cd | 36 cd | 54.7 bcd | 51.8 c |
| Significance | | | | | | | | | | | | | | | | | | | | | |
| B | | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Zn | | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| B × Zn | | *** | *** | *** | *** | ** | ** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | ** |

^aMeasured with a chlorophyll meter (SPAD 502; Minolta, Tokyo, Japan).

^y(no. of nuts/no. of initial flowers) × 100.

^x1 g = 0.0353 oz, 1 kg = 2.2046 lb.

^wn = 30 nuts/tree; 1 cm = 0.3937 inch.

^v(kernel wt/nut wt) × 100.

^zB0, B1, and B2 = 0, 174, and 348 mg·L⁻¹ B, respectively; Zn0, Zn1, and Zn2 = 0, 1050, and 1750 mg·L⁻¹ Zn, respectively; 1 mg·L⁻¹ = 1 ppm. Concentrations are in applied foliar sprays.

***, **Significant at P ≤ 0.001 or 0.0001, respectively.

percent, and husk thickness were increased by foliar application of Zn, B, or both, with the highest values consistently achieved by application of 174 mg·L⁻¹ B and 1050 mg·L⁻¹ Zn simultaneously. Applications of B at 174 mg·L⁻¹ markedly increased yield and quality characteristics, while the simultaneous application of 1050 mg·L⁻¹ Zn further enhanced yield. Application of Zn alone at 1050 mg·L⁻¹ resulted in a small but still significant increase in yields and nut size. Application of B at 348 mg·L⁻¹, although superior to 0 mg·L⁻¹ B application, was always less effective than 174 mg·L⁻¹ B application.

Although combined Zn and B sprays had a positive effect on fruit set in both years, the interaction between B and Zn differed between years. In 2008, the highest fruit set was obtained from 174 and 1050 mg·L⁻¹ concentration of B and Zn, respectively, while in 2009, the highest fruit set was obtained with 174 mg·L⁻¹ B and 1050 mg·L⁻¹ Zn and 174 mg·L⁻¹ B and 1750 mg·L⁻¹ Zn (Table 2).

Discussion

The value of spring foliar B and Zn for fruit set and yield has been demonstrated in many fruit and nut trees (Nielsen et al., 2004) and has been reported in nonrefereed publications for walnut grown in California (Brown et al., 1999; Ramos, 1997). Here, we demonstrate a clear positive effect of B and Zn applied as individual spray applications and a synergistic effect of these elements when applied in combination on walnut yield and quality parameters. For most parameters measured here, optimal response to foliar Zn occurred at 1050 mg·L⁻¹ Zn and was enhanced by the coapplication of B at 174 mg·L⁻¹.

To our knowledge, this is the first report of the benefit of foliar B and Zn on pollen germination in walnut trees. Our results in walnut are in general concurrence with those of Nyomora et al. (1997) in almond, who reported that the highest pollen germination and fruit set were observed when B was sprayed at 490 mg·L⁻¹ in 1993 and 735 mg·L⁻¹ in 1994 in ‘Butte’. Lee et al. (2009) reported the highest in vitro pollen germination in pear was achieved at 100 and 300 mg·kg⁻¹ B, and in field trials in pear (Lee et al., 2009), 200 mg·L⁻¹ B resulted in the highest

Table 3. Effects of Zn as zinc sulfate and B as boric acid treatment applied in spring and fall in two growing season on leaf nutrient concentration in 10-year-old persian walnut trees. Leaf samples were washed with distilled water, dried at 65 °C (149.0 °F) for 48 h, ground to pass through 1-mm (0.04 inch) sieve. N was measured according to Kjeldahl's method, P by vanado-molybdo-phosphoric method, K by Evenhuis and De Waard method, Zn and Fe by atomic absorption spectrometer; and B by curcumin spectrophotometer.

| | | 2009 | | | | | |
|-------------------------|-----|--|------------------------------|----------|---------|---------|------------------------------|
| Treatments ^y | | B (mg·kg ⁻¹) ^z | Zn (mg·kg ⁻¹) | N (%) | K (%) | P (%) | Fe (mg·kg ⁻¹) |
| B0 | Zn0 | 150.7 f ^c | 19.81 e | 1.53 e | 1.37 d | 0.16 e | 174.7 e |
| B0 | Zn1 | 184.0 e | 23.44 d | 2.10 d | 1.53 cd | 0.14 e | 192.2 de |
| B0 | Zn2 | 193.7 c | 26.79 c | 2.43 bcd | 1.72 bc | 0.12 e | 213.9 c |
| B1 | Zn0 | 211.0 cd | 26.18 c | 2.04 d | 1.60 bc | 0.27 cd | 205.8 cd |
| B1 | Zn1 | 255.5 ab | 26.51 c | 2.47 ab | 1.60 bc | 0.31 ab | 247.1 b |
| B1 | Zn2 | 227.2 cd | 29.87 b | 2.87 a | 1.59 bc | 0.22 cd | 262.7 ab |
| B2 | Zn0 | 210.2 cd | 25.61 c | 2.27 cd | 1.59 bc | 0.36 a | 254.2 b |
| B2 | Zn1 | 240.2 bc | 30.22 b | 2.65 abc | 1.76 b | 0.23 d | 248.2 b |
| B2 | Zn2 | 271.7 a | 34.88 a | 2.57 abc | 1.95 a | 0.21 d | 271.7 a |
| Significance | | | | | | | |
| B | | *** | *** | *** | *** | *** | *** |
| Zn | | *** | *** | *** | *** | *** | *** |
| B × Zn | | ** | ** | ** | ** | ** | ** |

