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RESEARCH ARTICLE

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Soil and foliar applied boron in olive: tree crop growth and yield, and boron remobilization within plant tissues

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Abstract

Boron (B) has great importance in the fertilizer recommendation programs of olive, since B deficiency is a frequent nutritional disorder. This paper reports results of the olive response to applied B from two 3-year field trials (ExpF1, ExpF2) and two pot experiments (ExpP1, ExpP2). The ExpF1 was installed in a 3-year-old orchard and the ExpF2 consisted of planting young trees with the aboveground biomass yield being recorded. In ExpP1, B was applied to the soil or as a foliar spray to the whole of the canopy. The ExpP2 consisted of the application of B to selected parts of the canopy. The concentration of B in the existing tissues receiving the spray directly and in the new shoots developing after the B application was determined. The performance of the crop, including olive yield, did not increase in ExpF1 with soil-applied B. However, dry matter yield of young plants in ExpF2 significantly increased with B application. The application of B, both as a soil fertilizer and as a foliar spray, significantly increased the B concentration in all tissues and in all experiments. From ExpP1, soil applied B proved to be a more powerful tool of delivering high amounts of B to the plant than foliar spray. From ExpP2, B was shown to be mobile in the tree to some extent, although the mobility appears to be cultivar-dependent. In 'Arbequina' the older leaves that received the foliar spray showed higher B levels than the young leaves that developed thereafter, whereas in 'Cobrançosa' this difference was not observed.

Additional keywords: boron fertilization; boron mobility; field trials; *Olea europaea*; olive yield; pot experiments.

Authors' contributions: IQF and MA performed all plant and soil analyses carried out in the lab, and MAR the design of field experiments, cropping practices related to field and pot experiments, soil and plant sampling. All authors wrote, read and approved the final manuscript.

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Introduction

Boron (B) is an essential element to higher plants. Boron's essentiality has been demonstrated since 1923 and is thought to have played an important role in the evolution of vascular plants due to its functions relating to the cell wall (Power & Woods, 1997). In higher plants, a significant fraction of the total B is complexed as *cis*-diol esters in cell walls associated with cell wall pectins (Power & Woods, 1997; Blevins & Lukaszewski, 1998; Broadley *et al*., 2012). According to Broadley *et al*. (2012), B is not a constituent of enzymes and is probably not directly involved in enzyme activation. It is probably its role in the cell wall and the interactions with the plasmalema that cause a 'cascade effect' with implications for several metabolic pathways.

B deficiency is more widespread in the world than that of any other micronutrient (Shorrocks, 1997; Gupta, 2007). The olive tree is considered to be a plant with high sensitivity to B deficiency (Freeman *et al*., 2005; Fernández-Escobar, 2017), although it is also accepted that the olive tree tolerates high levels of B in the soil (Freeman *et al*., 2005; Therios, 2009; Fernández-Escobar, 2017) or added in irrigation water (Chatzissavvidis *et al*., 2004). Observations from California allowed Freeman & Carlson (2005) to state that, along with nitrogen (N), B is the element most likely to be deficient in olive. Also in Trás-os-Montes, NE Portugal, the probability of B deficiency occurring in olive can only be compared to that of N, which makes B an important element in annual fertilization programs (Arrobas & Moutinho-Pereira,

2009; Arrobas *et al*., 2010). B is still, along with N, the element that trees most readily overcome nutrient deficiencies following applications to the soil (Gregoriou & El Kholy, 2010).

For most plant species, B is poorly mobile in the plant, due to restricted mobility in the phloem (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998; Bryson *et al*., 2014). The most common symptom of B deficiency in plants of restricted B mobility is the destruction of the active growing meristematic parts, which is related to the role of B in cell walls (Hu & Brown, 1997; Wimmer & Eichert, 2013). However, in some plant species B can be carried in the phloem in quantities sufficient to meet the needs of the plant. Thus, B appears to be the only nutrient that has restricted mobility in several species and is quite mobile in others (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998). In some species of the subfamilies Prunoideae and Maloideae, and in some brassicas, B is highly mobile (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998; Wimmer & Eichert, 2013). In the olive tree, B is considered to be poorly mobile (Gregoriou & El-Kholy, 2010) and in some cultivars B deficiency causes the death of the growing points (Arrobas & Moutinho-Pereira, 2009; Arrobas *et al*., 2010), a typical symptom of a non-mobile element. However, studies of Delgado *et al*. (1994) with cv. 'Manzanillo' showed that B applied to the leaves at the time of anthesis, increased the B concentrations in leaf blades, petioles, bark of the bearing shoot, and flowers and fruits three days after treatment, which suggest that B is mobilized from young leaves during anthesis to supply the requirements of flowers and young fruits. Perica *et al*. (2002) in a study with 'Manzanillo' also demonstrated that B can be remobilized from leaves of all ages. Liakopoulos *et al*. (2005) observed higher concentration of mannitol in B deficient plants of cv. 'Manaki' in comparison to the control, and concluded that, at low B supply, increased mannitol concentrations maintain B remobilization from source leaves to B-demanding sink leaves, since B is freely translocated from mature organs to young tissue in species where photoassimilates are translocated in the phloem as sugar alcohols.

