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SHORT NOTE

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Boron uptake by ectomycorrhizas of silver birch

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Abstract Boron (B) is an essential micronutrient for plants but it is thought not to be essential for fungi. We studied whether the extraradical mycelia of *Paxillus involutus* in symbiosis with silver birch (*Betula pendula*) take up B and transport it to the host plant. We grew mycorrhizal plants in flat microcosms with a partitioning wall, below which there was only extraradical mycelium. A boric acid solution enriched in ^{10}B was applied to these mycelia. Increased $^{10}B/^{11}B$ isotope ratios were subsequently measured in birch leaves, stems, and roots plus mycorrhizas in the upper compartment. Boron was therefore taken up by the mycorrhizal mycelia and transported to the host plant in this species combination.

Keywords *Betula pendula* · Boron uptake · Ectomycorrhiza · *Paxillus involutus* · Stable isotopes

Introduction

Boron (B) is an essential micronutrient for higher plants, but it is thought not to be essential for fungi. The best known function of B in plants is in the formation and stabilisation of the cell wall through cross-linking with pectic polysaccharides in the rhamnogalacturonan-II region (Matoh 1997). This function, as most of the other biological and soil-chemical properties of B, is due to its high affinity to form complexes with *cis*-diol groups (Power and Woods 1997).

The uptake of B by mycorrhizas has not been demonstrated, and it could be argued that it does not occur. Lewis (1980) presented a hypothesis that the evolution of the vascular structures in higher plants was related to B, because of the role of B in lignification. Most carbohydrates typical of lower plants and micro-organisms have high affinity for B binding, which would cause B immobilisation. In contrast, in vascular plants sucrose became the major carbohydrate for translocation. As sucrose has very low affinity for B, the mobility of B in the xylem and the function of B as a plant nutrient became possible (Lewis 1980). It is consistent with this hypothesis that ectomycorrhizas do not take up B, because fungal carbohydrates, such as mannitol, would complex with B and prevent it from moving into plant cells.

However, there are higher plant species with mannitol as a major carbohydrate in phloem translocation, and it has now been shown that, rather than sequestering B in an immobile form, mannitol, sorbitol and other sugar alcohols have a particular role in B mobility (reviewed by Brown and Shelp 1997). Sugar-alcohol containing plant species are able to redistribute B in the phloem, whilst in plant species with mostly sucrose translocation, B is one of the most immobile nutrients. Hu et al. (1997) demonstrated the presence of soluble mannitol-B-mannitol complexes in the phloem sap of celery (*Apium graveolens*). In view of the mobility of B-sugar alcohol complexes in plants, such complexes could potentially be translocated in the mycorrhizal fungus and the fungus-plant interface of the mycorrhiza. It is also possible that free boric acid is translocated within mycorrhizal mycelia, as the mycelia are known to take up significant amounts of water (Duddridge et al. 1980; Boyd et al. 1986).

If B were immobilised when it comes into contact with fungal carbohydrates, the B concentrations of sporocarps of mycorrhizal fungi should be negligible, as all constituents of sporocarps are translocated through below-ground mycelia. However, we have found high B concentrations in sporocarps of *Paxillus involutus* (Fr.) Fr. in

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the field; for example, 35 mg kg^{-1} in a site where the B concentration in current Norway spruce [*Picea abies* (L.) Karst.] needles was about 10 mg kg^{-1} (T. Lehto and A. Lavola, unpublished data). The hypothesis for this study is that extraradical mycelia of *Paxillus involutus* take up B, and that this B is translocated to the aboveground parts of the host plant.

We studied the B uptake of mycorrhizal silver birch [*Betula pendula* (L.) Roth.] by applying boric acid enriched in ^{10}B to external mycelia of *Paxillus involutus*, growing in a compartment separated by a wall from the root system.

