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Effect of boron spray at different phenological stages on fruit set, mineral composition, and carbohydrates content of three olive cultivars

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ABSTRACT

Purpose: Boron deficiency can be a limiting factor for the flowering and fruit production of olive orchards. The appropriate time for foliar spray needs to be found in each environment. **Research method:** Effect of foliar spray (350 mg L^{-1} from boric acid) after fruit harvest (Stage 1), during flower bud differentiation (Stage 2), and the anthesis (Stage 3) alone or in combination, on fruit set percentage, leaf and inflorescence boron, zinc, and iron concentration, soluble carbohydrates content in leaves of three commercial olive cultivars was investigated. **Findings:** Results showed, the maximum fruit set percentage, the highest leaf and inflorescence boron content, was found in the Koroneiki cultivar when boron sprayed two times at stages 1+3 and stages 2+3. The highest leaf and inflorescence zinc content was found when boron sprayed two times at stages 2+3 on the Koroneiki cultivar. The highest leaf and inflorescence iron content was found in Baladi and Mission cultivars, respectively, when sprayed at stage 2 or two times at stages 1+3. Olive cultivar Baladi showed the highest leaf fructose, sucrose, and glucose content when boron sprayed at stage 2 alone or three times at stages 1+2+3. **Research limitations:** It can be useful to study different concentrations of foliar spray and other cultivars. **Originality/Value:** The response of different olive cultivars was not the same. It seems that boron spray at the appropriate time increased fruit set percentage, especially in Koroneiki. Therefore, more research is needed to find the best fertilization program in olive orchards of Golestan province.

INTRODUCTION

Olive (*Olea europaea* L.) is an important commercial fruit tree that grows in Mediterranean countries with moderate weather conditions and mild winters (Turktas et al., 2013). Depending on its cultivar, the olive fruit is used for producing olive oil or canned products (Jasrotia et al., 2013). It is recognized as a valuable source of nutrients both in table fruit and oil. In recent years, due to possessing high oil nutritional value and tolerance to environmental stresses, the cultivation of olive trees has been expanded in various regions in Iran.

The olive tree suffers from different problems such as alternate bearing, incomplete flowers, fruit drop, and insufficient fruiting and crop yield (Erel et al., 2013). The most critical factors contributing to the flowering, fruit-set, and fruit production are water, nutrients, and environmental conditions during the flowering stage (Rapoport et al., 2011).

A balanced fertilization program of macro-and micronutrients is essential for access to high production with good quality (Sawan et al., 2001). Boron is not only involved with enzymatic and photosynthetic activities but also takes part in the reproductive system and carbohydrate translocation mechanism. Boron has also contributed to pollen germination and pollen tube growth and stability (Ganie et al., 2013). Boron is also involved in cell wall strength and development, cell division, sugar transport, hormones development, RNA metabolism, respiration, indole acetic acid (IAA) metabolism, and as part of the cell membranes (Camacho et al., 2008). According to previous studies, the need for boron is more pronounced during the generative growth stage rather than the vegetative growth stage. Sarrwy et al. (2012) characterized the role of boron in various processes, including hormone transfer, synthesis of proteins, sugar transfer, and metabolism of carbohydrates. Indirect evidence of phloem boron remobilization from olive leaves during flowering has been reported, but boron remobilization has not been determined during other stages of olive fruit development (Perica et al., 2001).

Olive oil quality is usually determined by the catabolic and anabolic processes taking place during fruit development and ripening. Previous studies implemented the biochemical process impacts on oil accumulation and their precursors during fruit ripening in olive cultivars (Seyyed Nejad & Niroomand, 2007). They reported that the carbohydrates substances are exported from leaves to fruits and then transformed into a fatty acid by several enzymatic steps. Tekaya et al. (2016) characterized those soluble sugars (mannitol, glucose, sucrose, and fructose) concentrations were significantly influenced by foliar fertilization of micronutrients. Because foliar fertilization induced a significant increase of total sugars in treated leaves. The accumulation of carbohydrates, photosynthesis products, in fertilized leaves suggests an improvement of photosynthetic capacity that may be related to the increase of chlorophyll concentration and some mineral elements, especially N, Fe, Mg, Mn, and Zn. Many abiotic stresses can influence sugar metabolism and phloem transport in the plant, including drought, nutrient deficiency, salt stress, and low temperatures (Peuke, 2010).

Hegazi et al. (2015) have studied the effect of time and concentration of boron foliar application (at the following concentrations: 0.00, 100, 200, 300, 400, 500 mg L^{-1}) on olive trees (cv. Frantoio) flowering and fruit set, applied on November, December, and January. Results indicated that boron application significantly improved flowering, fruit set, fruit yield, and fruit quality compared with the control. The highest increment of all mentioned traits was achieved at boron application rate of 200 mg L^{-1} applied in January.