Despite the importance of B to olive and the frequent observation of deficiency symptoms, few studies have been carried out showing a positive response of olive to B applications. Nevertheless, Soyergin (2010) reported that the application of B to the soil and by foliar spray increased the concentration of B in plant tissues and olive yield. Rodrigues *et al*. (2011), in a study in which olive trees were maintained for four years without B fertilization, recorded reduction in soil B availability, in leaf B concentration and in olive yield. On the other hand, Toker & Yavuz (2015) observed that B application to olive trees (cv. 'Ayvalik') led to a better olive oil quality by improving fatty acid composition, total phenol content, and major volatile compounds either in olive trees with or without B deficiency.

In this study the response of the olive tree to the application of B to the soil from field trials, and the response of the plant to the application of B to the soil or by foliar sprays from pot experiments, were evaluated. Three working hypotheses were set: i) the olive tree responds to the application of B; (ii) foliar application is equally effective as the application of B to the soil; and iii) B can be remobilized from the leaves to the growing parts of the plant.

Material and methods

Site characterization

This research involved two field and two pot experiments. The first field experiment (ExpF1) was established in a 3-year, rainfed olive grove. The second field experiment (ExpF2) consisted of a plantation of young plants purposely for this work. In pots, the first experiment (ExpP1) included three treatments: the application of B to the soil, B applied as a foliar spray and a non-fertilized control. In the second experiment (ExpP2) B was applied by foliar spray to specific parts of the canopy of two cultivars ('Arbequina' and 'Cobrançosa') to evaluate the mobility of B between different plant parts.

The experiments were carried out in Bragança, Northeast Portugal (41° 48'N; 6° 44'W). The region benefits from a Mediterranean climate whose yearly records of average air temperature and the accumulated precipitation (1980-2010) were 12.7 °C and 772.8 mm, respectively. The average air temperature and monthly rainfall recorded during the experimental period are shown in Figure 1. The pot experiments took place in a greenhouse with a twin wall polycarbonate cover, side and zenith openings, and a shade screen to reduce exposure to heat.

In the total of field and pot experiments, six different soils were used, two corresponding to the field trials (ExpF1 and ExpF2) and four corresponding to the pot experiments, three for the first experiment (ExpP1.1, ExpP1.2 and ExpP1.3) and one for the second (ExpP2). Some of the properties of these soils determined from samples taken at the 0-20 cm soil layer at the beginning of the experiments are shown in Table 1.

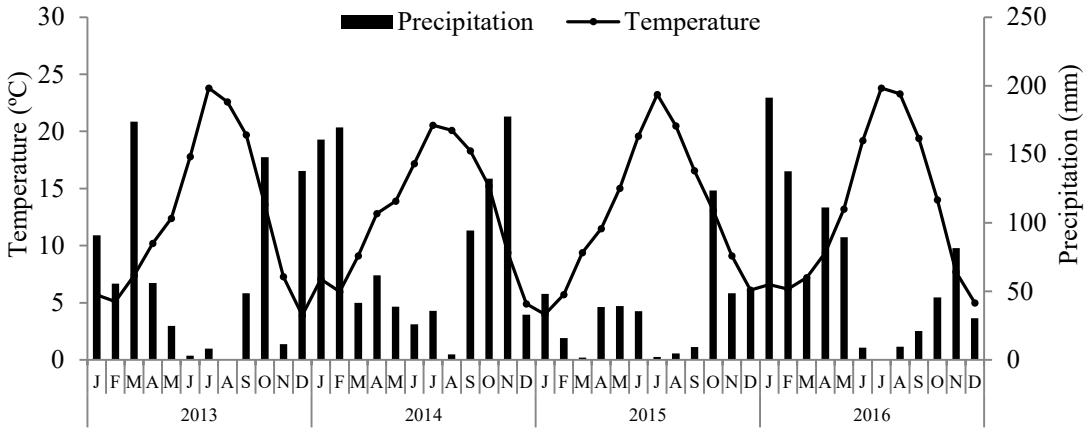

Figure 1. Monthly precipitation (mm) and mean monthly temperature (ºC) recorded during the experimental period at the meteorological station of Santa Apolónia farm in Bragança.

Experimental designs and management of the assays

Field trial 1 (ExpF1)

The first field trial (ExpF1) was installed in March 2013 in a 3-year-old 'Cobrançosa' olive orchard, spaced at 7 m × 6 m and rainfed managed. The soil is classified as Eutric Regosol (WRB, 2014) and some of its properties are shown in Table 1. The trial was organized in a completely randomized design with two treatments: soil applied B, and non-B fertilized control. The experimental design also included three replicates, each composed of four trees and, therefore, 12 trees per treatment. All experimental trees received a basal fertilization plan with N, as ammonium nitrate (34.5% N), phosphorus (P), as single superphosphate (18% P_2O_5), and potassium (K), as potassium chloride (60% K_2O). B was applied as borax (11% B) in the soil applied B treatment. Fertilizers were applied annually in early spring around the base of each tree. P and K were applied within a square area of 16 m^2 (2 m from trunk of each quadrant) while N and B fertilizers were applied within a square area of 4 m^2 (1 m from the trunk for each quadrant). N, P, K and B were respectively applied at the rates of 48, 70,

Table 1. Results of initial analyses of the soils used in the field trials (ExpF1, ExpF2) and in the pots experiments (ExpP1.1, ExpP1.2, ExpP1.3 and ExpP2).

