Effect of boron fertilization and phenological period on boron content in olive leaves

Efeito da adubação com boro e do período fenológico sobre o conteúdo de boro em folhas de oliveiras

Efecto de la fertilización con boro y el período fenológico sobre el contenido de boro en hojas de olivo

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Abstract

Boron (B) is one of the most important nutrients in olive growing, and in Brazil there is still a lack of studies that evaluated the effect of fertilization with B for olive trees. Thus, the aim of the study was to verify whether soil and foliar boron fertilization influenced the B leaves content, during the flowering period and the beginning of fruiting. Two olive groves were evaluated in the central region of the state of Rio Grande do Sul, in Caçapava do Sul (CS) and São Gabriel (SG), adopting a bifactorial experimental design with soil B doses (0, 25, 50 and 100 g) and presence/absence of foliar B. Boron contents were quantified before fertilization (T0), after soil fertilization (T1) and after foliar fertilization (T2). Fertilization with B in the soil and foliar did not increase the levels of foliar B. However, it was observed that B concentration decreased in T1 (flowering) compared to T0 (pre-flowering), with a reduction in mature leaves of CS and SG of approximately 45% and 34%, respectively. Boron concentration increased again in T2 (initial fruit development), regardless of fertilization, statistically differing from T1, in both olive groves. Higher B content was observed in CS in all evaluated periods, possibly being associated with higher concentration of B in the soil. The present study reinforces the hypothesis that in olive trees there is remobilization of B from the leaves to attend the metabolic needs of reproductive structures, especially in floral development.
Keywords: Granular fertilizer; Foliar fertilizer; Arbequina; Flowering; Initial fruit development.

Resumo

O boro (B) é um dos nutrientes mais importantes na olivicultura, sendo que no Brasil ainda uma escassez de estudos que avaliaram o efeito da adubação com B para oliveiras. Deste modo, o objetivo do trabalho foi verificar se a adubação do solo e foliar com boro influenciou os teores foliares de B, durante o período de floração e início da frutificação. Foram avaliados dois olivais na região central do estado do Rio Grande do Sul, em Caçapava do Sul (CS) e São Gabriel (SG), adotando-se um planejamento experimental bifatorial com doses de B solo (0, 25, 50 e 100 g) e presença/ausência de B foliar. Foram quantificados os teores de B antes da adubação (T0), após a adubação no solo (T1) e após a adubação foliar (T2). A adubação com B no solo e foliar não aumentou os teores de B foliares. Entretanto, observou-se que os níveis de B diminuíram em T1 (floração) em comparação com T0 (pré-floração), com redução nas folhas maduras de CS e SG de aproximadamente 45% e 34%, respectivamente. A concentração de boro aumentou novamente em T2 (inicio da frutificação), independente da adubação, diferindo estatisticamente de T1 em ambos os olivais. Maior conteúdo de B foi observado em CS em todos os períodos avaliados, possivelmente sendo associados com maior concentração de B no solo. O presente estudo reforça a hipótese de que em oliveiras há

remobilização de B das folhas para atender as necessidades metabólicas de estruturas reprodutivas, em especial no desenvolvimento floral.
Palavras-chave: Adubo granular; Adubo foliar; Arbequina; Floração; Desenvolvimento inicial do fruto.

