

Quantitative relationships between boron and mannitol concentrations in phloem exudates of *Olea europaea* leaves under contrasting boron supply conditions

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Received: 17 June 2008 / Accepted: 3 February 2009
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Abstract Boron remobilization (BR) occurs in plants that form and export phloem–mobile borate–polyol complexes. Previous studies demonstrate that a quantitative relationship exists between polyol translocation and BR. Here we investigate if mannitol translocation is quantitatively related to BR in olive plants thus allowing acclimation to boron limiting conditions. Plants were cultivated under different boron supply ranging from adequate (23 μM) to insufficient or zero supply (0.5 μM or 0 μM). Measurement of boron in the leaf phloem sap exudates (B_P) of olive leaves of low or zero boron supply treatments showed that, whereas boron was remobilized, its absolute amounts in the phloem were lower compared to the control. However, BR from source leaves at 0.5 μM or 0 μM was maintained at relatively high levels in regard to the amounts of

boron available in the cells of these leaves, indicating a strategy of the source leaves to remobilize boron by depleting their cell sap boron pool. Concurrently, in the above treatments, leaf phloem mannitol (M_P) was up to two-fold higher, resulting in a up to five-fold higher ratio of mannitol to boron in the phloem (M_P/B_P), compared to the control. Furthermore, both M_P and M_P/B_P were negatively correlated with cell sap boron concentration of the leaves indicating the trend for BR under boron limitation. It is concluded that, in this plant species, mannitol concentrations in the leaf phloem may be involved in the promotion of BR under inadequate external supply of boron.

Keywords Borate–polyol complexes · Boron remobilization · Cellular boron · Mannitol translocation · *Olea europaea* L. · Phloem sap exudate

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Responsible Editor: Richard Bell.

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Abbreviations

BR	Boron remobilization
M_P	Mannitol concentration in phloem sap exudates
B_P	Boron concentration in leaf phloem sap exudates
B_C	Boron concentration in cell sap (plus apoplastic fluid)
B23/B05/ B0	23 μM /0.5 μM /0 μM boron nutrient solution treatments

Introduction

A considerable number of plant species are characterised by their inability to remobilize acquired leaf boron to growing organs. On the contrary, in boron-remobilizers, boron can be redistributed to younger plant parts to meet the demands of the growing sinks (Brown and Hu 1996; Brown and Shelp 1997; Cakmak and Römheld 1997). As initially hypothesized by Raven (1980), in these plants, boron remobilization (BR) is owed to the formation of stable, phloem-mobile diester complexes of borate with appropriate low molecular weight ligands (Bellaloui et al. 1999; Brown et al. 1999). Up to now, borate complexes with polyols (sugar-alcohols) mannitol and sorbitol have been demonstrated to be present in the phloem (Hu et al. 1997; Penn et al. 1997; Dannel et al. 2002). Through remobilization, plants can supply boron to demanding sinks which is particularly important under low external supply (Liakopoulos et al. 2005; Matoh and Ochiai 2005; Leite et al. 2007; Huang et al. 2008). This mechanism is well-integrated since the above mentioned prerequisites for growth (photoassimilates (including polyols) and, under low external boron supply, remobilizable boron) are translocated via a common pathway, the source-to-sink phloem conduit.

Recently, Jiang et al. (2008) provided detailed information concerning boron recycling between xylem and phloem owed to the formation of boron-mannitol complexes and they demonstrated that a quantitative relationship exists between polyol translocation and BR. In view of these findings, the dynamics of BR could be described by studying the portion of boron that may be allocated for the formation of these complexes under contrasting boron supply. Given the plethora of soluble borate-complexing molecules of the cellular environment (Loomis and Durst 1992; Power and Woods 1997), it is assumed that virtually all cellular boron exists in complexed form (Power and Woods 1997). On the other hand, a considerable percent of cellular boron may be uncomplexed depending on boron supply and the abundance of complexing agents in the cells of a particular species (Pfeffer et al. 1999). Therefore, the relative abundance of polyols in the cell sap would be expected to be a major factor determining the potential of boron export (Bellaloui et al. 2003). In olive (*Olea europaea*), a boron remobilizing species

(Delgado et al. 1994; Perica et al. 2001), both mannitol concentrations in cells of source leaves and the rate of mannitol export increase under low-boron supply, while a considerable rate of BR is also maintained under these conditions (Liakopoulos et al. 2005). Therefore, increased mannitol concentrations in the phloem could be involved in the promotion of BR under low-boron conditions in olive. Although the existence of polyols is related to boron mobility (Brown and Hu 1996; Bellaloui et al. 1999; Brown et al. 1999; Bellaloui et al. 2003; Lehto et al. 2004a; Lehto et al. 2004b; Jiang et al. 2008), BR has not been quantitatively related to polyol translocation under different boron regimes.