Soil properties	ExpF1	ExpF2	ExpP1.1	ExpP1.2	ExpP1.3	ExpP2
pH (H_2O)	5.8	5.5	4.7	4.9	4.8	4.9
pH (KCl)	4.6	4.6	4.0	3.8	3.9	3.4
[1]Extractable P (mg P_2O_5 kg^{-1})	87.9	93.4	17.2	14.5	15.8	18.3
[1]Extractable K (mg K_2O kg^{-1})	102.0	114.0	92.0	59.0	75.5	53.0
[2]Extractable B (mg kg^{-1})	0.5	0.6	0.7	0.6	0.7	0.1
[3]Easily Oxidizable C (g kg^{-1})	8.7	8.7	27.5	7.5	17.5	9.5
[4]Total organic C (g kg^{-1})	25.6	27.3	54.6	27.3	41.0	28.0
[5]Exchang. K ($Cmol^+$ kg^{-1})	0.2	0.3	0.2	0.1	0.1	0.1
[5]Exchang. Na ($Cmol^+$ kg^{-1})	0.4	0.4	0.3	0.3	0.3	0.7
[5]Exchang. Ca ($Cmol^+$ kg^{-1})	7.2	8.5	1.7	2.8	2.2	4.7
[5]Exchang. Mg ($Cmol^+$ kg^{-1})	2.2	2.6	0.8	1.0	0.9	1.4
[5]Exchang. acidity ($Cmol^+$ kg^{-1})	10.7	11.9	4.4	4.6	4.5	9.1
[6]Clay (%)	14.5	14.6	13.2	7.8	10.5	11.5
[6]Silt (%)	27.7	29.2	26.4	9.4	17.9	19.4
[6]Sand (%)	57.8	56.2	60.4	82.8	71.6	69.1
Soil classification (WRB, 2014)	Eutric regosols		---------- Dystric Leptosols ----------			

[1] Egner-Riehm. [2] Hot water, azomethine-H. [3] Walkley-Black. [4] Incineration. [5] Ammonium acetate. [6] Robinson pipette.

133 and 1.2 g tree^{-1} yr^{-1}. Single superphosphate (18% P_2O_5), potassium chloride (60% K_2O), ammonium nitrate (34.5% N) and borax (11% B) were the fertilizers used. Weed control was performed by the application of a non-selective glyphosate-based herbicide (360 g L^{-1} active ingredient, 4 L of herbicide per hectare) applied once a year in early April.

In the ExpF1, the trees trunk diameter was periodically measured at 40 cm in height and the canopy volume estimated by measuring its maximum height and width in two directions (North/South and East/West) assuming an ovoid shape of the canopy. The canopy volume (CV) was estimated using the equation $CV = 2/3\ \pi\ R^2\ (L + S)$, where R is the mean radius of the two measurements of plant width, L is the distance between the point of greatest width of the canopy and the top (2/3 of the height of the canopy) and S the distance between the point of greatest width of the canopy and the base of the canopy (1/3 of the total height of the canopy). Pruning wood weight was also used as an index of tree growth as the trees were pruned annually in winter. The trees were harvested and annual olive yields recorded. Sub-samples of 100 fruits were weighed for fruit size evaluation. From this sub-sample, 20 random fruits were separated into pulp and pit, which, once recorded their fresh and dry weights, were used for the determination of pulp/pit ratio and the elemental composition. Twice a year, in the resting period of winter, and in summer, at endocarp hardening, leaf samples were taken from non-bearing one-year-old shoots for evaluation of the nutritional status of plants. At the end of the study, soil samples were collected at three depths (0-5 cm, 5-10 cm, and 10-20 cm) to evaluate the effect of treatments on soil properties. In September 2016, turnip (*Brassica rapa* var. *rapa* L.) was sown beneath the canopy within polyvinyl chloride rings to be used as a biological indicator of the availability of B to the plants. This method was developed by Rodrigues *et al*. (2010) and successfully used to measure soil N availability in the field, and was tested here to assess soil available B.

Field trial 2 (ExpF2)

The ExpF2 started in May 2014. The ExpF2 was arranged in a completely randomized design similar to that of ExpF1, with two treatments, soil B application and a non-B fertilized control and three replicates per treatment. Young rooted plants (20-30 cm high) of cv. 'Cobrançosa' were planted spaced 1 m within the row and 6 m apart. Each one of the three replicates per treatment was composed of 10 consecutive plants. All experimental trees were also subject to an annual fertilization plan with N, P and K. The fertilization was done in rectangles of 40 m^2 for P and K (10 m on the line and 2 m on both sides of the line) and 20 m^2 for N and B (10 m on the line and 1 m on each side of the line). N, P, K and B were applied at rates of 200, 175, 332 and 6 g per experimental unit (40 m^2 for P and K and 20 m^2 for N and B). The fertilizers used in this experiment were those previously described in ExpF1. In the first year of this experiment the soil was tilled after planting. In the following years the ground was maintained by a non-selective herbicide as in ExpF1. In the first year the plants were watered three times during the summer to reduce the risk of death. In the following years the plants were kept without irrigation.

In the ExpF2, twice a year, in the resting period of winter and in summer, leaves were sampled to evaluate the nutritional state of the plants. In October 2016, the aerial biomass of four plants from each plot was cut at aboveground level and weighed fresh. A sub-sample was also weighed, oven-dried at 70 ºC and weighed again after being separated into leaves and stems. Each part of the plants was then analyzed for elemental composition.