In another study, Spinardi and Bassi (2012) have investigated the effects of foliar boron application (0, 262, 525, or 1050 mg L^{-1}) applied about one week before anthesis on fruit set, shotberry set, and on in vitro pollen germination in Casaliva, Frantoio, and Leccino cultivars. Results showed boron treatments increased significantly fruit set in Frantoio and Casaliva, but not in Leccino. Their results highlight the beneficial effect of boron treatments immediately before anthesis, possibly by affecting positively the fertilization process and subsequent plant source-sink relations linked to fruitlet retention.

Also, the impact of foliar application of zinc and boron solutions (twice at full bloom, and 15 days later with zinc sulfate (2.5 kg m^{-3}), Boric acid (2.5 kg m^{-3}), and the combination of these chemicals) on fruit set percentage, fruit physical properties, and intake rate of the elements by leaf and fruit of three olive cultivars (Keylet, Koroneiki, and Mission), were assessed by Saadati et al. (2016). The result indicated that boric acid spray increased the initial fruit set (76.9%), while spraying with zinc sulfate + boric acid caused significant increase of final fruit set, and number of fruits during the harvest (8.2% and 8%, respectively). Nutrient solution spraying significantly increased boron and zinc concentration in leaf and fruit.

This study aimed to evaluate the influence of boron foliar spray in different phenological stages on leaf soluble carbohydrates, leaf and inflorescence mineral composition, and fruit set of three olive cultivars to find the appropriate time of foliar spray and compare the behavior of different cultivars in Golestan province.

MATERIALS AND METHODS

Experimental site

This study was conducted through 2017–2018 at an olive orchard located at the agricultural and natural resources research and education center, Golestan province, Iran, with longitude: 54°27 E, latitude: 36°50 N, 155 m above sea level. The average annual rainfall of the region is 600 mm, with minimum and maximum temperatures of 7.3°C and 34.8°C, respectively. The physical and chemical characteristics of the soil are shown in Table 1.

Plant materials and fertilization treatments

The experiment was performed on ten years old olive cultivars; Koroneiki, Baladi, and Mission. The trees were planted at 5 × 6 m distances apart, and the irrigation system was trickle irrigation. Trees with uniform vigor were selected for foliar spray treatments.

The experimental design was a randomized, completed block design arranged in a factorial. Three replicates were used, and each replicate consisted of one tree. Foliar spray of boron with 350 mg L^{-1} from boric acid source (62 mg boron L^{-1}) was applied at different phenological stages; after fruit harvest in autumn (stage 1), during flower bud differentiation in winter (stage 2), anthesis in spring (stage 3), and the combination of stages 1 and 2, stages 1 and 3, stages 2 and 3 and stages 1, 2 and 3. Tween 20 (0.5%) was used as a wetting in all treatments. Control was distilled water with a 0.5% wetting agent.

Plant measurements

Fruit set

At full bloom, from April to May, four branches of each tree in different directions were randomly selected. Then the number of flowers on the marked stems was counted. The percentage of the final fruit set was calculated according to the following formula (1) (Saadati et al., 2016):

$$\text{Fruit set \%} = \frac{\text{Number of fruit set}}{\text{Total number of flower}} \times 100 \quad (1)$$

Leaf and inflorescence mineral composition

About 100 full mature leaves and inflorescences from each olive tree were collected at the anthesis stage, from April to May. The samples were washed with tap water first and second washed with distilled water. After that, samples were dried at 70°C for 24 h to reach constant weight, ground, and finally digested. The digested solution was used to determine boron, zinc, and iron both in leaves and inflorescence. Boron was determined by the curcumin method (Dible et al., 1954), and zinc and iron were analyzed by Atomic Absorption Spectrometer (Kalra, 1998).

Leaf carbohydrates content

The selected leaf samples from each tree were washed, and the method of Omokolo et al. (1996) was used to extract soluble sugars. In this method, 40 mg of fresh leaf tissue was mixed with 5 mL of 80% ethanol and placed in a hot water bath at 70 ° C for 10 minutes. The alcoholic extracts were centrifuged for 15 minutes at 1000 rpm, and the resulting clear solution, containing soluble sugars, was transferred to a beaker. The above operation was repeated four more times on the tissue residue. Finally, the alcoholic extract was concentrated with heat, and its volume reached one-fifth. The concentrated alcoholic extract was then mixed with chloroform in a ratio of 1: 5 and centrifuged for 10 minutes at a rate of 2000 rpm, and the upper phase was separated and used to measure various soluble sugars as following:

Glucose content was calculated using dinitrosalicylic acid by spectrophotometry at 575 nm (Miller, 1959). Sucrose content was determined according to the Antron method by spectrophotometry at 620 nm (Handel, 1968). Fructose content was calculated by the resorcinal method by spectrophotometry at 520 nm (Ashwell, 1957). Light absorption was read by Unico 2800 UV / VIS spectrophotometer.