^z1 mg·kg⁻¹ = 1 ppm.

^yB0, B1, and B2 = 0, 174, and 348 mg·L⁻¹ B, respectively; Zn0, Zn1, and Zn2 = 0, 1050, and 1750 mg·L⁻¹ Zn, respectively; 1 mg·L⁻¹ = 1 ppm. Concentrations are in applied foliar sprays.

^xWithin columns, means followed by the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

, *Significant at $P \leq 0.001$ or 0.0001 , respectively.

pollen production. Optimal B concentrations for application to nut trees have been variously reported as 300 mg·L⁻¹ in hazelnut (Serdar et al., 2005; Silva et al., 2003), 490 mg·L⁻¹ in almond (Nyomora et al., 1997), and 756 mg·L⁻¹ in pistachio [*Pistacia vera* (Brown et al., 1995b)]. Here, we observed maximal yield and quality parameters with a B application between 174 and 348 mg·L⁻¹, which is consistent with prior results in most fruit and nut trees, though lower than the optimum suggested for pistachio (Brown et al., 1995b).

The benefits of foliar B on yield in walnut can be partially explained by an increase in pollen germination rates. Boron applied at 174 mg·L⁻¹ increased pollen germination at all rates of applied Zn, while foliar Zn applications were only effective when applied at 1050 mg·L⁻¹ in combination with 174 mg·L⁻¹ B. Application of Zn in the absence of B was ineffective at increasing pollen germination.

Conclusion

The results provided here confirm that in a walnut orchard with low concentration of available Zn and B in soil and leaves, foliar application of B and Zn at 174 mg·L⁻¹ B and 1050

mg·L⁻¹ Zn resulted in a consistent improvement in vegetative growth and reproductive performance of trees. The results also verify observations made in numerous tree species that concentrations of 150–400 mg·L⁻¹ B and 1000–2000 mg·L⁻¹ Zn are optimal for foliar application of these elements. This study also suggests that combined application of B and Zn are synergistic.

Literature cited

Brown, P.H., L. Ferguson, and G. Picchioni. 1995a. Boron boosts pistachio yields. *Fluid J.* 4:11–13.

Brown, P.H., Q. Zhang, and J. Grant. 1995b. Improving walnut zinc nutritional status by foliar spray. *Walnut Res. Rpt., Walnut Mktg. Board, Modesto, CA.*

Brown, P.H., S. Perica, L. Hendricks, K. Kelley, J. Grant, S. Sibbett, and H. Hu. 1999. Foliar boron application to decrease PFA, increase fruit set and yield in walnut. *Walnut Res. Rpt., Walnut Mktg. Board, Modesto, CA.*

Evenhuis, B. and W. De-Waard. 1980. Principles and practices in plant analysis. United Nations Food Agr. Organization (FAO). *Soil Bul.* 38:152–163.

Hafez, O.M. and I.M. El-Metwally. 2007. Efficiency of zinc and potassium sprays alone or in combination with some

weed control treatments on weeds growth, yield and fruit quality of 'Washington Navel' orange orchards. *J. Appl. Sci. Res.* 3:613–621.

Kaya, C. and D. Higgs. 2002. Response of tomato (*Lycopersicon esculentum*) cultivars to foliar application of zinc when grown in sand culture at low zinc. *Sci. Hort.* 93: 53–64.

Lee, S.H., W.S. Kim, and T.H. Han. 2009. Effects of post-harvest foliar boron and calcium applications on subsequent season's pollen germination and pollen tube growth of pear (*Pyrus pyrifolia*). *Sci. Hort.* 122:77–82.

Lieten, P. 2002. Boron deficiency of strawberries grown in substrate culture. *Acta Hort.* 567:451–454.

Luza, J.M. and V.S. Polito. 1991. Progammy and chalazogamy in walnut (*Juglans regia*). *Bot. Gaz.* 152:100–106.

Marschner, H. 1986. Functions of mineral nutrients: Macronutrients, p. 195–267. In: R.J. Haynes (ed.). *Mineral nutrition of higher plants.* Academic Press, Orlando, FL.