Pot experiment 1 (ExpP1)

The ExpP1 was a study of olive response to applied B with three treatments: soil applied B; foliar applied B; and control, non-B fertilization. The experiment was arranged as a randomized block design in which the blocks consisted of three different soils (ExpP1.1, ExpP1.2 and ExpP1.3) whose properties are shown in Table 1. Young rooted olives plants, ~20 cm height, of cv. 'Cobrançosa' were planted in pots filled with 3 kg of dry soil sieved in 2 mm mesh and mixed with 200 ml of perlite. Six pots (replicates) were used for each combination of soils and fertilizer treatments. Leaf B treatment consisted of annual application of 0.04 mL of Tradebor (11% w/w B-ethanolamine) split into two applications during the growing season by completely spraying the entire canopy of the plant. Soil B treatment consisted of annual applications of 0.29 g of borax (11% B) also divided into two applications. A basal fertilization plan with N (0.80 g pot^{-1} yr^{-1}), P (0.35 g pot^{-1} yr^{-1}) and K (0.66 g pot^{-1} yr^{-1}) was also applied. In the foliar B treatment, the mouth of the pots was protected with cardboard circles to prevent B reaching the soil. The experiment was set up in March 2015 and involved two growing seasons with shoot cutting at the end of the first cycle (February 2016) and recovering a soil sample and the plant biomass divided into root, stem and leaves at the end of second growing season (February 2017).

Pot experiment 2 (ExpP2)

The ExpP2 involved three B treatments: B applied to the entire canopy; B applied to approximately half of the branches, with the other protected with plastic bags; and control, without B application. Foliar fertilization consisted of total annual application of 0.04 mL of Tradebor (11% B) split into two applications with a one-week interval to enhance B absorption. In this experiment the mouth of the pots subjected to foliar B application was also protected with cardboard circles so that the soil did not receive B from foliar application. In these partially treated plants, the sprayed branches were marked. In all plants of the experiment, the shoots were tagged at the end of each growing point to separate treated from untreated tissues in future sampling. This experiment was installed in March 2016. In February 2017, the plants were removed from the soil and separated into roots, fraction of old stems existing up to the second foliar application of B, fraction of leaves present until the second B application, fraction of stems that developed after the second application of B and fraction of leaves that developed after the second application of B. In the case of partially treated plants, the sprayed and non-sprayed branches (stems and leaves) were also separated as well as the old and young (which had developed respectively before and after the application of B) tissues (leaves and stems) of the sprayed branches. All tissues were taken to the laboratory for B determination.

Laboratory determinations

After drying and sieving, soil samples from field and pot experiments were submitted to analytical determinations: pH (H_2O, KCl); easily oxidizable carbon (C) determined by the Walkley-Black method and total organic C by incineration; cation exchange capacity (ammonium acetate, pH 7.0); extractable P and K (ammonium lactate); extractable B (azomethine-H); and clay, silt and sand fractions by the Robinson pipette method (Houba *et al*., 1989).

Tissue samples (leaves, stems, roots, fruit pulps and pits) were oven-dried at 70 ºC and ground. Tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry [calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn)] methods (Walinga *et al*., 1989), after tissue samples were digested with nitric acid in a microwave.

Data analysis

Data was subjected to analysis of variance according to the experimental design of each of the experiments. When significant differences were found ($\alpha < 0.05$) and the factor present more than two treatments, the means were separated by the multiple range test Tukey HSD ($\alpha = 0.05$). To facilitate the interpretation of results and for graphical representation purposes, in some situations the mean confidence intervals ($\alpha = 0.05$) were also estimated.

Results

The application of B to the soil did not produce a significant response in olive yield in the ExpF1 (Table 2). The accumulated olive yield over the three years was 2.47 and 2.46 kg tree^{-1}, respectively in the -B and + B treatments, although there was a tendency for yield increase with the application of B in the last years of the trial. Other yield components, such as fresh fruit weight and pulp/pit ratio, also did not significantly vary between fertilizer treatments. However, pulp and pit B concentrations were significantly higher in B fertilized treatments in the three years of recording. The concentrations of other nutrients in pulp and in pit did not significantly vary between B treatments and were not shown.

Table 2. Olive yield, fruit biometry and B concentration in pulp and pit in experimental trees of ExpF1.

	2014		2015		2016	
	-B	+B	-B	+B	-B	+B
Olive yield (kg tree^{-1})	0.56 a	0.19 a	0.95 a	1.06 a	0.96 a	1.21 a
Fresh weight per fruit (g)	4.26 a	4.66 a	3.49 a	3.67 a	1.41 a	1.97 a
Pulp/pit ratio (dw)	1.27 a	1.27 a	1.99 a	1.86 a	1.39 a	1.49 a
Pulp B (g kg^{-1})	7.5 b	12.9 a	8.8 b	20.8 a	5.8 b	18.0 a
Pit B (g kg^{-1})	8.0 b	11.1 a	6.5 b	9.9 a	5.7 b	10.2 a

Within each year, means followed by the same letter are not significantly different ($\alpha < 0.05$).