Statistical analysis

The data were statistically analyzed by SAS software version 9 SAS 9.0. Mean data were compared using the least significant difference (LSD) method at the 5% probability level.

RESULTS AND DISCUSSION

Fruit set percentage

Analysis of the variance of fruit set percentage in three different olive cultivars is shown in Table 2. The results showed that boron foliar spray, depending on phenological stages, increased fruit set percentage in all three olive cultivars than the control. However, the Koroneiki cultivar responded positively better than two other cultivars. The means comparison showed that the maximum fruit set percentage was found in Koroneiki cultivar when boron sprayed two different times; after fruit harvest in autumn and during anthesis in spring (Stage 1+ 3) and or during flower bud differentiation in winter and anthesis (Stage 2+3) (Fig. 1).

It was demonstrated that boron increased the pollen germination and pollen grain survival, which results to increase productivity in olive trees; however, the adaptability of the cultivars to the climate of each region should not be neglected (Iqbal et al., 2019). Boron also increases pollen grain viability and growth rate of the pollen tube (Hegazi et al., 2015). Perica et al. (2001) found that fruit set percentage in olive increased by the response to all boron applications, but cited that more significant effect of boron on flower formation than on final fruit set suggests that a dilution effect may have occurred and that the optimum concentration during flowering may not be maintained as fruits grow. However, the fruit set percentage may not be correlated with the number of perfect flowers.

Leaf and inflorescence boron concentration

Analysis of the variance of leaf and inflorescence boron concentration in three different olive cultivars is shown in Table 2. The results demonstrated that the maximum leaf boron content (63 ppm) was found in the Koroneiki cultivar when sprayed two times at both flower bud differentiation and anthesis (Stage 2+3) (Fig. 2). The maximum boron concentration in inflorescence was found in Koroneiki cultivar when sprayed two different times; after fruit harvest in autumn and anthesis in spring (Stage 1+ 3) and or during flower bud differentiation in winter and anthesis (Stage 2+3) with 51.4 and 50.9 ppm respectively (Fig. 3).

Due to the olive leaf analysis standards, the optimum amount of boron in leaves is 19-150 ppm, and boron deficiency appears less than 14 ppm (Tabatabaei, 2013). Foliar boron spray significantly increased leaf and inflorescence boron content in some treatments of all three olive cultivars. In agreement with this finding, previous studies also showed that boron spray had a positive effect on the flowering and fruit yield of olive trees, even when the boron deficiency was not observed (Spinardi & Bassi, 2012). The results also showed a positive relationship between the boron content of leaf and inflorescence, because boron can easily transfer from leaves to inflorescence during flowering time (Perica et al., 2001).

Leaf and inflorescence zinc

Dependent on phenological stages and olive cultivars, leaf and inflorescence zinc content of olive cultivars significantly affected by boron spray (Table 2). The highest leaf zinc content (66.25 ppm) was found when boron sprayed two times at both flower bud differentiation and anthesis (Stage 2+3) in the Koroneiki cultivar (Fig. 4). Boron spray also increased zinc content in the olive inflorescence. The highest zinc content was found in the Baladi cultivar when boron sprayed during flower bud differentiation (Stage 2) or two times after fruit harvest in autumn and at anthesis in spring (Stage 1+3) with 24.99 and 25.28 ppm, respectively. In Mission cultivar, the highest zinc content was found at anthesis (Stage 3) and during flower bud differentiation, and at anthesis (Stage 2+3) with 27.69 and 26.76 ppm, respectively. In Koroneiki, the highest inflorescence zine content was found when boron spray was done two times, both at flower bud differentiation and at anthesis (Stage 2+3) with 30.13 ppm.

Olive leaf analysis standards show that the optimum zinc content in leaves is 10-24 ppm and zinc deficiency appears less than eight mg kg^{-1} (Tabatabaei, 2013). In the current study, dependent on phenological stages, boron spray affected leaf and inflorescence zinc concentration. Overall, boron spray positively increased the zinc content of leaf and inflorescence in some treatments. In agreement with our finding, Jasrotia et al. (2013) also reported an increase in zinc concentration with increasing the boron content. They found that boron spray can increase the zinc content of leaves in olive especially during the flowering, and after that with movement of zinc from leaves to inflorescence, improves the flower productivity. In contrast, a previous study showed that increasing the internal content of boron by foliar boron application reduced leaf zinc content (Fageria et al., 2002), and there is a negative relationship between boron and zinc (Tabatabaei, 2013).