Resumen

El boro (B) es uno de los nutrientes más importantes en la olivicultura, y en Brasil aún faltan estudios que evalúen el efecto de la fertilización con B para los olivos. Así, el objetivo de este estudio fue verificar si la fertilización foliar y del suelo con boro influyó en los niveles foliares de B, durante el período de floración y el inicio de la fructificación. Se evaluaron dos olivares en la región central del estado de Rio Grande do Sul, en Caçapava do Sul (CS) y São Gabriel (SG), adoptando un diseño experimental bifactorial con dosis de B al suelo (0, 25, 50 y 100 g) y presencia/ausencia de foliar B. Los contenidos de B se cuantificaron antes de la fertilización (T0), después de la fertilización al suelo (T1) y después de la fertilización foliar (T2). La fertilización con B en el suelo y foliar no incrementó los niveles de B foliar. Sin embargo, se observó que los niveles de B disminuyeron en T1 (floración) en comparación con T0 (prefloración), con una reducción en hojas maduras de CS y SG de aproximadamente 45% y 34%, respectivamente. La concentración de boro volvió a aumentar en T2 (inicio de la fructificación), independientemente de la fertilización, diferenciándose estadísticamente de T1 en ambos olivares. Se observó un mayor contenido de B en CS en todos los períodos evaluados, posiblemente asociado con una mayor concentración de B en el suelo. El presente estudio refuerza la hipótesis de que en el olivo se produce una removilización de B desde las hojas para cubrir las necesidades metabólicas de las estructuras reproductivas, especialmente en el desarrollo floral.
Palabras clave: Fertilizante granular; Fertilizante foliar; Arbequina; Floración; Desarrollo de frutos inmaduros.

1. Introduction

Olive cultivation is one of the oldest agricultural practices in the world, with archaeological records in the region of Palestine, Israel, Jordan and Syria from 6500 BP, followed by the Crete region of Greece from 6000-5500 BP (Langgut et al., 2019). Later, olive growing expanded to other regions of the Mediterranean and currently spread to the five continents, such as Australia, China and Brazil (Lucena et al., 2017). Rio Grande do Sul is the state with the largest olive oil producer in the country, followed by the Serra da Mantiqueira region, with emphasis on the states of Minas Gerais, São Paulo and Rio de Janeiro (IBRAOLIVA, 2022). From 2010 to 2022, olive oil production in Rio Grande do Sul followed an upward trend, with fluctuations in production due to climatic conditions and possibly alternation of production, and in 2022 a record harvest of 448,500 l of olive oil was reported (João, 2022).

According to the Agroclimatic and Edaphoclimatic Zoning, about 7.4 million hectares of the territory of Rio Grande do Sul has recommended edaphoclimatic conditions for the cultivation of olive trees (Alba et al., 2013). In 2022 the area with olive cultivation covered about 6000 ha in Rio Grande do Sul (Ambrosini et al., 2022). Most information on olive cultivation is based on the Mediterranean region, however, for the establishment of the culture in Brazil, research is fundamental with the aim of adapting cultural practices to regional conditions such as adapted cultivars, pest and disease control, pruning, fertilization and liming, among others.

Regarding fertilization, boron (B) stands out as one of the most important micronutrients for olive trees, whose deficiency is recurrent in olive groves (Bender et al., 2018). Boron contributes to the formation of cell wall structure, reproduction and other processes of primary and secondary metabolism of plants (Raven et al., 2001). Due to its importance, boron needs to be continuously absorbed by roots and translocated to plant tissues through the vascular system (Blevins and Lukaszewski, 1998). For olive trees, a high demand for boron has been observed, especially during the period of bud differentiation and flowering, and the application of fertilizer based on B is recommended, with doses suggested in Brazil of 25-40 g B via soil/plant or foliar fertilization of 0.1% B before flowering (Mesquita et al., 2012).

Studies on the effect of boron fertilization on olive trees have shown an increase in B concentration in leaves after fertilization, as well as in buds, flowers and increase of fruit set (Delgado et al., 1994; Perica et al., 2001a; Ateyyeh & Shatat, 2006; Hegazi et al., 2018; Ferreira et al., 2019; Pascović et al., 2019). Despite the increasing number of publications considering national olive oil (Minuceli et al., 2021; Gonçalves et al., 2022) and its by-products (Ripoll et al., 2022; de Souza et al. 2022), there are few studies on nutrient recommendation and fertilization in Brazil for olive trees (Mesquita et al., 2012;

CQFS-RS/SC, 2016, Bender et al., 2018; Tiecher et al., 2020, Figueiredo et al., 2022; Figueiredo, 2022). Thus, the aim of the study was to evaluate whether soil and foliar boron fertilization modified the levels of B in adult olive tree leaves, during the flowering period and the beginning of fruiting.

