Title page

Comparative boron nutritional diagnosis for olive based on July and January leaf samplings

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Abstract

In this work, diagnosis of boron (B) nutritional status based on leaf B concentrations were compared for the most common leaf sampling times for olive trees, January and July. For this purpose field experiments were conducted over four years (2003-06) in two rainfed olive groves located in Mirandela and Bragança, in north-eastern Portugal. Leaf samples were collected in January and July and analysed for B by standard procedures. Fruit harvest occurred in December of each year.

The crops followed typical alternate fruiting cycles. During the four years of the study, mean olive yields in the Bragança orchard, for instance, fluctuated, yielding 3.6, 28.1, 5.5 and 22.7 kg tree^{-1}. Yield variation per individual tree was also great. In the Bragança orchard and in the 2004 harvest, for instance, yields ranged from 1.2 to 52.7 kg tree^{-1}. Leaf B concentrations also varied greatly between individual trees. In the Bragança orchard in the July sampling of 2004, values for individual trees varied from 12.2 and 23.7 mg B kg^{-1}. From a total of 16 scatterplots generated from the relationship between leaf B concentrations and olive yields, 10 significant linear relationships were established; six of them were related to July sampling dates and four were related to January dates. The number of significant linear relationships established between leaf B concentration and olive yield was used as a criterion of the accuracy of the B nutritional diagnosis, since this represented the lowest experimental variability. By using this criterion, the July sampling date was proved as better B nutritional diagnosis, although the difference from January sampling date was not sufficient to disregard this. Leaf B concentrations were consistently higher in July than in January. Averaged across the four years of the study in both orchards, the difference was 4.3 mg B kg^{-1}. This difference should be taken into account in the interpretation of leaf analysis results when B levels are close to the deficient critical concentration.

Keywords: biennial bearing; leaf boron analysis; leaf sampling date; olive (*Olea europaea* L.); rainfed olive orchards

INTRODUCTION

The olive is widely grown in North-eastern Portugal having high economic and social significance, particularly in the southernmost part region where this crop is the most important in terms of harvested area and overall profit. The topography of this region is characterized by an irregular relief, with high potential for soil erosion. Most olive orchards are grown on Leptosols derived from schist (Figueiredo et al., 2002). These soils are shallow with low water-holding capacity. They are acidic, poor in organic matter and containing low levels of macronutrients. The climate is Mediterranean-type with a pronounced hot and dry summer season. Almost all the orchards (≈ 95%) are grown rainfed under a severe drought stress (Bacelar et al., 2007). In general, the production of these orchards is low characterized by alternate-bearing cycles.

Boron is an essential element for higher plants (Marschner, 1995). Clear evidence of B deficiency has been reported in many countries for several crops (Shorrocks, 1997). Dicots have higher B requirements compared to monocots (Asad et al., 2001), being B deficiency in olive a common nutritional disorder (Tsadilas and Chartzoulakis, 1999; Soyergin et al., 2002; Rodrigues and Arrobas, 2008). Probably because B deficiency is widespread in olive orchards, olive is considered a high-demand plant for this nutrient (Fernández-Escobar, 2001). However, olive is also a crop much less sensitive to higher B levels than other commercial tree crops (Freeman et al., 2005). A lack of available B is prevalent in most soils of NE Portugal. Vale (1988) showed that

73.5% of soil samples collected in all the NE Portugal territory demonstrated B deficiency symptoms in radish (*Raphanus sativus* L.) grown in pot experiments.

In NE Portugal, visible symptoms of B deficiency are common in commercial olive orchards. Short branch growth and chlorosis on the apical portion of the olive leaves in young tissues are the most common symptoms of B deficiency. Chronic symptoms of B deficiency include branch dieback, bushy vegetation and parthenocarpic and malformed fruits. Hidden B nutritional disorders are certainly present in many orchards, considering that B levels are considered deficient for olives below 14 mg kg^{-1} and at an adequate level ranges from 19 to 150 mg kg^{-1} (Fernández-Escobar, 2001; Freeman et al., 2005). Over the last 10 years, approximately 50% of the leaf samples from commercial orchards received by the Agrarian School of Bragança for laboratory analysis have had B concentration levels below the adequate range (unpublished).

Standard leaf nutrient concentrations for olive have been reported in many books and manuals on soil and plant analysis (Mills and Jones, 1996; Santos, 1996; Jones, 1998; Fernández-Escobar, 2001; LQARS, 2006). Although some authors consider July (in the northern hemisphere) the most appropriate time for leaf sampling (Mills and Jones, 1996; Fernández-Escobar, 2001), others consider the wintertime as an adequate period for diagnosing the nutritional status of the olive (Brito, 1976; Bouat, 1987; Santos, 1996; Jordão et al., 1999; Soyergin and Katkat, 2002; LQARS, 2006).