Here the response of BR under boron limitation as well as the involvement of mannitol in BR promotion is examined. Olive plants were hydroponically cultivated under three boron supply levels, ranging between adequate (23 μM boron in nutrient solution) and insufficient or zero-supply (0.5 or 0 μM). Typically, BR is assessed by monitoring changes in leaf boron between sources and sinks during the experimental period (reviewed by Brown and Shelp 1997; see also Lehto et al. 2000; Perica et al. 2001; Bellaloui et al. 2003; Lehto et al. 2004a; Lehto et al. 2004b). In the present study boron and mannitol concentrations were measured in leaf phloem exudates of source leaves (see also Huang et al. 2008). Using this straightforward approach, the relationships between BR, cellular boron and phloem mannitol concentrations were quantitatively investigated.

Materials and methods

Plant culture

Grafted plants of *Olea europaea* L. cv. 'Manaki' (approx. 17 cm height above root-stem interface) were grown hydroponically in 2 l pots using quartz sand as the supporting medium in a growth chamber according to the experimental practice reported previously (Liakopoulos et al. 2005). Environmental conditions were: photoperiod 16.5/7.5 h, air temperature 25/18°C and relative air humidity 50/60% (day/night). Light intensity was 450–550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PAR) at plant height, provided by sodium vapour lamps (VIALOX NAV-T400 4Y, OSRAM, GmbH, Munich, Germany). Plants were supplied with either

full composition half-strength Hoagland nutrient solution (boron supplied as boric acid at 23 μM , control treatment, B23) or boron insufficient half-strength Hoagland nutrient solution (boron supplied as boric acid at 0.5 or at 0.0 μM , low-boron and zero-boron treatments, B05 or B0 respectively), for a period of 20 days. Above zero boron concentrations (control and low-boron treatments) were chosen based on previous studies and preliminary experiments (Liakopoulos et al. 2005; Stavrianakou et al. 2006). Each pot/plant received six irrigations per 24 h (67 ml of nutrient solution per irrigation). The exact composition of the nutrient solution is reported elsewhere (Liakopoulos et al. 2005).

Sampling and measurements

Twenty four plants were assigned to each treatment. At harvest, leaves were divided into two groups according to their developmental stage/age, as described in Table 1. Depending on the particular analysis, leaves from three to 24 plants were randomly pooled to yield the final replicates (see figures for details). Statistics were performed using JMP v. 7.0 (SAS Institute, Cary, NC, USA). Significant differences between treatments were determined by one-way ANOVA and post-hoc comparisons by least significant difference ($P < 5\%$).

Collection of cell sap and phloem exudates

Samples of cell sap (100–400 μl) were collected from batches of leaves (ca. 10–20 leaves per sample) by applying pressure with a mechanical tool. Samples collected by this technique represent a mixture of intracellular fluids and apoplastic fluid. Samples were

Table 1 Classification of leaf samples according to their developmental stage

	Leaf surface area at harvest (cm^2) ^a	Description
Young	3.98	Young leaves, fully expanded at harvest, non existing at the beginning of the experiment
Mature	4.57	Mature leaves, fully expanded at the beginning of the experiment

^a Mean of all experimental treatments. Sample leaf area per treatment/leaf age is shown in Table 3

centrifuged (16,000 $\times g$, 5 min) and the supernatants were stored at -20°C until analyzed. Leaf phloem exudates were collected by the EDTA-enhanced exudation technique (King and Zeevaert 1974, with modifications by Flora and Madore 1993). Leaf samples (each sample comprised 20 to 24 leaves) were placed in plastic vials and the petioles were immersed in 1.5 ml of exudation solution (20 mM Na_2EDTA , pH adjusted to 7.0 with KOH). Leaves were exposed at 45° angle to incident light and the duration of exudation was 8 h. After 4 h of exudation, 1 ml of water was added in each vial to replace losses due to evaporation. Afterwards, the volume of each exudate solution was measured and the exudates were stored at -20°C until analyzed. Since the EDTA-enhanced exudation technique does not yield direct quantitative data because the volume of phloem sap collected is unknown (Fisher and Frame 1984), carbohydrate or boron content in the exudation solution was expressed on a leaf water basis; i.e., moles of translocated molecule per unit of leaf cell sap volume of each sample used to collect the exudates and per hour of exudation. This expression allowed the subsequent expression of the relative rate of BR as moles of translocated boron per mole of boron available in the leaf cells (see Results).