2. Methodology

2.1 Study area

The assay was conducted in two commercial olive orchards in the central region of the state of Rio Grande do Sul, in the municipalities of Caçapava do Sul (CS) (30°24'50"S, 53°27'55"W) and São Gabriel (SG) (30°05'10.6"S, 54°36'35.2"W), from July to November 2019. The climate is characterized as subtropical Cfa in both municipalities, according to the Köppen classification. Accumulated precipitation during the evaluated period was 716 mm and 701 mm, respectively for Caçapava do Sul and São Gabriel, while the minimum and maximum temperatures recorded were 8.4-25.9 °C and 7.9-28.5 °C for the aforementioned locations (Rio Grande do Sul, 2019). Olive trees (*Olea europaea* L.) of the Arbequina cultivar were selected, due to their great expressiveness in Brazilian olive orchards. The olive trees in CS were ten years old, planted 5 x 3 m apart in Regolithic Neosol. In SG, the olive trees were seven years old, spaced 7 x 5 m apart in Dystrophic Red Argisol. The olive groves received fertilizers in accordance with the olive fertilization recommendations for Brazil (CQFS-RS/SC, 2016; Tiecher et al., 2020), with the exception of the source of boron.

2.2 Experimental design

The experiment was carried out in a two-factor design with randomized blocks and split plots (n=3). Factor A, in the main plots, consisted of the application of boron to the soil at rates of 0, 25, 50 and 100 g per tree of granular fertilizer, while factor B, in subplots, consisted of the presence and absence of application of foliar fertilizer. Each tree consisted of an experimental unit, totaling 24 trees in each field experiment. The same experimental design was replicated in the two olive orchards.

The fertilizer applied via soil was Ulexite, which presented 10% total B and 6% B soluble in citric acid. The fertilizer rates were broadcast around the trunk, in a square area of 4 m^2 (2 m x 2 m) below the canopy. For foliar B fertilizer, in turn, the commercial product Bortrac™ (10.9% B) was sprayed, in a single application, using 10 mL of Bortrac™ and 5 mL of adjuvant in 1.5 L of aqueous solution per tree, as indicated by the manufacturer. Samples of mature leaves were collected in three periods: in pre-flowering, before application of B via soil (July) (T0); at flowering, 45 days after fertilization with Ulexite (August) (T1); at the beginning of fruit development, 18 days after application Bortrac™ (November) (T2). From each tree, 100 leaves were collected, washed with distilled water, dried at 50°C until constant weight and ground (18 mesh).

2.3 Chemical analysis

For the determination of boron in the leaves, B was initially extracted by burning in a muffle furnace at 600°C and was determined by colorimetry using azomethine-H (Tedesco et al., 1995). In total, 144 samples were evaluated, considering the two olive groves and the three times analyzed.

2.4 Statistical analysis

The boron content in leaves was statistically evaluated through factorial analysis of variance (blocks; treatments with B in the soil; presence of foliar fertilization; time sampling) with comparison of means by Tukey test ($p<0.05$). Analyzes were performed using the statistical program Sisvar version 5.8. Differences in leaf B concentration between olive groves were evaluated by Mann-Whitney, using the SigmaPlot version 11.0 program. Median values and quartiles were graphically represented by box-plot, with the aim of evaluating the amplitude of data distribution.

3. Results and Discussion

The boron content in leaves in both olive groves, in all treatments and sampling times was between 23.6 - 67.8 $mg.kg^{-1}$ (average values; Table 1). The values fell within the threshold range of foliar levels considered adequate for olive trees, between 19 and150 $mg.kg^{-1}$ (Mesquita et al., 2012; CQFS-RS/SC, 2016).

Table 1 – Boron concentration in mature olive leaves ($mg.kg^{-1}$ DW) submitted to soil and foliar fertilization in Caçapava do Sul (CS) and São Gabriel (SG) olive groves, in three sampling time: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development.