The standards for interpretation of olive leaf analyses have been supported for early studies mostly carried out in irrigated orchards in California, grown for the table fruit industry (Chapman, 1966; Hartmann et al., 1966; Beutel et al., 1983). Despite the relevance of these previous works, only very limited studies exist, particularly regarding the relationship between the B nutritional status of olives and factors such as dry-farming and alternate-fruiting cycles, which may influence plant growth and yield as well as leaf B concentration. Based on a four year field trial carried out in rainfed conditions, this study compared (i) the leaf B concentrations in January and July with the leaf standards reported in the literature for these periods; and (ii) the accuracy of the diagnoses obtained from the January and July leaf samples.

MATERIALS AND METHODS

Site Description

The field experimentations were carried out in two commercial olive orchards located in Mirandela (41º 31' N; 7º 12' W) and Bragança (41º 48' N; 6º 44' W) in north-eastern Portugal. Mirandela represents the main centre of olive production in the region. Bragança represents the northern limit of the expansion of the crop. Mirandela is located at 250 m altitude and its climate is warmer and dryer than Bragança. The mean annual temperature and precipitation are 14.2 ºC and 520 mm, respectively. Bragança is located at 690 m altitude, and registers 11.9 ºC and 741 mm for the mean annual temperature and precipitation, respectively (INMG, 1991). Both the Mirandela and Bragança orchards are planted on Leptosols derived from schist. Selected soil properties of both the olive orchards are presented in Table 1. The Mirandela orchard is a rainfed young plantation (13 years old in 2001) of the cv. 'Cobrançosa', spaced at 7 m x 6 m. The Bragança orchard is an old plantation of more than 60 years, of the cv. 'Cobrançosa', spaced at 7 m x 7 m grown under rainfed conditions.

Crop Management

The Mirandela orchard has been managed since 2001 under three different ground-cover systems corresponding to three separated plots: conventional soil tillage using a scarifier (two tillages yr^{-1} in spring), the herbicide Mascot 600 SC® (diuron +

glyphosate + terbutilazine) applied in February, and the herbicide Roundup® (glyphosate) applied in early April. The Bragança orchard has also been managed under three ground-cover systems: sheep-walking, conventional soil tillage, and the herbicide Roundup®.

A compound 10:10:10 (10% N, P_2O_5 and K_2O) fertiliser was applied annually at a rate of 1 kg per tree in the Mirandela orchard plus 5.5 g B per tree as borax. In the Bragança orchard after March 2002, 1.5 kg per tree per year of a compound 10:10:10 fertiliser was applied plus 7.7 g B per tree per year as borax. All fertilisers were applied beneath the trees' canopy. The incorporation in soil was only performed on conventional soil tillage plots. The Mirandela orchard was pruned (≈ 33% leaf area removed) in February 2002 and in April 2006 while the Bragança orchard was pruned in March 2001 (≈ 33% leaf area removed), March 2003 (≈ 15% leaf area removed) and March 2006 (≈ 25% leaf area removed).

Sampling and Analytical Procedures

Groups of similar trees in terms of vigour and canopy volume were marked in each plot of both orchards in October 2001. In the Bragança orchard, ten trees per plot were selected while the Mirandela orchard, twelve. The olive yields were recorded annually in December for each tree. Leaf samples were also collected during the experiment from each one of the pre-selected trees.

Leaf samples were taken from the middle of last season's shoots of the pre-selected trees in January and July, from January 2003 to July 2006, following standard procedures (Fernández-Escobar, 2001; LQARS, 2006). The leaf samples were oven-dried at 70 ºC and ground to pass through a 1 mm^2 mesh. Leaf boron concentration was determined colorimetrically using the azomethine-H method after dry combustion with CaO (Walinga et al., 1989).

Data Analysis

Correlation analysis between leaf B concentration and olive yield revealed that the better fit obtained with a linear model ($P < 0.05$) in all cases. The differences in leaf B concentration between the groups of the most and least productive trees of each orchard were analysed by comparing their mean confidence limits ($P < 0.05$). The differences in leaf B concentrations between samples taken in July and January were analysed as paired plots, using the t test ($P < 0.05$).