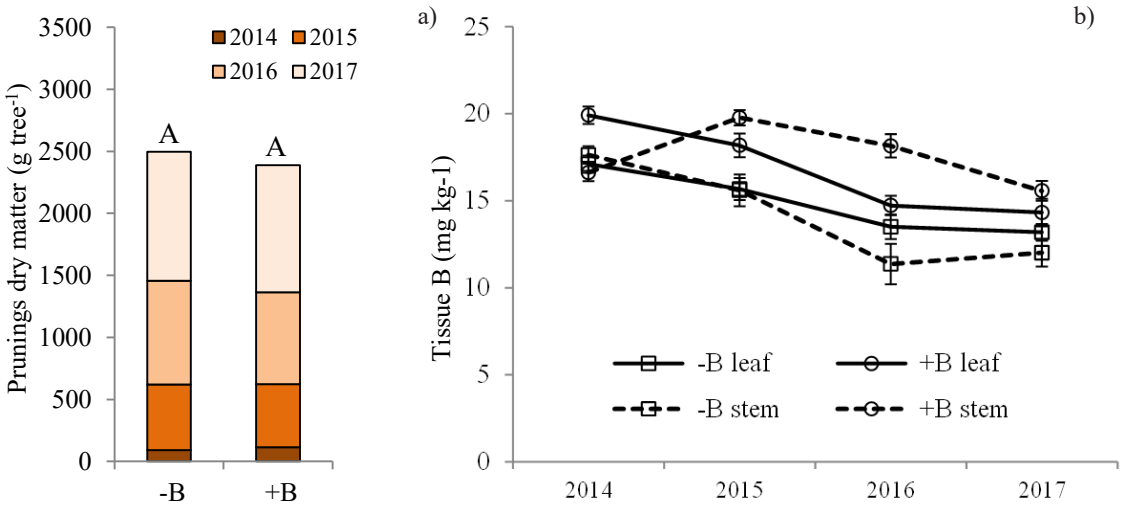

Figure 2. Pruning wood weight (a) and B concentration in pruning wood (b) from four consecutive growing seasons as a function of soil B application in ExpF1. a) capital letters above the columns is the result of analysis of variance (no significant differences between B treatments, $\alpha < 0.05$) for accumulated dry matter of four consecutive prunings; b) the error bars are the mean confidence intervals ($\alpha = 0.05$).

Pruning wood weight (Fig. 2) and other variables related to plant performance, such as trunk diameter and canopy volume (data not shown) did not show significant differences between -B and +B treatments. However, the concentration of B in the pruned material (leaves and stems) was significantly higher in +B treatment. The concentration of other nutrients analyzed in leaves and stems of pruned material did not show significant differences between treatments.

In general, the analysis performed on the leaves harvested on the dates and by the standard procedure for this species revealed higher B concentrations in the +B treatment in comparison to the control (Fig. 3). In both +B and –B treatments leaf B concentrations were lower in winter samplings. The unfertilized treatment often presented average values of B in leaves close to the lower limit of the sufficiency range. Foliar analysis for macronutrients (N, P, K, Ca, Mg) and other micronutrients (Cu, Fe, Zn and Mn) revealed no significant differences or consistent trends between fertilizer treatments, and data are not shown.

The application of B to the soil in ExpF1 increased soil extractable B (2.56 mg kg^{-1}) compared to the control (0.53 mg kg^{-1}) (Table 3). B content in the soil decreased significantly from the upper (2.17 mg kg^{-1}) to the deeper layers (1.34 and 1.13 mg kg^{-1}, respectively at 5-10 and 10-20 cm). Turnip, grown as a bioindicator for soil B availability, showed significantly higher values of dry matter (22.0 g $plot^{-1}$), tissue B concentrations (29.7 mg kg^{-1}) and B removal (0.65 mg $plot^{-1}$) in +B treatment in

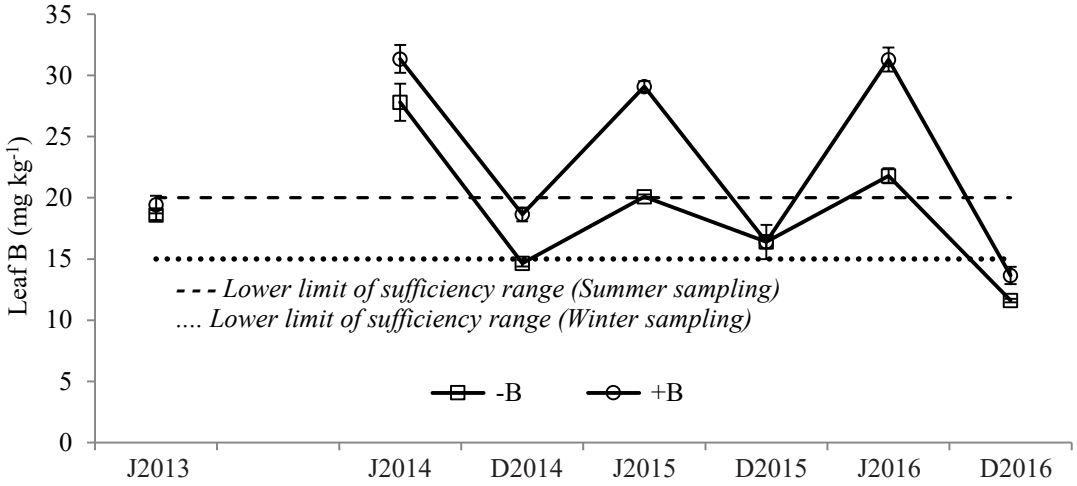

Figure 3. Leaf B concentration from samples taken in December (D), during the resting period of winter, and in late July (J) from B treated and untreated olive trees of ExpF1. The error bars are the mean confidence intervals ($\alpha = 0.05$).

Table 3. Soil B (mg kg^{-1}) status as a function of soil B application (-B, +B) and soil depth (0-5, 5-10, 10-20 cm) and turnip performance grown in B treated and untreated soils of ExpF1.

B treatment	Dry matter yield (g pot^{-1})	Tissue B (mg kg^{-1})	B recovery (mg pot^{-1})	Soil B (mg kg^{-1})
-B	8.3 b	24.4 b	0.23 b	0.53 b
+B	22.0 a	29.7 a	0.65 a	2.56 a

For each group of results, means followed by the same letter are not significantly different ($\alpha < 0.05$).

comparison to control (8.3 g plot^{-1}, 24.4 mg kg^{-1} and 0.23 mg plot^{-1}, respectively) (Table 3).