Measurement of boron concentration

Total leaf boron was measured photometrically using the azomethine colour reaction, according to Banuelos et al. (1992) with modifications (Liakopoulos et al. 2005). Boron in cell sap and leaf phloem exudate were measured using the chromotropic acid/HPLC method according to Matoh et al. (1997) with modifications (Stavrianakou et al. 2006). Standard solutions and reagents were prepared using Ultrapure water (Merck KGaA, Darmstadt, Germany; max $[\text{B}] < 0.2 \text{ ng g}^{-1}$) and sample handlings were done using polypropylene tubes and beakers. Samples of phloem exudate were processed for boron determination according to Stavrianakou et al. (2006). Due to small volumes of the cell sap samples, measurements were done using small volumes down to 100 μl . Final samples were prepared by diluting by the appropriate volume of Ultrapure water to a final 1000 μl volume, according to the initial protocol (Matoh et al. 1997). In all cases, the concentration of the dilute sample was within the limits of the reference curve. Since the samples were

not acidic (see Matoh et al. 1997), no addition of NaOH was carried out (Stavrianakou et al. 2006).

HPLC analysis of carbohydrates in leaf phloem sap exudates

Analyses were performed on a Jasco HPLC system (Jasco Corporation, Tokyo, Japan). Samples were filtered (Chromafil, RC-20/25, Macherey-Nagel) and aliquots (20 μ l) were injected on a Nucleosil Carbohydrate 10 μ m (250 \times 4 mm) analytical column (Macherey-Nagel) kept at 40°C. The mobile phase (HPLC grade acetonitrile 70% in water, Labscan, Dublin, Ireland) was delivered at 2 ml min⁻¹ flow rate. Eluents were detected with a RI-930 refractive index detector and chromatograms were captured in a PC system running Borwin Chromatographic Software, v. 1.21.60 (JMBS Development, Fontaine, France). Peaks were identified with comparison to pure standards and quantitative analysis was made according to reference curves.

Results

Effect of the experimental treatments on plant growth and leaf boron

Total boron of leaves ranged between adequate and deficient levels responding to boron treatments. Both treatments which were regarded as inadequate for growth (B05 and B0) resulted in equally low levels of leaf boron (Table 2). The reduction of leaf boron in the B05 and B0 treatments was more evident in young compared to mature leaves. Young leaves showed higher boron concentrations compared to older leaves when the supply of boron was adequate

for growth (B23) while this pattern was reversed under boron deficiency (Table 2). Boron treatments caused a considerable hampering of growth that was evident both in the growth rate and in the plant biomass at harvest. Among the plant parts, shoot (biomass and length) and leaves (total leaf area per plant) were more severely affected (Table 3). Soluble boron in the mesophyll cells (B_C), determined in the isolated cell sap of the leaves, was drastically reduced in young leaves due to the experimental treatments. On the other hand, mature leaves retained comparably higher amounts of B_C despite the restriction of boron supply from the substrate (Fig. 1a).

Relationship between leaf boron status and boron remobilization

BR from young leaves (B_P , concentration of boron in the leaf phloem sap exudate) was reduced in B05 and B0 treatments compared to controls (B23) while B_P from mature leaves was similar between all boron treatments (Fig. 1b). Moreover, B_P of mature leaves was higher compared to young leaves indicating that mature leaves contribute considerably to BR, especially when external boron supply is low. It is worth noticing that while B_C was drastically reduced due to the low-boron treatments, remobilizable boron was only reduced by about half in the corresponding treatments (compare Fig. 1a and b). To describe the distribution of boron among cellular allocation and remobilization, the ratio of phloem exudate to leaf cell sap boron was calculated (B_P/B_C , Fig. 1c). Since B_C is considered as the boron pool that is potentially remobilizable (Dannel et al. 2000; Dannel et al. 2002) and B_P is the remobilizable boron, a relative rate of BR can be estimated from the above ratio. It is clear

Table 2 Concentration and content of boron in leaves of different developmental stages after each treatment