Fertilization/	CS			SG		
Time sampling	T0	T1	T2	T0	T1	T2
0 g [a]	58.6±2.9 [c]	29.2±1.3	65.2±4.1	34.7±1.5	24.3±1.4	42.7±2.9
25 g	57.8±5.0	32.1±1.7	67.8±3.1	36.0±1.5	25.4±1.6	43.4±2.4
50 g	58.0±3.3	32.3±1.2	63.9±3.5	41.0±3.0	24.6±1.4	48.5±2.8
100g	52.6±1.9	31.4±0.8	66.2±1.5	37.4±4.6	24.1±2.0	48.9±3.9
	NS	NS	NS	NS	NS	NS
No foliar	55.2±1.9	31.3±1.0	64.0±2.2	39.5±2.6	25.5±1.1	46.8±1.2
With foliar [b]	58.2±2.8	31.2±0.9	67.6±2.2	35.0±1.0	23.6±1.0	45.2±3.0
	NS	NS	NS	NS	NS	NS
Ulexite x Bortrac™	NS	NS	NS	NS	NS	NS

[a] Grams of ulexite per tree. [b]Bortrac™ (10.9% B), in a rate of 10 mL of commercial productin 1.5 L of aqueous solution/tree. [c]Mean values ± SE are presented (n = 9). Source: Authors (2023).

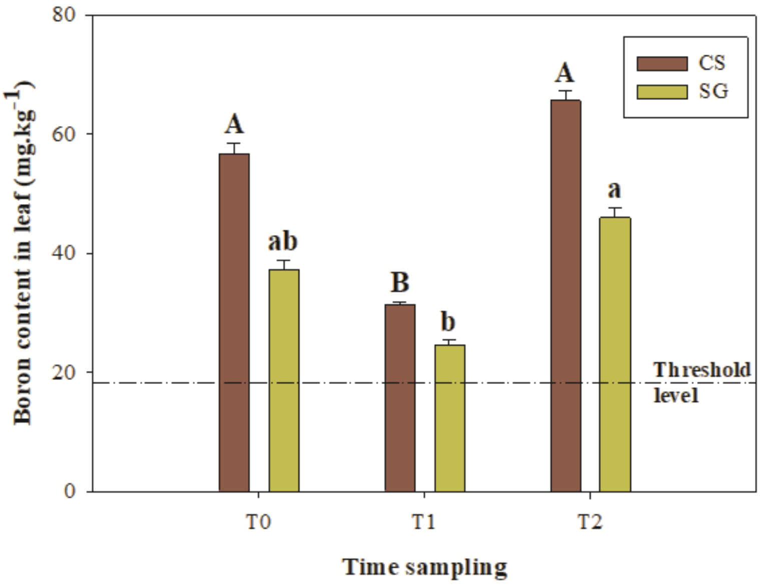
Boron concentration in leaves did not differ statistically among soil and leaf B treatments (Table 1). There was no significant difference between blocks, as well as the interaction of factors. It is important to highlight that before fertilization (T0), olive trees were nourished in relation to boron content, especially in CS (Table 1). In general, trials with fertilization for adult plants were conducted with olive trees with B deficiency or close to the limit of deficiency, as reported by Delgado et al. (1994) (19 ppm), Perica et al. (2001a) (17 ppm) and Hegazi et al. (2018) (13.4 ppm). In the present study, it was decided to carry out the experiments in olive trees without evident B deficiency in order to be connected to the reality of most olive growers currently in south of Brazil. It is worthy to note that no evidence of toxicity by B excess was noticed, even in the treatment with the largest rate of soil B fertilizer joined by foliar fertilization.

The absence of increase in leaf B content may be associated with the interval of time between the B fertilization and leaf sampling. The period of 45 days after broadcasting the non-soluble B-ulexite in the soil may be short to evidence increases in leaf B content because the nutrient uptake may be slow compared to the sink effect to the flowering demand. In the other side, the interval of 18 days to sprayed foliar B was within the period evaluated in the literature for olive trees, considering the effect of foliar fertilization with B, reported between 14-30 days (Perica et al., 2001a; Hegazi et al., 2018; Pasković et al., 2019).