From the ExpF2, results of leaf B concentration, dry matter yield and B removal in the aboveground biomass are presented in Figure 4. Leaf B concentration was significantly higher in the fertilized plots as of the second sampling date (in July 2016 leaf B concentrations were respectively 29.9 and 20.9 mg kg^{-1} in the +B and -B treatments). The application of B to the soil led to a significant increase in dry matter yield, with total values (leaves + stems) varying from 365.5 to 515.5 g tree^{-1} respectively in the +B and -B treatments. B removal in plant tissues was also significantly higher in the fertilized plots due to the cumulative effect of higher B concentration in the tissues and higher dry matter yield. The total accumulated values (leaves + stems) varied from 4.0 to 7.5 mg tree^{-1}, respectively in the -B and +B treatments.

In ExpP1 three treatments were established: soil B (Bs), leaf B (Bf) and control (B0) and three different soils, which were included in the experimental design as blocks. The analysis of variance to the different variables analyzed did not show significant differences among blocks, so that in Table 4 only the results of the effect of the B treatments were included. The application of B to the soil resulted in significantly higher B concentrations in tissues (leaves, stems and roots) than with foliar application, although the latter gave significantly higher values than the control. In 2017 the Bs treatment showed B concentrations in leaves, stems and roots, respectively of 117.9, 124.3 and 124.5 mg kg^{-1}, Bf of 25.6, 26.8 and 32.6 mg kg^{-1} and B0 of 14.5, 13.1 and 11.5 mg kg^{-1}. Soil B was significantly higher in Bs treatment in comparison to Bf and B0. Despite the significant difference found in soil B levels and tissue B concentrations between treatments, no significant differences were found in dry matter yield (leaves, stems or roots) between B fertilized treatments and control.

The concentration of B in the leaves of ExpP2 is shown in Table 5. In the plants that received B in the whole canopy, cv. 'Arbequina' showed significant differences in leaf B concentration, the higher values being recorded in the old treated leaves (29.8 mg k^{-1}) in comparison to the young leaves (23.0 mg kg^{-1}). In the partially treated plants of 'Arbequina', the branches which had received foliar B also showed significant higher leaf B concentration in old leaves

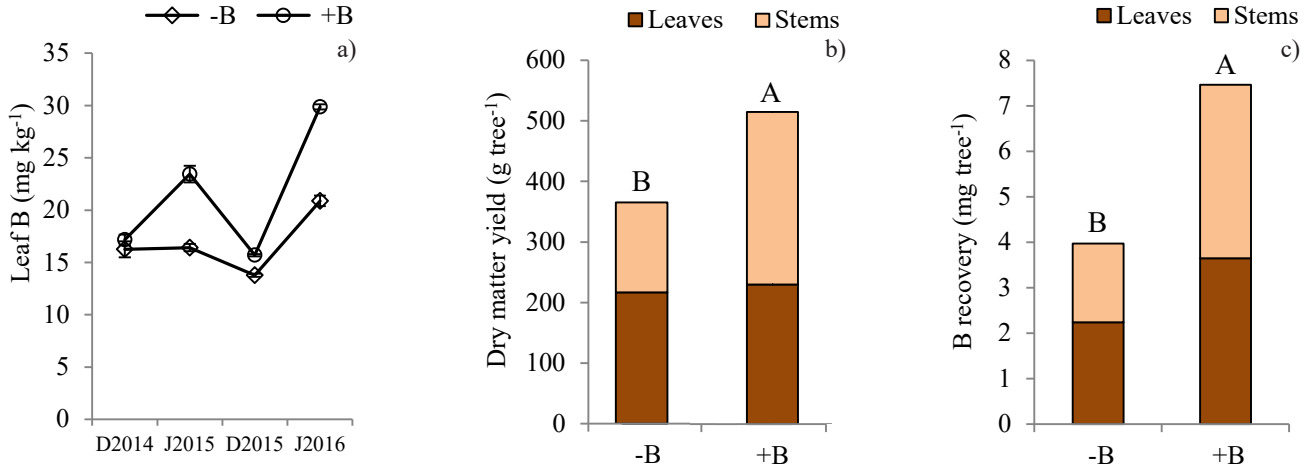

Figure 4. Leaf B concentration (a) in four sampling dates in December (D) and July (J), dry matter yield (b) and B recovery (c) in above ground biomass as a function of B treatments after three growing seasons. Error bars (a) are the mean confidence intervals ($\alpha = 0.05$); and capital letter above the columns (b, c) is the result of analysis of variance ($\alpha < 0.05$) for accumulated (leaves + stems) dry matter yield or B recovery, respectively.

Table 4. Tissue B concentration, soil B and dry matter yield as a function of B treatments (Bs, soil B; Bf, foliar B; B0, non-fertilized control) from a pot experiment (ExpP1).