	B23		B05		B0			
Young								
Boron concentration (mg B kg ⁻¹ d.w.)	15.52 \pm 1.25	a	7.15 \pm 0.83	(-54%)	b	7.65 \pm 0.66	(-51%)	b
Boron content (μ g leaf ⁻¹)	1.52 \pm 0.13	a	0.97 \pm 0.13	(-37%)	b	0.96 \pm 0.08	(-37%)	b
Mature								
Boron concentration (mg B kg ⁻¹ d.w.)	11.57 \pm 0.62	a	9.78 \pm 0.44	(-15%)	b	9.83 \pm 0.52	(-15%)	b
Boron content (μ g leaf ⁻¹)	1.13 \pm 0.07	a	1.11 \pm 0.05	(-1%)	a	1.13 \pm 0.05	(0%)	a

Data are means of five replicates (each replicate from three plants; one leaf per plant) \pm s.e. Percents in parentheses are differences of each treatment compared to the control (B23). Different letters denote statistically significant differences between treatments ($P < 0.05$)

Table 3 Growth rates (a) (during a 10-day period at the middle of the 20 days experiment) and plant growth parameters (b) (at harvest)

	B23		B05		B0		
(a) Plant growth rate							
Shoot length (cm day ⁻¹ plant ⁻¹)	0.184±0.052	a	0.109±0.028	(-41%)	a	0.081±0.030	(-56%) a
Leaf length (mm day ⁻¹ leaf ⁻¹)	0.238±0.067	a	0.159±0.062	(-33%)	a	0.108±0.030	(-55%) a
Leaf number (leaves day ⁻¹ plant ⁻¹)	0.044±0.017	a	0.030±0.009	(-32%)	a	0.015±0.008	(-66%) a
(b) Plant parameters at harvest							
Young shoot fresh mass (g plant ⁻¹)	1.02±0.12	a	0.45±0.05	(-56%)	b	0.29±0.03	(-72%) b
Young leaves fresh mass (g plant ⁻¹)	3.68±0.46	a	3.09±0.37	(-16%)	ab	2.27±0.20	(-38%) b
Root fresh mass (g plant ⁻¹)	7.57±0.36	a	4.99±0.32	(-34%)	b	3.92±0.32	(-48%) c
Young shoot length (cm)	17.24±1.57	a	9.04±0.72	(-48%)	b	7.58±0.58	(-56%) b
Young leaves total area (cm ² plant ⁻¹)	87.0±4.1	a	44.5±1.6	(-46%)	b	35.0±2.2	(-58%) c
Young leaves area (cm ² leaf ⁻¹)	5.03±0.11	a	3.43±0.07	(-32%)	b	3.49±0.09	(-31%) b

Data for (a) are means of five replicates (plants)±s.e. Data for (b) are means of 10–12 replicates (plants)±s.e. Percents in parentheses are differences of each treatment compared to the control (B23). Different letters denote statistically significant differences between treatments ($P<0.05$)

that inadequate boron supply caused a notable shift in soluble boron usage since remobilization to growing sinks had precedence over maintenance (storage) of soluble boron reserves in leaf tissues, judged by the high values of the B_P/B_C ratio (Fig. 1c). This phenomenon was more pronounced in young leaves, especially those of the B05 treatment (Fig. 1c). The examination of the relationship between B_C and B_P (Fig. 2) showed that the amount of remobilizable boron depends on its concentrations in the mesophyll cells of the corresponding leaves. However, this relationship was much stronger in the leaves of the B05 and B0 treatments compared to the control (Fig. 2).

Relationship between mannitol translocation and boron remobilization

Boron treatments of B05 and B0 caused leaf age-dependent effects in concentrations of mannitol in the phloem sap exudates (M_P). In particular, low boron supply caused an up to two-fold increase in M_P in young leaves, while no notable effect on M_P concentrations of mature leaves was observed (Fig. 3). This effect was specific for mannitol since phloem sucrose concentrations were virtually stable between all treatments (data not shown). The above behavior resulted in an up to three-fold increase in the mannitol to sucrose ratio in the phloem (Fig. 3, insert). As shown

in Fig. 4a, M_P concentrations were related to B_C of the corresponding leaves, but only under boron limitation. In B05 and B0 treatments, very low cellular boron concentrations were related to M_P concentrations since the latter were increased by ca. 45–75%. On the other hand, M_P was held relative constant when B_C was not at deficient levels (i.e. B23, Fig. 4a).