RESULTS

Olive Yields and Leaf Boron Concentrations in the Biennial Cycle of the Olive

Olive yields differed greatly even though between different trees from the same orchard. In the Mirandela orchard, olive yield ranged between 3.6 and 11.8, 0.1 and 7.6, 0.0 and 8.8 and 1.2 and 14.2 kg $tree^{-1}$ in 2003, 04, 05 and 06, respectively. The average values of the Mirandela orchard were 7.4, 2.1, 4.6 and 6.4 kg $tree^{-1}$ in 2003, 04, 05 and 06, respectively. Years 2003 and 2006 are considered as ‘on’ years, 2004 as ‘off’ year and 2005 as a ‘fair’ year. In the Bragança orchard, the olive yields per tree varied from 0.0 and 12.6, 1.2 and 52.7, 1.4 and 19.7 and 2.3 and 46.2 kg $tree^{-1}$ in the harvests of 2003, 04, 05 and 06, respectively. The average values of the orchard were 3.6, 28.1, 5.5 and 27.7 kg $tree^{-1}$. Similarly the years 2003 and 05 are considered as ‘off’ years while 2004 and 06 as ‘on’ years.

Leaf B concentrations varied also greatly between trees for both sampling dates. In the Mirandela orchard in January, leaf B concentrations for the individual trees ranged from 11.6 to 15.0, 13.9 to 18.9, 12.6 to 18.5 and 11.4 to 18.8 mg kg^{-1} in 2003, 04, 05 and 06, respectively. In July, leaf B concentrations varied between 15.1 and 23.4, 13.1 and 23.5, 14.4 and 24.1 and 13.5 and 22.2 mg kg^{-1} in 2003, 04, 05 and 06, respectively. In the Bragança orchard, leaf B concentrations in January ranged from 9.5 to 13.6, 8.8 to 13.9, 11.5 to 20.1 and 14.2 to 20.2 mg kg^{-1} in 2003, 04, 05 and 06, respectively. The values in July varied between 12.2 and 20.6, 12.2 and 23.7, 14.3 and 24.5 and 17.4 and 34.4 mg kg^{-1} for the four consecutive years.

Relationship between Leaf Boron Concentrations and Olive Yields

In figure 1 the relationship between leaf B concentration and olive yield are presented (Fig. 1). From this figure it is obvious that three of the five significant linear relationships were based on data from 'on' years and two were based on data from 'off' years, three of the five significant linear relationships corresponded to the July sampling date and the others to the January sampling date. From the Bragança experiment, five similar significant linear relationships between leaf B concentration and olive yield were also found (Fig. 2).

Leaf Boron Concentrations in the Groups of Productive and Unproductive Trees

Based on the individual olive trees yields in the four harvests, two groups of the five most and least productive trees were established. In the Mirandela trial, these groups represented 14% of the total number of trees included in the experiment (36), whereas in the Bragança trial, these groups represented 17% of the trees in the study (30). The mean leaf B concentration and the mean confidence limit of each group of trees were estimated for all sampling dates being presented in figure 3.

In both experiments, the groups containing the most productive trees often showed higher leaf B concentrations for both the January and the July sampling dates. From the eight leaf samplings made for the Mirandela experiment, four of the differences in mean leaf B concentrations between the most and least productive trees groups were statistically significant ($P < 0.05$). Three of them were associated to ‘on’ years and one only to an ‘off’ year. The sampling dates of July and January registered two cases with significant differences. The group with the most productive trees from the Bragança orchard showed significantly higher leaf B concentrations than the least productive group on six cases. Significant differences were observed for three ‘on’ years and also for three ‘off’ years. Regarding the sampling dates, July sampling registered four cases while January only two.

Leaf Boron Concentrations for July and January Samples

The differences in leaf B concentrations between July and January for samples collected from the same trees were estimated over the four year period and in both locations. The results are shown in figure 4, which also shows the mean confidence limits for each population point. The mean July values were significantly higher compared to January for all the years, in both sites. In the Mirandela experiment, the mean values of the difference of the leaf B concentrations in July and January were 6.8, 1.9, 3.0 and 2.5 mg B kg^{-1} in 2003, 04, 05 and 06, respectively. In the Bragança experiment, the respective values were 4.3, 5.8, 4.8 and 7.1 mg B kg^{-1} in the four consecutive years.

DISCUSSION

Olive Yield Pattern and Leaf Boron Concentration

Both the orchards showed typical alternate-fruiting cycles during the four years of the study. The biennial-bearing in olive is common and it is very well documented (Lavee et al., 1986; Rallo et al., 1994; Rallo and Cuevas, 2001), especially under poor growing conditions. Lavee et al. (1983) stated that under rainfed conditions, yields during the 'off' years can be so low that growers find it uneconomic to harvest the small crops. In rainfed orchards, soil water availability in summer is one of the most limiting factors for crop growth and yield in the Mediterranean basin (Orgaz and Ferreres, 2001). This is certainly an important factor influencing the production pattern in our experiments.