Leaf and inflorescence iron content

Leaf and inflorescence iron content was significantly affected by olive cultivars and spray time (Table 2). The means comparison showed that the highest leaf iron content was 985.8 ppm when boron spray applied during flower bud differentiation in winter (Stage 2) in Baladi cultivar, and the highest amount of inflorescence iron was 175.8 ppm in Mission cultivar when sprayed two different times; after fruit harvest in autumn and anthesis in spring (Stage 1+ 3) (Fig. 6 and 7).

Table 1. Physical and chemical properties of soil in the experimental site

Depth (cm)	pH	EC(ds/m)	S.P (%)	Sand (%)	Silt (%)	Clay (%)	Texture	T.N.V (%)	OC (%)	N (%)	P (ppm)	K (ppm)
0-30	7.7	0.85	43	28	50	22	Silty loam	5.9	1.3	0.11	8.9	407

Table 2. Analysis of variance (means of square) of boron, zinc, and iron in the leaf and inflorescence of olive cultivars

Source of variation	df	Fruit set	Boron		Zinc		Iron	
			Leaf	Inflorescence	Leaf	Inflorescence	Leaf	Inflorescence
Block	2	0.25	0.02	1.91	0.13	0.61	0.006	0.33
Cultivar (C)	2	10.9**	423.5**	1078.14**	1264.38**	58.04**	154925.36**	24420.36**
Treatment (T)	7	4.53**	123.48**	87.39**	496.4**	42.35**	87913.02**	5780.73**
C × T	14	5.83**	161.59**	128.06**	274.50**	25.79**	124801.65**	4810.27**
Error	46	0.77	0.38	19.32	20.09	29.41	21.504	16.64
c.v.	-	20.21	1.5	1.97	2.002	3.32	0.24	0.75

**Significant at 1% probability level.

Table 3. Analysis of variance (means of square) of fructose, sucrose, and glucose in the leaf of olive cultivars

Source of variation	df	Fructose	Sucrose	Glucose
Block	2	0.00000052	0.004	0.008
Cultivar (C)	2	0.00012**	0.052**	0.058**
Treatment (T)	7	0.000021**	0.013**	0.018**
C × T	14	0.000064**	0.039**	0.056**
Error	46	0.00000046	0.002	0.004
c.v.	-	4.1	6.5	7.4

**Significant at 1% probability level.

The optimum range of iron in olive leaves is 90-124 ppm, and iron deficiency usually appears when iron content decreases below 40 mg kg^{-1} (Tabatabaei, 2013). Since Koroneiki and Mission cultivars outperformed the Baladi cultivar in terms of reproductive and fruiting characteristics, they were expected to have higher amounts of iron in the leaves, but leaf analysis showed different results. Under the boron treatment, the Baladi cultivar showed higher leaf iron content than two other olive cultivars. However, iron content in inflorescence was higher in Mission and Koroneiki cultivars than Baladi.

Tabatabaei (2013) also suggested that during flowering, reproductive organs act as strong sinks for nutrients elements that move towards the flowers. However, a contrast report showed that an antagonistic effect of boron on iron and boron treatment reduced iron content. But in agreement with this finding, Gurel and Basar (2016) found that boron can increase the internal iron and zinc content, and results to increase metabolism in the leaves, and has a positive effect on the flowering quantity and quality.

Leaf carbohydrates

Analysis of variance of leaves carbohydrates content in three different olive cultivars is summarized in Table 3. As the results showed, carbohydrates content of leaves was significantly affected by boron spray. The highest fructose and sucrose content of leaves were found in the Baladi cultivar when boron sprayed during flower bud differentiation in winter (Stage 2) with 0.025 and 0.921 mg g^{-1}, respectively (Fig. 8 and 9). The highest glucose content was also found in Baladi cultivar when boron sprayed one time during flower bud differentiation in winter (Stage 2) or three times; after fruit harvest in autumn, during flower bud differentiation in winter, and anthesis in spring (Stage1+2+3) with 1.086 and 0.99 mg g^{-1}, respectively (Fig. 10).