Given the chemical composition of the mannitol–borate complex, the molecular ratio of the two components in the leaf phloem was examined (M_P/B_P). When M_P/B_P was plotted versus B_C , it was apparent that boron deprivation of the leaves affects the M_P/B_P ratio and consequently remobilization (Fig. 4b). Under normal boron status of the leaves (i.e. B23), the M_P/B_P ratio was between 5 and 38. The M_P/B_P ratio considerably increased (up to five-fold on average) when boron was deprived from the leaf tissues (Fig. 4b). Furthermore, it is of physiological importance to examine if increased M_P concentrations promotes BR from the corresponding leaves. Consequently, the B_P/B_C ratio, considered as a measure of relative BR, was examined versus M_P (Fig. 5). According to the results, the B_P/B_C ratio was strongly correlated with M_P in both low-boron treatments (B05 and B0) but not in controls (B23). Therefore, increased concentrations of mannitol in the leaf phloem were associated with the relative remobilization of leaf boron.

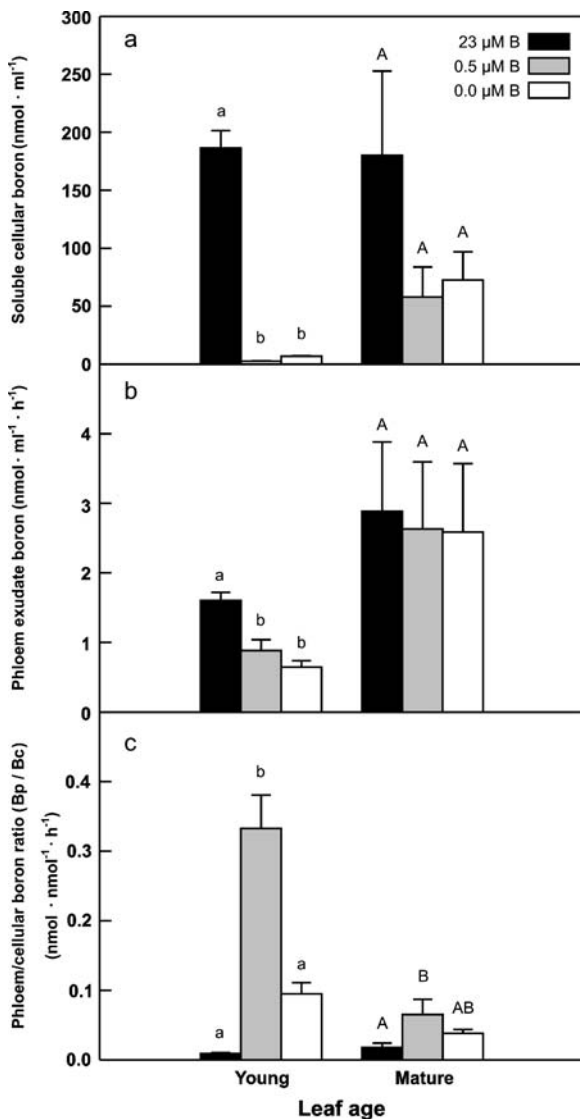


Fig. 1 **a** Soluble cellular boron concentration, **b** boron concentration in phloem exudates, and **c** ratio of boron concentration in phloem exudates to the corresponding leaf soluble cellular boron concentration (B_P/B_C) of leaves of different developmental stages after each treatment. Data are means of four replicates (each replicate from 12–24 plants; one leaf per plant) \pm s.e. Different letters above bars denote statistically significant differences between treatments ($P < 0.05$)

Discussion

Boron allocation between storage and remobilization depends on boron regime

According to the experimental practice followed and the results of the present study, hydroponic culture of

olive trees in nutrient substrates differing in the concentration of boron, resulted in the development of boron deficiency in B05 and B0 treatments, while B23 treatment served as the B-adequate control (see also Liakopoulos et al. 2005). The levels of boron measured in the leaves either as total leaf boron or as soluble cellular boron (Table 2 and Fig. 1a) and the visible deficiency symptoms (data not shown) and the hampering of growth recorded as the production of new plant biomass during the experimental period (Table 3), indicate that both B05 and B0 treatments were inadequate for plant growth, although treatment B05 resulted in considerably milder effects in growth parameters.