McGranahan, G.H., D.G. Voyiatzis, P.B. Catlin, and V.S. Polito. 1994. High pollen loads can cause pistillate flower abscission in walnut. *J. Amer. Soc. Hort. Sci.* 119:505–509.

Memon, N.N., M. Qasim, and M.J. Jaskani. 2009. Effect of various corm size on the vegetative, floral and corm yield attributes of gladiolus. *Pakistan J. Agr. Sci.* 46:13–19.

Momeni, A. 2003. An appraisal of land resources of Iran: A contribution to a project on framework for sustainable agricultural development strategy in Iran under the auspices of FAO. United Nations Food Agr. Organization (FAO) Representation, Tehran, Iran.

Neilsen, G.H., D. Neilsen, E.J. Hogue, and L.C. Herbert. 2004. Zinc and boron nutrition management in fertigated high density apple orchards. *Can. J. Plant Sci.* 84:823–828.

Nyomora, A.M., S. Nyomora, and P.H. Brown. 1997. Fall foliar application boron increases tissue boron concentration and nut set of almond. *J. Amer. Soc. Hort. Sci.* 122:405–410.

Ostrowska, A., S. Gawlinski, and Z. Szczubialka. 1991. *Metody Analizy i Oceny Wlasciwosci Gleb i Roslin.* Instytut Ochrony Srodowiska, Warsaw, Poland.

Rajput, C.B.S., B.P. Singh, and H.P. Mishra. 1976. Effects of foliar application of boron on mango. *Sci. Hort.* 5:311–313.

Ramos, D.E. 1997. *Walnut production manual.* Div. Agr. Natural Sci., Univ. California, Oakland, CA.

- Serdar, U., A. Horuz, and T. Demir. 2005. The effects of B-Zn fertilization on yield, cluster drop and nut traits in hazelnut. *J. Biol. Sci.* 5:786–789.
- Shrestha, G.K., M.M. Thompson, and T.L. Righetti. 1987. Foliar applied boron increase fruit set in ‘Barcelona’ hazelnut. *J. Amer. Soc. Hort. Sci.* 121:412–416.
- Silva, A.P., E. Rosa, and S.H. Haneklaus. 2003. Influence of foliar boron application on fruit set and yield of hazelnut. *J. Plant Nutr.* 26:561–569.
- Solar, A. and F. Stampar. 2001. Influence of boron and zinc application on flowering and nut set in ‘Tonda di Gifoni’ hazelnut. *Acta Hort.* 556:307–309.
- Sotomayor, C., H. Silva, and J. Castro. 2000. Effect of boron plus zinc foliar spray on fruit setting of two almond cultivars. *Acta Hort.* 591:437–440.
- Storey, J.B. 2007. Zinc, p. 411–437. In: A.V. Barker and D.J. Pilbeam (eds.). *Handbook of plant nutrition*. CRC Press, New York.
- Umer, S., S.K. Bansal, P. Imas, and H. Magen. 1999. Effect of foliar fertilization of potassium on yield, quality and nutrient uptake of ground nut. *J. Plant Nutr.* 22: 1785–1795.
- Usenik, V. and F. Stampar. 2002. Effect of application of zinc plus boron on sweet cherry fruit set and yield. *Acta Hort.* 594:245–249.
- Ute, K. and S. Clemens. 2005. Functions and homeostasis of zinc, copper, and nickel in plants. *Topics Current Genet.* 14: 215–271.
- Wang, Q., L. Longdou, W. Xiaoqin, L. Yiqin, and L. Jinxing. 2003. Boron influences pollen germination and pollen tube growth in *Picea meyeri*. *Tree Physiol.* 23:345–351.
- Wojcik, P. 2007. Vegetative and reproductive responses of apple trees to zinc fertilization under conditions of acid coarse-textured soil. *J. Plant Nutr.* 30: 1791–1802.
- Wojcik, P. and W. Treder. 2006. Effect of drip boron fertigation on yield and fruit quality in a high-density apple orchard. *J. Plant Nutr.* 29:2199–2213.
- Zheng, W., M.M. Pi, and W.D. Liu. 1989. A study on the effects of boron on the carbon metabolism of Ramie. *J. Huazhong Agr. Univ.* 8:354–360.
- Ziaeiian, A.H. and M.J. Malakouti. 2001. Effects of Fe, Mn, Zn and Cu fertilization of wheat in the calcareous of Iran, p. 840–841. In: W.J. Horst, M.K. Schenk, A. Bürkert, N. Claassen, H. Flessa, W.B. Frommer, H.E. Goldbach, H.W. Olf, V. Römheld, B. Sattelmacher, U. Schmidhalter, S. Schubert, N. von Wirén, and L. Wittenmayer (eds.). *Plant nutrition: Food security and sustainability of agro-ecosystems through basic and applied research*. Kluwer Academic Publisher, Dordrecht, The Netherlands.