B treatment	Tissue B (mg kg^{-1})					Soil B (mg kg^{-1})
	Leaves		Stems		Roots	
	2016	2017	2016	2017	2017	2017
B0	14.6 c	14.5 c	16.2 c	13.1 c	11.5 c	0.44 b
Bs	67.8 a	117.9 a	70.1 a	124.3 a	124.5 a	6.26 a
Bf	30.4 b	25.6 b	29.7 b	26.8 b	32.6 b	1.28 b
	Dry matter yield (g pot^{-1})					
B0	12.1 a	14.3 a	11.4 a	23.6 a	22.2 a	
Bs	11.9 a	14.8 a	11.1 a	24.3 a	21.3 a	
Bf	13.2 a	14.4 a	11.7 a	26.6 a	25.2 a	

In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

(30.9 mg kg^{-1}) in comparison to recently developed leaves (23.6 mg kg^{-1}). In 'Cobrançosa' there were no significant differences in B concentration between old and young leaves in total or partially treated plants. In both cultivars, treated plants displayed significantly higher leaf B concentrations than the plants of the control treatment.

In Table 6 results are presented of ExpP2 of B concentration in old stems (present at the time of foliar B application), young stems (later developing) and roots for the two cultivars 'Arbequina' and 'Cobrançosa'. The young stems tended to show higher B levels than the old stems, namely in the plants totally or partially treated, with significant differences in cv. 'Cobrançosa'. From plants totally treated old and young stems showed concentrations of B of 24.5 and 32.9 mg kg^{-1}, respectively. From partially treated plants, old and young stems of the treated branches showed average B concentrations of 22.4 and 29.8 mg kg^{-1}, respectively. In 'Arbequina' the concentration of B in the stems did not show significant differences related to the age of the tissue. In the roots, B concentrations were significantly higher in the totally or partially fertilized plants compared to the plants of the control treatment, either in 'Arbequina' or in 'Cobrançosa'.

Table 5. B concentration (mg kg^{-1}) in the leaves receiving foliar spray (old) and in those developed after foliar B application (young) as a function of B treatments of ExpP2.

	Arbequina		Cobrançosa	
	Old leaves	Young leaves	Old leaves	Young leaves
Control	16.7 c	16.9 c	16.6 b	21.0 ab
Wholly treated plants	29.8 a	23.0 b	25.0 a	27.8 a
Partially treated plants				
Protected shoots	23.3 b	20.6 bc	21.0 ab	24.2 ab
Sprayed shoots	30.9 a	23.6 b	26.2 a	27.9 a

Separately for each cultivar, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

Discussion

From ExpF1 it was not possible to find a significant response to applied B in variables such as trunk diameter, canopy volume, pruning wood, olive yield and pulp/pit ratio. However, the concentration of B in all the analyzed tissues (pulp, pit, stems and leaves of pruning wood and leaves) significantly increased in the fertilized treatment in comparison to the control. These results seem to fit a typical situation where B application increases nutrient concentration in tissues but does not cause physiological changes in the plant at a level that could affect its agronomic performance including productivity. The lower limit of sufficiency range for B in summer sampling is set at 19 mg kg^{-1} and visible symptoms of B deficiency usually only appear at values below 14 mg kg^{-1} (Freeman *et al*., 2005; Gregoriou & El-Kholy, 2010; Fernández-Escobar, 2017). In a previous study carried out in Greece, Tsadilas & Chartzoulakis (1999) have found that critical soil B concentration for olive is close to 0.33 mg kg^{-1} when extracted by hot water, value that is lower than those recorded in the soil of ExpF1 at the beginning (Table 1) and at the end (Table 3) of the experimental period. Thus, in this study, even in the control plots, it seems that the severity of deficiency would never have been high enough to cause a significant difference in tree growth and yield.

In the ExpF2, dry matter yield significantly increased with the application of B to the soil. Also the

Table 6. B concentration (mg kg^{-1}) in the stems receiving foliar spray (old) and in those developed after foliar B application (young) and in the roots as a function of B treatments of ExpP2.

	Arbequina			Cobrançosa		
	Old stems	**Young stems**	**Roots**	**Old stems**	**Young stems**	**Roots**
Control	18.1 b	20.6 b	12.0 b	18.2 cd	24.4 bc	11.7 b
Wholly treated plants	29.3 a	31.5 a	21.4 a	24.5 bc	32.9 a	15.5 a
Partially treated plants			19.2 a			15.9 a
Protected shoots	24.9 ab	29.1 a		17.8 d	29.3 ab	
Sprayed shoots	25.1 ab	29.6 a		22.4 cd	29.8 ab	

Within each cultivar, for old and young stems, and separately for roots, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

concentration of B in the tissues (stems and leaves) as well as the B recovered in the aboveground biomass increased in response to the applied B. All the international literature on the subject identifies B as a usual nutritional disorder in olive (Freeman *et al*., 2005; Therios, 2009; Arrobas & Moutinho-Pereira, 2009; Gregoriou & El-Kohly, 2010; Fernández-Escobar, 2017), although studies that show increased growth of olive trees or olive yield by the application of B are not abundant. Nevertheless, Soyergin *et al*. (2010) observed an increase in olive yield through the application of B to the soil or as a foliar spray. Rodrigues *et al*. (2011) reported a reduction in olive yield from a field trial in which the olive trees were maintained without application of B for four years in comparison to B fertilized trees. The results of this trial also contribute to the experimental evidence of the importance of B to olive in the early stages of development.

In ExpF1, leaves taken by the standard procedure of the fertilizer recommendation programs, showed higher B concentrations in summer sampling in comparison to winter sampling. This finding had already been reported by other researchers (Fernández-Escobar *et al*., 1999; Chatzissavvidis *et al*., 2005; Arrobas *et al*., 2010; Rodrigues *et al*., 2012) and has led to the establishment of different sufficiency ranges for B, depending on whether sampling is done in summer, at endocarp hardening, or in winter, during the resting period of olive (LQARS, 2006; Therios, 2009). In any case, it seems that summer sampling better reveals situations of different availability of B in the soil than winter sampling, since the difference in leaf B concentrations from B treated and untreated plants was greater in summer sampling.