In this experiment, two leaf categories were chosen for measurements (Table 1). According to the experimental setup, expansion of young leaves occurred entirely within the experimental period (Table 1). Therefore, young leaves are thought to be acclimated to the boron regime imposed by the treatments and the differences in boron concentration between young leaves (Table 3) can be largely attributed to the differences in boron supply from the external medium and remobilization from existing leaves. Moreover, boron in young leaves of the B0 treatment can be considered to originate predominantly through remobilization from older leaves, because no boron was supplied from the external medium (Liakopoulos et al. 2005). Differences in total leaf boron between mature leaves of the three treatments were limited

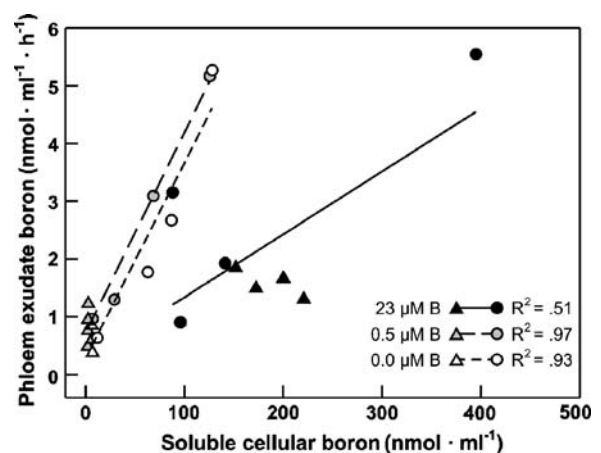


Fig. 2 Linear regression between soluble cellular and phloem exudate boron concentration. For each treatment, data from both leaf developmental stages (triangles: young; circles: mature) were used

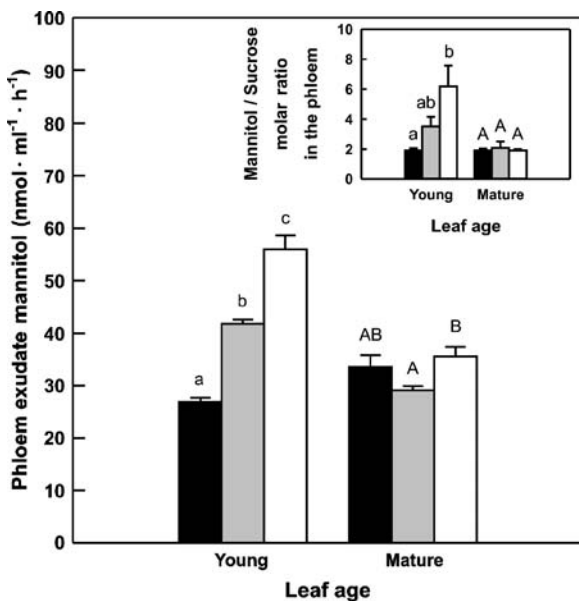


Fig. 3 Mannitol concentration in phloem exudates and mannitol to sucrose molar ratio in phloem exudates (*insert*) of leaves of different developmental stages after each treatment. Data are means of four replicates (each replicate from 12–24 plants; one leaf per plant) \pm s.e. Different letters above bars denote statistically significant differences between treatments ($P < 0.05$)

(Table 3) indicating that boron amounts acquired during development at plant nursery (before the experiment) primarily determined final boron concentrations and that the experimental treatments did not considerably affect this parameter. This is particularly evident if boron content per leaf is examined (Table 2). Compared to the total leaf boron concentrations, soluble cellular boron concentrations were more severely affected by the treatments. In fact, boron deprivation primarily affects the soluble boron fraction (Hu and Brown 1994; Matoh and Ochiai 2005).

Typically, experiments that include the measurement of BR are conducted by the use of the isotopic tracer ¹⁰B and the subsequent enrichment of sink tissues in this isotope. Recently, Huang et al. (2008) employed the use of both stable boron isotopes and direct measurements in xylem and phloem saps to discriminate newly acquired and remobilizable boron (through a phloem to xylem transport) supply to growing sinks in white lupin. In the present study, BR was assessed by the concentration of boron in leaf phloem sap exudates. This approach was chosen as most appropriate in order to depict the relationships

between boron status of the source tissues, remobilizable boron and mannitol translocation.

According to B_P concentrations, leaves of all experimental treatments were able to remobilize boron, although at different amounts (Fig. 1b), showing that BR is sustained under boron limiting conditions in accordance to previous findings (Liakopoulos et al. 2005). Mature leaves exported larger amounts of boron compared to young (Fig. 1b) probably because their soluble boron pool was also larger (Fig. 1a). These leaves were developed during boron sufficient conditions (before the treatments) and their high boron content may therefore have an important effect on the capacity for BR, in accordance to previous findings (Matoh 1997; Leite et al. 2007).