Overall, a higher content of carbohydrates was found in Baladi cultivar than two other cultivars. Furthermore, foliar spray of boron, especially during anthesis, reduced the carbohydrate content of leaves. These results also showed that flowers act as a strong sink for carbohydrates, and during the flowering time, too many carbohydrates translocated from leaves to flowers. Boron facilitates the translocation of carbohydrates in the plant. Therefore, it expects treatments that increase boron content in tissues, especially when sprayed during flowering, accelerated carbohydrate translocation from leaves to flowers. In agreement with these findings, Sarrwy et al. (2012) also found that boron facilitated the transport of sugars in plants. Ganie et al. (2013) reported that boron fertilization both increased the synthesis of internal carbohydrates and translocation of carbohydrates from leaves to flowers and fruits. Therefore, increase in fruit set percentage by boron treatment is because of more transport of sugars from leaves to inflorescence (Sarrwy et al., 2012).

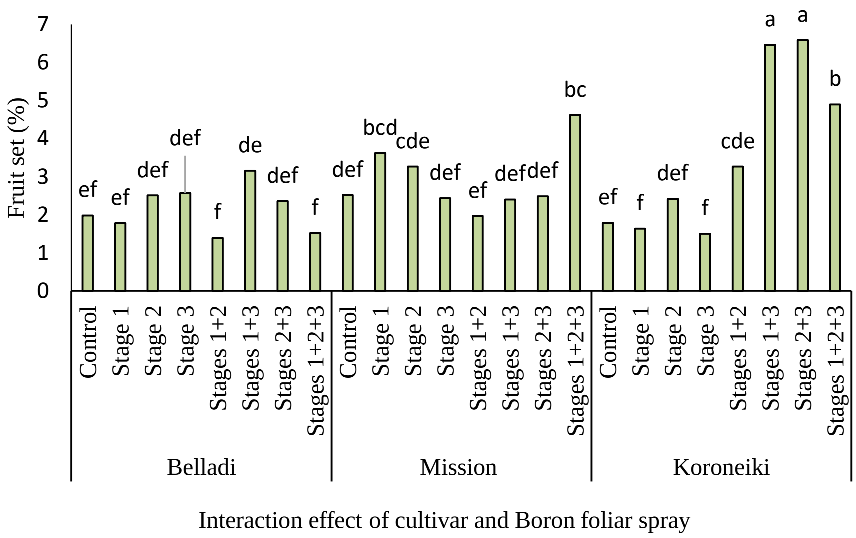

Fig. 1. The effect of boron foliar spray in different phenological stages on fruit set percentage of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

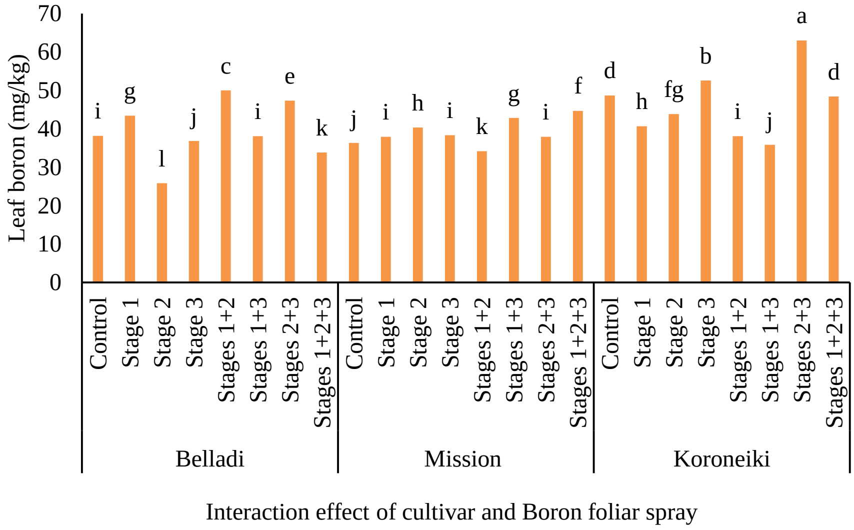

Fig. 2. The effect of boron foliar spray in different phenological stages on leaf boron concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