The differences in crop yield between trees from the same orchard and year were very high. For instance, the yields of the individual trees of the Bragança trial in the harvest of 2004 ranged from 1.2 to 52.7 kg $tree^{-1}$. Similarly, leaf B concentrations varied greatly among trees in the same field. These results may be explained by the combined effect of several factors, including differences in the yield potential of the individual trees, the situation of each tree in its biennial-bearing cycle and the spatial variability in soil properties. López-Granados et al. (2004), for instance, found a great spatial variability for leaf nutrients N, P, K and B in a 30 ha olive orchard located in Southern Spain. According to López-Granados et al. (2004), the variation detected for leaf nutrient concentration was high enough to justify the implementation of a site-specific fertilisation programme.

Relationship between Leaf Boron Concentration and Olive Yield

The relationship between the concentration of a given nutrient in a plant part and the growth or yield can be considered under three main zones: the *zone of deficiency*, where the yield increases sharply, but there is only a small change in nutrient concentration in plant tissues; the *transition zone*, where both nutrient concentration and yield increase simultaneously; and the *adequate zone*, where the nutrient concentration increases without a correspondent increase in growth and yield (Munson and Nelson, 1973). In olive leaves, B is considered deficient below 14 mg kg^{-1} and is in the adequate range between 19 and 150 mg kg^{-1}, for the sampling period of July (Fernández-Escobar, 2001; Freeman et al., 2005). Similar standards were also reported for the winter period (Santos, 1996; MADRP, 2000; LQARS, 2006), and for the entire season (Jones, 1998). In the present study, soil B levels were particularly low (Table 1). As a consequence, leaf B levels were often below the adequate range, and even below the critical deficient limit, for July and also for winter sampling dates (Figs. 1 and 2). The low leaf B levels found in these orchards were certainly the main reason for the linear relations recorded between leaf B concentration and olive yield. Under these conditions, a significant linear relationship for a given scatterplot shows a lower experimental variability compared to plots in which data did not fit the linear model. When a significant linear relationship is found, the scatterplot provides a better diagnosis of the B nutritional status of the trees.

The relationship between leaf B concentration and olive yield was statistically significant for ten scatterplots from a total of sixteen tested (Figs. 1 and 2). When the significant linear relations were considered according to the production level, six of them were associated with 'on' and four were associated with 'off' years. It seems therefore that the B nutritional status of a tree affected significantly crop productivity. Regarding the two sampling periods, six significant linear regressions were related to July and four were related to January leaf sampling dates. Thus, it seems that a slightly better B nutritional diagnosis may be achieved by leaf analysis in July than in January.

Considering the four consecutive years (2003-06), a slight increase in leaf B concentration was recorded in both orchards. This was obviously due to the regular B supply. Annually, in early spring, 5.5 and 7.7 g B $tree^{-1}$ was applied to the younger (Mirandela) and the older (Bragança) orchards, respectively. These rates based on the integrated olive production norms for the Portuguese rainfed orchards (MADRP, 2000). The tenuous increase observed in leaf B concentration throughout the years indicated that the rates of applied B were not adequate to quickly alleviate B deficiency. Fernández-Escobar (2001), for instance, recommends 25 to 40 g B $tree^{-1}$ (≈ 5 to 8 kg ha^{-1}) to correct B deficiency in olive. Mortvedt and Woodruff (1993) reported general values for arable crops and vegetables in the range of 0.25 to 3 kg B ha^{-1}, depending on crop requirements and method of application. Fernández-Escobar (2001) recommendations for olive seem to agree with the results of this study.