In ExpF1 soil B availability was determined by a chemical extraction method and by using turnip as bioindicator of the availability of B in the soil. Both methods revealed increased availability of B in the soil in the plots that received B as a fertilizer. Unlike the olive trees, the turnip showed a significant increase of biomass by the application of B, likely due to the smaller extension of the root system, which makes it more dependent on the B applied, and/or because brassicas are plants of high B requirements (Pan *et al*., 2012; Thapa *et al*., 2016). The results also showed a higher concentration of B in the topsoil (2.17 mg kg^{-1}) in comparison to the deeper 5-10 cm (1.34 mg kg^{-1}) and 10-20 cm (1.13 mg kg^{-1}) soil layers. Thus, although B is generally considered to be mobile in the soil and subject to leaching (Hu & Brown, 1997), this soil was able to retain some B in the upper layer, probably associated with organic matter (25.6 g kg^{-1} organic C) and clay fraction (14.5% of the mineral fraction), since these are important properties responsible for the retention of B in the soil (Goldberg *et al*., 2005; Gupta, 2007; Havlin *et al*., 2014).

In ExpP1 significantly higher tissue B concentrations were found in the treatment of soil applied B in comparison to the application of B as a foliar spray. In the non-fertilized control the values were significantly lower than in both the fertilized treatments. Despite the great difference in B concentration in the tissues between treatments, no significant differences were observed in biomass yielded, either in leaves, stems or roots. Perhaps B levels in plant tissues did not reach sufficiently low values to reduce productivity as suggested for ExpF1. Roots, stems and leaves reached equivalent B concentrations, which is probably due to the fact that the majority of B is present in the apoplast (Matoh, 1997; Miwa & Fujiwara, 2010).

In ExpP2, B was applied as a foliar spray to the whole of the canopy and only to some branches, in a study conducted with 'Arbequina' and 'Cobrançosa'. Leaves and stems previously sprayed (older) and those that were developed after the application of the foliar spray (young), as well as the roots, were analyzed for B concentration. The treated plant, wholly or partially, exhibited higher B concentrations in tissues than the control plants. In 'Arbequina', the young leaves, which developed after the treatment, showed lower B levels

than the older leaves that received the B spray directly. In 'Cobrançosa' the old and young leaves showed similar B concentrations. This result seems to indicate some restriction on B mobility in 'Arbequina'and greater mobility in 'Cobrançosa'. The young and old stems did not show significant differences in B levels in 'Arbequina' and in 'Cobrançosa' young stems revealed higher B levels than the older stems, seeming to indicate that stems are able to retain high amounts of B that could not reach the leaves. The roots showed higher B concentrations in the fertilized plants, totally or partially, compared to the control. This result demonstrates some mobility of B in the plant, since when applied to the shoot the nutrient was able to reach the root. B is known to be an element of reduced mobility in plants (Shorrocks, 1997), although it has been shown to be highly mobile in some species, especially of the genera *Prunus*, *Malus* and *Brassica* (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998; Wimmer & Eichert, 2013). In olive B is generally considered to be poorly mobile (Gregoriou & El-Kholy, 2010). The symptoms of chlorosis and death of the growing points that some olive cultivars exhibit under B deficiency is seen as a typical symptom of an element of reduced mobility. Some studies, however, have suggested that B may have some mobility in olive (Delgado *et al*., 1994; Perica *et al*., 2002), probably associated to complexes that B forms with mannitol making it more mobile in the phloem (Liakopoulos *et al*., 2005). Interestingly, the symptoms of death of growing points seem not to be common to all the olive cultivars (Arrobas & Moutinho-Pereira, 2009). Thus, in general terms, this result seems to suggest that B may present some mobility in olive trees and also that the mobility of B in olive may be somehow dependent on the cultivar. The importance of the result is high since the effectiveness of foliar B applications is dependent on B mobility in the phloem.

In conclusion, in ExpF1 tree growth and olive yield did not increase in response to soil applied B, although in ExpF2 the aboveground biomass increased in B fertilized plots in comparison to the control. B concentration in all plant tissues increased significantly with the application of B. Thus, the results validate only partially the hypothesis that the olive tree responds to the application of B, and the lack of response in ExpF1 may be due to the fact that the levels of B in the tissues did not fall below the lower limit of the sufficiency range in the control treatment. In ExpF1 the leaves collected by the standard procedure revealed higher B concentrations in summer sampling than in winter sampling, which suggests that this aspect should be taken into account in the fertilization recommendation systems that accept as valid the two sampling dates.

The application of B to the soil increased the concentration of B in plant tissues much more than the application of B as a foliar spray, proving to be a more consistent way to correct a situation of B deficiency due to the greater amount of nutrient that can be supplied.

When B was applied as a foliar spray to specific parts of the canopy, the young leaves of 'Arbequina', that developed after the application of B, showed lower B levels than the older leaves that received B directly, which suggests some restriction on B mobility. In 'Cobrançosa', B appeared homogeneously distributed between young and old leaves suggesting higher mobility of B in this cultivar. However, B showed some mobility in both cultivars, as B levels in the roots increased after the application of B to the shoot. These results are important because they suggest that B mobility seems cultivar dependent, which raises doubts about the efficacy of using B as a leaf spray in the fertilization programs of olive orchards. Further studies on B should take the cultivar into account for a faster progress in the knowledge on B mobility in olive.

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