Materials and methods

Three isolates of *Paxillus involutus* were used in the experiment: J222, J224 and J235. All the isolates originated from fertile Norway spruce or Norway spruce/silver birch forests near Joensuu. Fungal cultures were grown in glass jars with an autoclaved mixture of vermiculite and unfertilised peat (120 ml: 30 ml), amended with 80 ml modified Melin-Norkrans medium (Mason 1980). Silver birch seeds were germinated aseptically on water agar after surface sterilisation with H_2O_2. When the fungus had colonised the vermiculite-peat, germlings were placed in the jars. After 8 weeks under fluorescent tubes, mycorrhizal seedlings were moved to flat polycarbonate microcosms (30×30 cm), with a barrier allowing the growth of external mycelium in a separate compartment from the root system (Finlay et al. 1988). A 2 mm layer of unfertilised and unlimed peat was used as a growth substrate, and there were 2–3 seedlings in each microcosm. The microcosms were in a walk-in growth room with continuous light and 22°C temperature. Initially, after planting the seedlings into microcosms, the relative humidity (RH) of the chambers was 90% and photosynthetic photon flux density 30 μmol m^{-2} s^{-1}. Gradually the light level was raised to 200 μmol m^{-2} s^{-1} and the RH reduced to 70%. The peat was watered as needed with deionised water. The only nutrient addition was a total of 0.28 mg P and 0.96 mg K, applied as a dilute solution of KH_2PO_4 and K_2SO_4 1 week before the treatments; peat soils are typically poor in P and K.

At 8 weeks after planting into microcosms, when the mycelium had grown to the isolated compartment, the uptake experiment was started. Control plants did not receive the N and B treatment, and their main purpose was to determine the background levels of the added isotopes. The ratio of the two B isotopes ^{10}B and ^{11}B is usually very constant in nature (Power and Woods 1997), and any increase in the controls would have indicated contamination in the growth room or an analytical error. Moreover, in a preliminary experiment we had applied ^{10}B in a lower compartment without any mycelium, with no change in the resulting proportion of ^{10}B in the plant in the microcosm. Initially there were three replicate microcosms for the B and N treatment, and two controls for each of the three *Paxillus*-birch combinations.

The treatment microcosms were treated with 20 μg ^{10}B and 1 mg ^{15}N in 20 ml double-deionised water. The B was added as $H_3{}^{10}BO_3$ (Sigma-Aldrich, 97% of the B as ^{10}B), and the ^{15}N was as $(^{15}NH_4)_2SO_4$, (Sigma-Aldrich, 98% of the N as ^{15}N). The B and N treatments were both in the same solution, which was applied with a pipette on the growing edge of the mycelial fans. Controls were treated with 20 ml double-deionised water. The purpose of the ^{15}N treatment was to control that the treated mycelia were viable and able to take up nutrients, and also to relate the N and B uptake to each other.

After 72 h, the shoot of each seedling was severed at the base of the topmost lateral root, and dried at 60°C. The root systems from the top compartment were harvested with forceps and scalpel, without rinsing with water. The root harvest was not quantitative, as 1 cm from the edge of the two compartments was left out of the harvest, and only the roots growing on top of the peat were taken. Leaves, stems and roots from the 2–3 plants in each microcosm were bulked and milled. For B analysis, a subsample was dried at 105°C, ashed, and digested in HNO_3 (Suprapur). Total-boron concentration was determined by ICP-AES (Thermo Jarrell Ash Iris Advantage Duo High Resolution; Thermo Jarrell, Franklin, Mass.) and the $^{10}B/^{11}B$ isotope ratios were determined with ICP-MS (Perkin-Elmer Sciex Elan 6000).

The N isotope ratios were analysed from a subsample of each leaf sample and of those stem samples that were large enough for both B and N analysis. There was not enough root material for the N analysis. The isotope ratio (as $\delta^{15}N$) and N concentration of a subsample were analysed with a stable isotope analyser (Europa Scientific 20/20, PDZ Europa, Northwich, UK) at the Waikato Stable Isotope Unit, University of Waikato, New Zealand. ^{15}N abundance is reported as:

$$\delta^{15}N = \left(R_{sample}/R_{standard} - 1\right) \times 1,000 \quad (1)$$

where $R_{sample}={}^{15}N/{}^{14}N$ in the sample and $R_{standard}={}^{15}N/{}^{14}N$ in the reference standard (atmospheric N_2).

Welch's two-sample *t*-test was used for testing the treatment differences, as it does not assume homogeneity of variance. Proportion of ^{10}B and $\delta^{15}N$ were analysed using logarithmic transformation. In the case of values of $\delta^{15}N$, the number 15 was added before taking the logarithm. Additionally, Pearson correlations were computed.

Results

There were no major differences between the three *Paxillus involutus* isolates, and therefore the data for all isolates were pooled. In the majority of the microcosms (each with 2–3 seedlings bulked) there was increased $\delta^{15}N$ and ^{10}B, but in three of the nine treated microcosms neither was elevated relative to the controls in any plant part. Accordingly, ^{15}N and ^{10}B were statistically rather well correlated, particularly in the leaves, for which there were more replicates on ^{15}N (df=7, r=0.69, P=0.041), but to some extent also in the stems (df=4, r=0.60, P=0.212). For roots, there were no data on N.