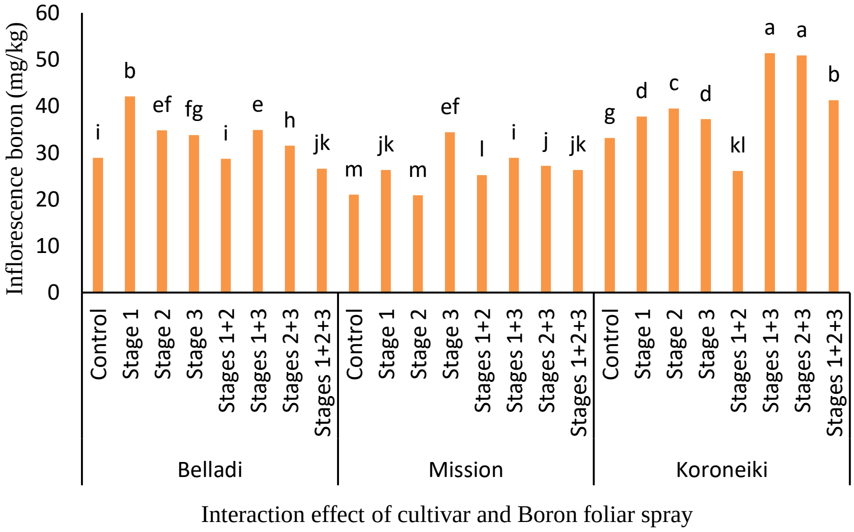

Fig. 3. The effect of boron foliar spray on inflorescence boron concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

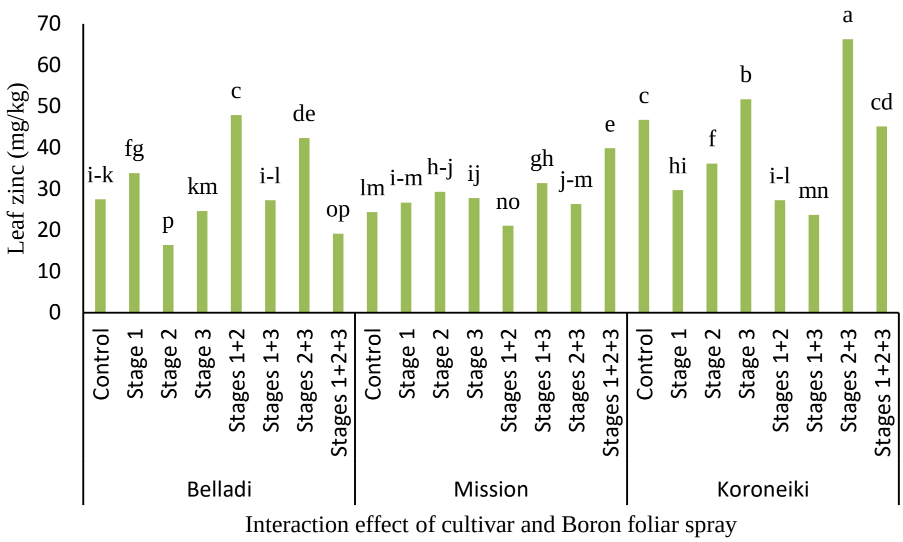

Fig. 4. The effect of boron foliar spray on leaf zinc concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

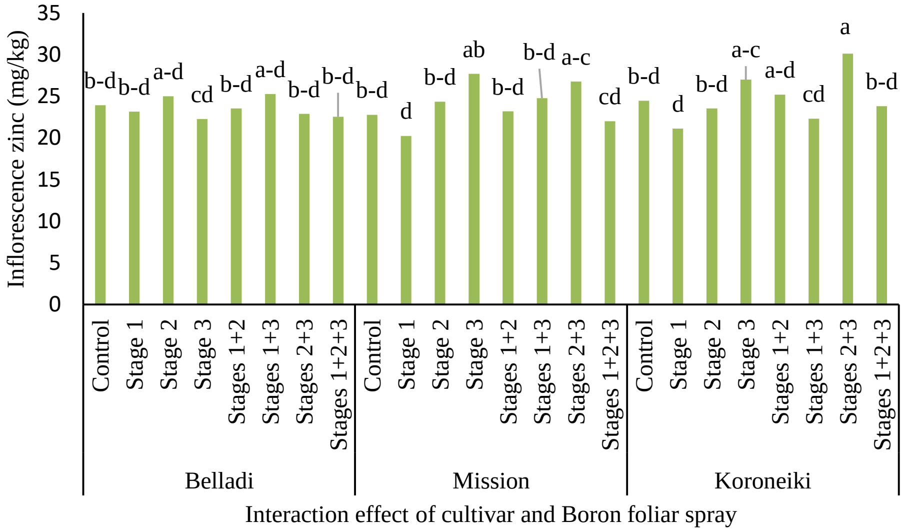

Fig. 5. The effect of boron foliar spray on inflorescence zinc concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