The relative rate of BR (as the ratio of phloem exudate to leaf cell sap boron) was extremely high in

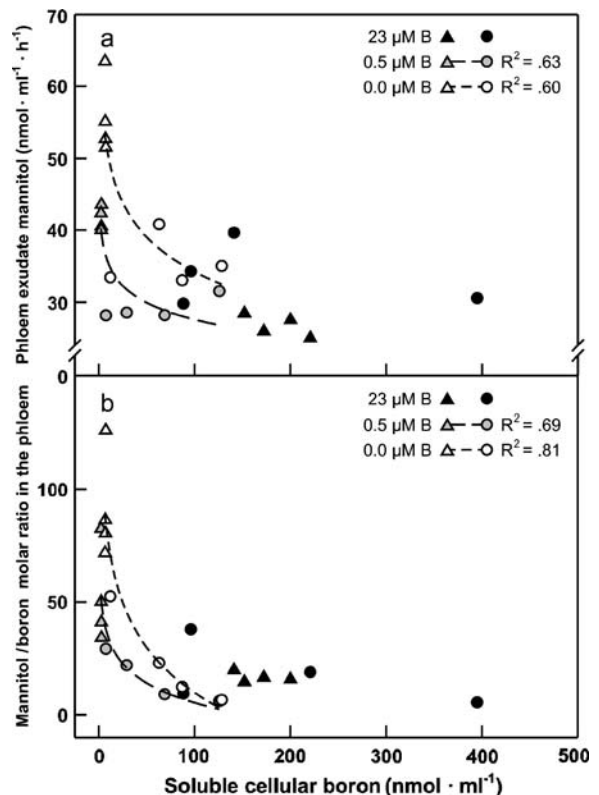


Fig. 4 Logarithmic regression between **a** soluble cellular boron concentration and mannitol concentration in phloem exudates and **b** soluble cellular boron concentration and the molecular ratio of mannitol to boron in phloem exudates. For each treatment, data from both leaf developmental stages (*triangles*: young; *circles*: mature) are used. Only statistically significant regressions ($P < 5\%$) are plotted

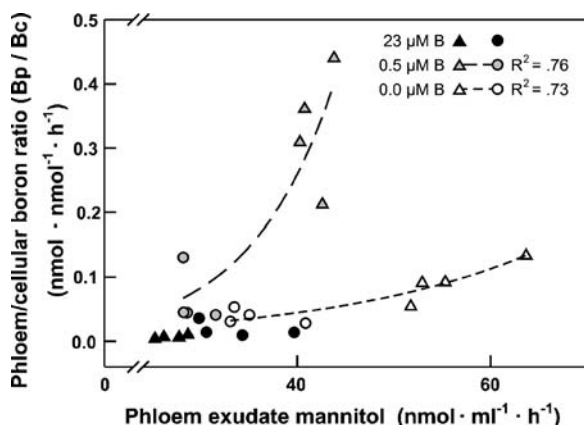


Fig. 5 Exponential regression between mannitol concentration in phloem exudates and the ratio of boron concentration in phloem exudates to the corresponding leaf soluble cellular boron concentration (B_p/B_c). For each treatment, data from both leaf developmental stages (*triangles*: young; *circles*: mature) are used. Only statistically significant regressions ($P < 5\%$) are plotted

B05 and B0 treatments suggesting that low-boron conditions promote BR (Fig. 1c). This response may be related to the essentiality of the maintenance of boron supply to growing sinks at low-boron conditions. In particular, leaves of the B05 treatment were more effective in contributing to BR (resulting in a higher rate of BR) compared to B0, which could be related to the supply of boron from the external medium and/or higher sink strength of the B05 treatment. Between leaf ages, young leaves showed higher values of BR rate compared to mature when boron was limiting suggesting that these leaves are more active in contributing to BR in expense of their soluble boron pool. Also, Matoh and Ochiai (2005) reported that, under boron limitation, younger sunflower leaves export newly taken-up boron to growing sinks more vigorously, eventually resulting in lower leaf boron concentrations than older leaves. Similarly, enhancement of boron remobilization was observed under boron limiting supply from source leaves of white lupin (Huang et al. 2008).