Considering the same experimental design as in the present study, Figueiredo (2022) did not observe differences in the fruit yield in the 2020 harvest in the evaluated olive groves. However, in the following harvest, there was a greater residual effect for the treatment with 100g Ulexite/no foliar on fruit yield. Such result indicate that fertilization of B via soil, using a non-soluble source as ulexite, may supply the element in a very slowly manner. Contrarily to our results, Ateyyeh and Shatat (2006) observed an increase in the concentration of B in olive leaves and inflorescences after application of foliar B, however they did not observe increase in fruit yield between fertilized and non-fertilized plants. The authors suggest that olive trees with adequate levels of B may not respond significantly to the application of the micronutrient.

Regarding the sampling time of leaf tissue, a significant difference was observed in the two olive groves. In CS, T1 (flowering) differed from the other periods, with lower levels of B in the leaves. In SG, T1 exhibited lower B content than T2 (initial fruit development), but did not differ from T0 (pre-flowering) (Figure 1).

Figure 1 – Boron concentration in mature olive leaves (mg.kg^{-1} DW) in Caçapava do Sul (CS) and São Gabriel (SG) olive groves, in three sampling time: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development. Different letters, in the same olive grove, differ statistically according to Tukey's test with p<0.05 (n=9).



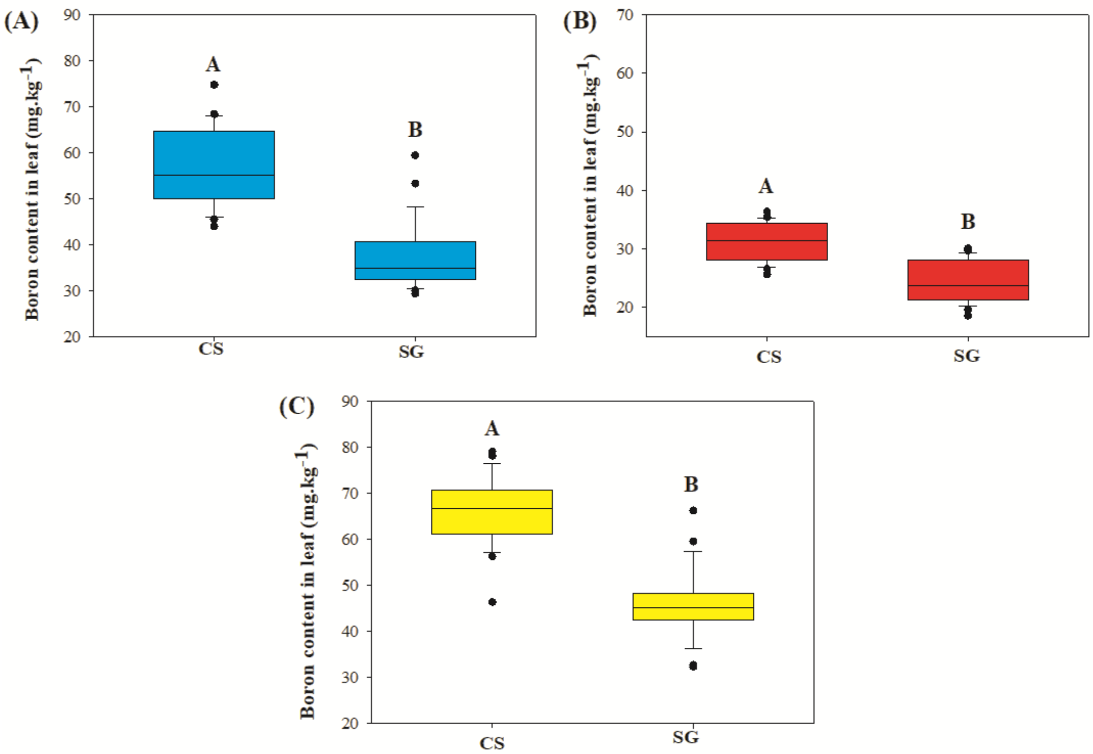
Source: Authors (2023).