Relationship between Leaf Boron Concentrations and Olive Productivity

The groups Mirandela and Bragança most productive trees (14% and 17%, respectively) had often significantly higher leaf B concentrations than the less productive groups. This result demonstrated that there was a clear influence of the leaf B levels on olive yield. In the 'on' years, more statistically significant differences occurred in leaf B concentrations between the most and least productive groups of trees. This could mean that in the years 'on' diagnosis of the B nutritional status is easier. A better diagnosis enables a prompt correction of a B deficiency to mitigate the alternate-bearing cycle of olive, since B is directly related to flowering and fruit set (Scott et al., 1943; Perica et al., 2002). From the leaf samples collected in July, six occasions were found in which the differences between the more and less productive groups' trees were statistically significant while from the samples collected in January only four statistically significant differences occurred out of a total eight. Thus, in this experiment the diagnoses made from the July leaf samples were slightly better than the diagnoses made from the winter samples, as was previously concluded by the number of significant linear regressions recorded. July is the only sampling period for leaf analysis suggested by Mills and Jones (1996) and Fernández-Escobar (2001). However, the potential of the winter sampling period for diagnosing the boron nutritional status of olive must not be disregarded, since it is of fundamental importance for enabling the fertilisation programme for the next season, especially when the fertilisers are applied to soil which is a common practice in rainfed orchards. Additionally, the winter sampling period is much longer, making it easier. If B deficiency is detected by leaf analysis in July the problem could only be mitigated by foliar B sprays. By July, the blossoming has already occurred and thus any improvement in the current year crop is uncertain.

Leaf Boron Concentrations in July and January Samplings

Leaf B concentrations appeared consistently higher in July compared to January. This may be attributed to the fact that the leaves taken in July were younger than those collected in January, since shoots develop faster in spring and in early summer as it was suggested by Delgado et al. (1994) and Fernández-Escobar (2001). The competition between fruits and leaves for B occurring late in summer may also be an additional reason for the lower B levels found in January. Since fertiliser-B was applied in early spring, available soil B would be higher for leaves which were developed in spring and early summer and collected in July. Low availability of soil water has been reported to promote B deficiency in plants during dry periods (Bell, 1997). The winter samples are composed of leaves that developed during summer and early autumn, a period of substantial water shortage, which may also influence the composition of leaves.

Higher leaf B concentrations in the summer compared to winter were previously reported by Fernández-Escobar et al. (1999), Rodrigues et al. (2001) and Sibbett and

Ferguson (2002). In this study, the difference in leaf B concentrations in July and January reached values that should be taken into account in the diagnosis of the B nutritional status of an olive orchard. Averaged across the four years and two localities, the mean of the differences was 4.3 mg B kg^{-1}. LQARS (2006) previously reported standards for the adequate zone ranging from 19 to 50 mg B kg^{-1} and 15 to 50 mg B kg^{-1} for the July and winter sampling periods, respectively. The results presented in this work support a difference of 4 mg B kg^{-1} reported by LQARS (2006) for the lower limit of the adequate zone.

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Table 1. Some properties of the initial (October, 2001) soil samples.

	Mirandela		Bragança	
Parameters	0-10 cm	10-25 cm	0-10 cm	10-25 cm
pH (soil:water, 1:2)	5.5	5.5	6.0	6.0
Organic C (Walkley-Black) (g kg^{-1})	2.9	0.8	8.4	2.2
Extractable P (Egner-Rhiem) (mg kg^{-1})*	21.8	23.1	36.2	11.4
Extractable K (Egner-Rhiem) (mg kg^{-1})*	53.1	42.3	75.5	58.1
Exchang. bases (ammonium acetate, pH 7)				
Ca ($cmol_c$ kg^{-1})	3.14	3.18	11.60	11.12
Mg ($cmol_c$ kg^{-1})	2.21	2.65	3.05	3.40
K ($cmol_c$ kg^{-1})	0.07	0.04	0.21	0.14
Na ($cmol_c$ kg^{-1})	0.11	0.11	0.13	0.12
Soluble B (Boiling-Water and Azomethine-H procedure) (mg kg^{-1})	0.12	0.10	0.33	0.13
Bulk density (Mg m^{-3})	1.5		1.6	
Texture (USDA)	Loam		Loam	

*Extracted by ammonium lactate plus acetic acid, buffered at pH 3.7.

List of figures

Figure 1. Relationship between leaf B concentrations in January (four left figures) and July (four right figures) and olive yields in December from the Mirandela orchard. From top to bottom, each pair of figures corresponds to the years 2003, 04, 05 and 06.

Figure 2. Relationship between leaf B concentrations in January (four left figures) and July (four right figures) and olive yields in December from the Bragança orchard. From top to bottom, each pair of figures corresponds to the years 2003, 04, 05 and 06.

Figure 3. Mean leaf boron concentrations of the 17% and 14% most (open rings) and least (closed circles) productive trees in Bragança (left figure) and Mirandela (right figure) orchards. Vertical bars are the mean confidence limits ($P < 0.05$).

Figure 4. Variation of leaf B concentrations between samples collected in July and January of the same year from the same tree in the Bragança (left figure) and Mirandela (right figure) orchards. Horizontal bars define the mean confidence limits ($P < 0.05$).