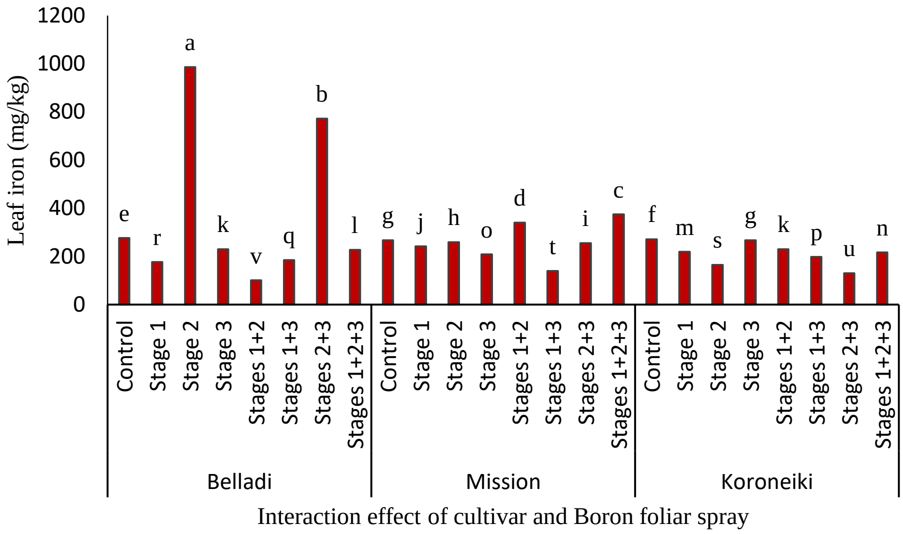

Fig. 6. The interaction effect of cultivar and boron foliar spray on leaf iron concentration of three different olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

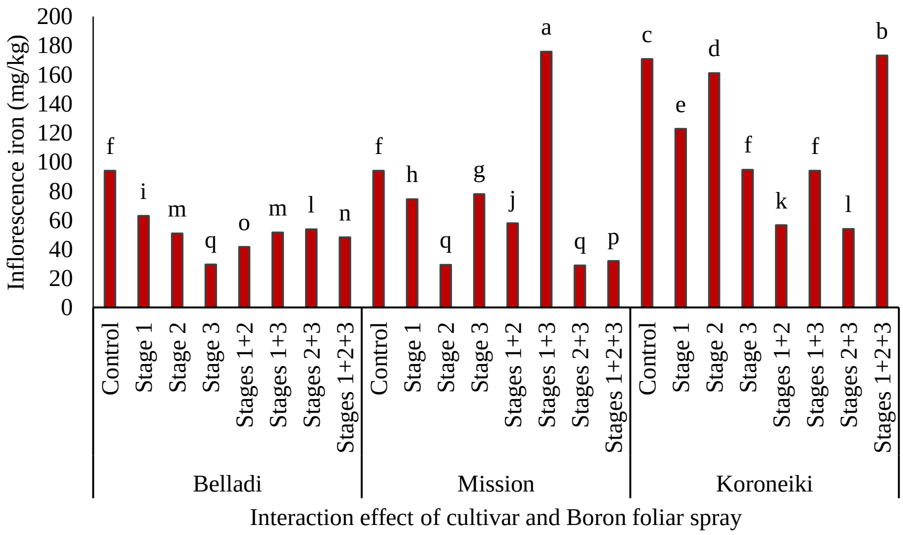

Fig. 7. The effect of boron foliar spray on inflorescence iron concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

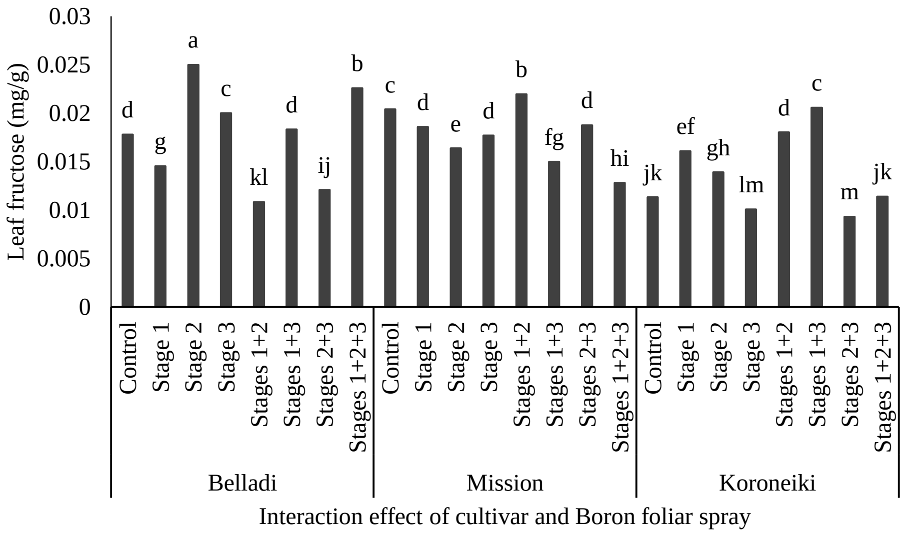

Fig. 8. The effect of boron foliar spray on leaf fructose concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