There was a reduction in boron concentration in mature leaves of CS and SG of approximately 45% and 34% respectively, at T1 compared to T0 (Figure 1). In the literature, it has been suggested that there is translocation of B from vegetative tissues to reproductive structures, especially flowers, so that such structures stock greater amounts of such micronutrient (Dell & Huang, 1997; Blevins & Lukaszewski, 1998). Studies with olive trees corroborate this hypothesis, since higher B contents were observed in reproductive structures, compared to vegetative structures (Perica et al., 2001a; Hegazi et al., 2018). In addition, a marked reduction in the levels of B in leaves during the anthesis period was reported (Delgado et al., 1994).

The retranslocation of B by the phloem has been associated with the formation of a complex of boron with transport molecules of sugars or polyols (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998). Perica et al. (2001b) in assays with B fertilization in olive trees found evidence of B remobilization from leaves of different ages to inflorescences and fruits, through the phloem with transport mediated by sugars, such as mannitol and glucose. Liakopoulos et al. (2005) in studies with nutritional deficiency of B in olive trees found evidence of mobilization of the micronutrient from mature leaves to young leaves, in addition to an increase in the concentration of mannitol in plants with deficiency. Ferreira et al (2019) also suggest mobility of B in olive trees, and the retranslocation of B is dependent on the cultivar and must be taken into account in boron fertilization. Du et al. (2020) in studies with B foliar fertilization in Citrus trees showed long-distance mobilization of B, through a B-sucrose complex, from leaves to roots.

It stands out that B content in leaves at T2 practically doubled compared to T1. Thus, it is suggested that the amount of B in immature fruits is sufficient to attend their metabolic demands. Boron concentration differed between olive groves at the three sampling times, with CS showing higher values than SG (Figure 2). It was found that in T0 and T2 the SG olive grove showed a smaller range of data variation than CS.

Figure 2 – Box-plots of boron concentration in leaves of mature olive leaves (mg.kg^{-1} DW) in Caçapava do Sul (CS) and São Gabriel (SG), in three sampling time: T0 = before boron application/pre-flowering (A); T1 = after soil boron application/flowering (B); T2 = after foliar boron application/initial fruit development (C). Each box-plot is represented by the minimum, maximum, lower horizontal line of the box (1st quartile), intermediate line (median, 2nd quartile), upper horizontal line (3rd quartile) and 'outliers' (points). Different letters indicate statistical differences according to the Mann-Whitney test with p<0.05 (n=27).



Source: Authors (2023).

Figueiredo (2022) analyzed the concentration of B in the soil at T0 and found values of 0.44 and 0.11 mg.kg^{-1} in CS and SG, respectively. In addition, the author observed that the soil in SG is more sandy, with a lower clay content (4.8 g.100g^{-1}) than in CS (7.4 8 g.100g^{-1}), making it more difficult for the roots to absorb B, factors that may have influenced the differences found in the two olive groves.

4. Final Considerations

Fertilization for adult olive trees with soil fertilizer at doses from 0 to 100 g and foliar fertilizer did not increase B content in relation to non-fertilized trees. The micronutrient concentration before fertilization showed adequate levels, with a marked reduction being observed in the flowering period, regardless of the treatment with B. At the initial fruit development, exhibited B concentration similar to pre-flowering. It is suggested mobilization of B from leaves to flowers, corroborating results in the literature for olive trees. Boron content was higher in CS than in SG and may be associated with higher levels of B in the soil.

Further studies with a greater number of fertilizer applications and longer sampling times, from the end of a season's harvest to the beginning of the next season, will help to understand the effect of B fertilization on olive trees in different phenological periods. Future researches are also suggested to evaluate higher doses of foliar B, which do not lead to toxicity, but which provide more pronounced effects on micronutrient concentration in different tissues of olive trees.

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