Mannitol translocation is correlated to boron remobilization depending on boron regime

Factors that may determine BR include the size of the soluble boron pool of the source leaf, mannitol concentration in leaf tissues and rate of export (which in turn affects mannitol concentration in the phloem

sap). It is evident that the concentration of soluble cellular boron affects the corresponding amounts of boron exported in the phloem (Fig. 2). This is expected if soluble cellular boron is considered as the source of remobilizable boron. It is notable, however, that the relation between phloem boron and soluble cellular boron concentrations was stronger under low-boron conditions compared to that under adequate boron supply (Fig. 2). This was particularly obvious after careful examination of the mature leaves. Mature leaves of B05 and B0 treatments were able to remobilize comparable boron amounts with those of mature leaves of the B23 treatment (Fig. 1b) despite their lower soluble cellular boron concentrations compared to the control (Fig. 1a). Therefore, it seems that yet more factors determine the relative rate of BR.

Leaf phloem mannitol (Fig. 3) could be considered as a principal factor determining BR since it complexes boric acid and renders it mobile in the phloem (Hu et al. 1997). Therefore, mannitol concentrations in the phloem could possibly influence the intensity of remobilization. It is not surprising therefore that BR from mature leaves was similar among treatments since mannitol translocation from mature leaves was also not affected by the low boron treatments (Fig. 3). However, in young leaves, higher mannitol concentrations in the leaves of boron deficient plants (Liakopoulos et al. 2005) could increase the rate of mannitol–borate complex formation and subsequently increase the relative intensity of BR despite the low pool size of soluble cellular boron of these leaves. Jiang et al. (2008) demonstrated that boron redistribution between barley and its hemiparasite *Rhinanthus minor* depends on the abundance of mannitol in the later. Furthermore, if more mannitol–borate complexes are continuously created in source tissues and loaded into the phloem under low-boron conditions compared to control, then the amounts of mannitol in the phloem should be increasing under boron deficiency. Indeed, this was reported earlier for olive plants grown under field conditions (Liakopoulos et al. 2005) and it was also observed in the present study for young leaves (Fig. 3, Fig. 4a). It is important to state that the above effect was specific for mannitol, since sucrose concentrations were unaffected, resulting in a significant increase in the mannitol to sucrose ratio in the phloem (Fig. 3, insert). The increase of phloem mannitol is a notable effect since one would

expect a reduction of sink strength due to the effect of boron deficiency on growth (see also Table 2).

The molar ratio of mannitol to boron in the phloem sap exudates was also increased as a response to boron starvation of the corresponding leaves (Fig. 4b) suggesting the existence of a mechanism that works for the maintenance of BR from source leaves even at low soluble cellular boron concentrations. Indeed, phloem mannitol concentrations were related with the relative rate of BR (Fig. 5). This may be a causal relationship indicating that mannitol export is able to uphold BR rates even when very low amounts of boron are present in the source leaves. However, the concentration of mannitol in the leaf cells rather than in the leaf phloem may control boron export since it is hypothesized that only mannitol–borate complexes created at the leaf cells are loaded into the phloem. Accordingly, previous studies have shown that increase of leaf phloem mannitol co-occurs with similar increases of mannitol in source-leaf cells (Liakopoulos et al. 2005). Nevertheless, apart from mannitol export, other physiological variables may be implicated to the determination of boron export from leaf cells. Considerable BR occurs in canola (Stangoulis et al. 2001) and white lupin (Huang et al. 2008) under boron limitation although these plants are not known to remobilize boron through polyols. Lehto et al. (2004b) showed that the polyol content is not the only factor determining the rate of BR by studying a wide range of plant species. Nevertheless, the present study indicates that the relationship between polyol content and BR appears to be more predictable within the same plant species.

Concluding, the present study showed that BR rates in olive are reduced by boron limitation but remained at high levels in relation to the soluble boron pool of the source leaves. As a consequence, cellular boron concentrations may fall to very low levels, indicating that the minimum amounts of boron required by already grown organs are very low (Brown and Hu 1997). According to the results, mannitol export increases in the leaf phloem, depending on boron starvation, and that this increase is related to BR. This relationship may be causal since boron in the olive tree is remobilized as a mannitol–borate complex and may signify an acclimation mechanism that operates under limiting boron supply.

Acknowledgements Authors thank Dr. Mariangela N. Fotelli for useful suggestions during manuscript preparation and Mr.

G. Kostelenos (Kostelenos Olive Nurseries) for the supply of plant material.

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