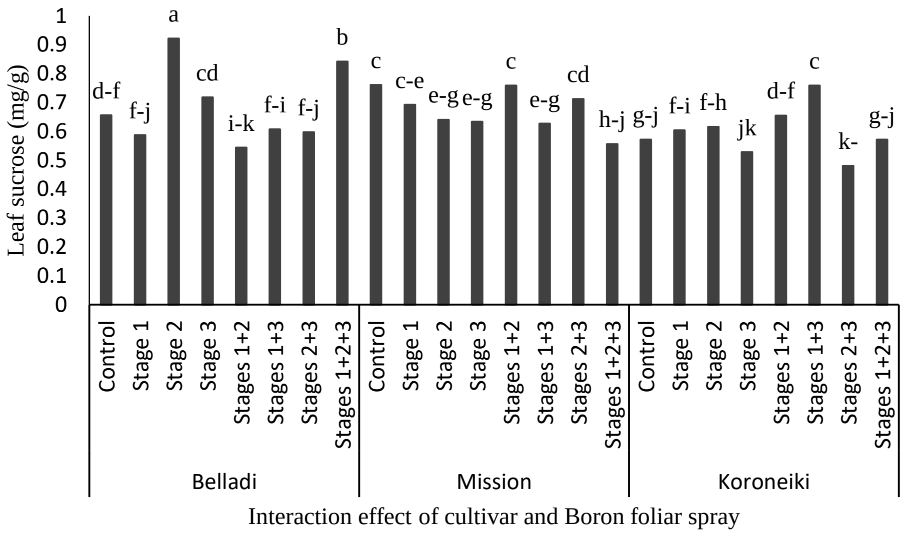

Fig. 9. The effect of cultivar and boron foliar spray on leaf sucrose concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

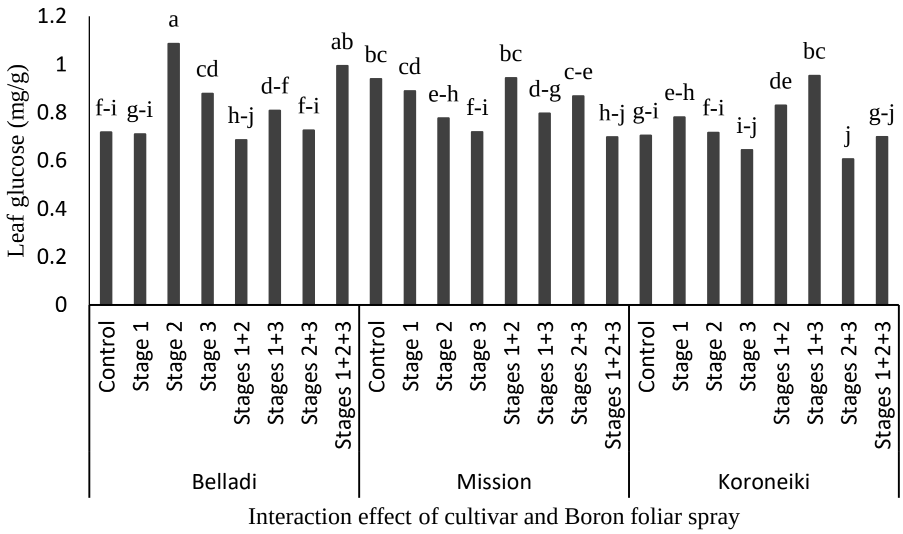

Fig. 10. The effect of boron foliar spray on leaf glucose concentration of three olive cultivars. *Columns with similar letters do not have a significant difference at the 5% level.

CONCLUSION

The results demonstrated that when boron content in olive plant tissues improved, photosynthesis-derived sugar transfer effectively towards the flowers and fruits and improved the final fruit set. However, the response of different olive cultivars was not the same. It seems foliar spray treatments combined together had more effects on studied characteristics. Koroneiki cultivar showed a more positive reaction, maybe because of its more adaptability to the environment. Also, considering that no apparent deficiency of minerals was observed in the control treatments, it can be said that foliar spray with boron and increasing the content of minerals in the optimal range can have a positive effect on plant yield and characteristics.

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Conflict of interest
The authors have no conflict of interest to report.

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