The purpose of the ^{15}N treatment was to verify that the mycelia in the microcosms were viable and able to take up nutrients. Therefore, *t*-tests were performed both with and without the three microcosms that showed no ^{15}N translocation. The data in the figure and table are without these three, hence n=6 for both treatment and control.

The plants from control microcosms had values that did not deviate much from the natural ^{10}B abundance, 19.9%, and the variability was very small. Therefore contamination between treatments did not occur, and the variability in the control was due to determination error. The ^{10}B proportion was considerably higher in the root systems of the treated plants than in stems, and lowest in leaves, marking the pathway of B in the plant (Fig. 1). A correlation between the ^{10}B proportion between roots and stems of the same plants was clear (df=7, r=0.94, P=0.0002), as it was between the roots and leaves (df=7, r=0.97, P<0.0001). No attempt was made to separate mycorrhizal fungus from the roots, or non-mycorrhizal parts of the root system, and a large part of the ^{10}B in the root systems may still have been in the fungus. Nevertheless, the aboveground stems, which were

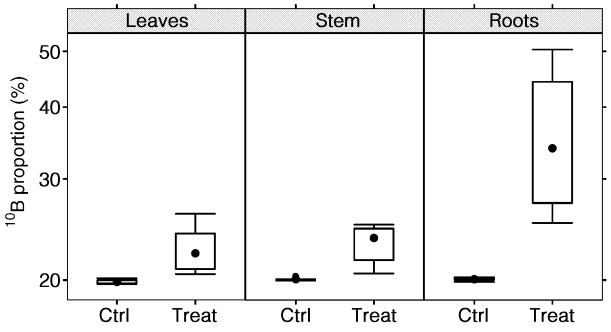

Fig. 1 Box plot of the percentage of ^{10}B of total B in birch leaves, stems, and roots in seedlings with and without ^{10}B treatment to external mycorrhizal mycelium. *Filled circles* Median values; n=6. Note the logarithmic scale

Table 1 Concentrations of boron (B) and nitrogen (N), proportion of ^{10}B of total-B, and δ ^{15}N in birch seedlings treated with corresponding markers in a mycelial compartment. n=6, means ±SE. Probabilities for treatment difference from Welch two-sample t-test

		Control	^{10}B- and ^{15}N-treated	P
B (mg kg^{-1})	Leaves	23.25±1.50	19.33±2.30	0.189
	Stems	9.92±1.27	7.90±0.95	0.249
	Roots	5.69±0.91	7.56±1.20	0.246
^{10}B (%)	Leaves	19.9±0.07	22.7±0.85	**0.017**
	Stems	20.1±0.05	23.2±0.71	**0.006**
	Roots	20.1±0.05	35.9±4.12	**0.005**
N (g kg^{-1})	Leaves	20.7±1.74	18.3±1.54	0.327
δ^{15}N	Leaves	−9.61±1.13	32.42±17.33	**0.015**

not in any direct contact with the mycelium, had a mean ^{10}B proportion of 23.2%, and the leaves, 22.7%, which were significantly higher than controls (Table 1).

When all nine treatment microcosms were included in the t-test, the mean values for the ^{10}B proportion were slightly lower: 31.1%, 22.2% and 21.8% in roots, stems and leaves, respectively. Each of these still differed significantly from the control ($P \leq 0.026$). However, P for δ^{15}N was 0.083 when all data were included, and the mean was 17.9.

The uptake of the stable-isotope markers did not significantly affect the total-B or the total-N concentration in any part of the seedlings analysed (Table 1), whether the analysis was carried out using all the plants or discarding the three with no ^{15}N and ^{10}B uptake.

Discussion

It is now 50 years since Melin (1952) first demonstrated uptake of ^{15}N by external mycelium in a *Suillus-Pinus sylvestris* symbiosis. Remarkable progress has been made since in the research on nutrient uptake, translocation and assimilation by ectomycorrhizas (Smith and Read 1997). Yet this is the first time that B uptake by external mycelia of an ectomycorrhizal fungus, and translocation to aboveground parts of plants, has been shown. The fungal carbohydrates of *Paxillus involutus* did not immobilise the B supplied, although it is possible that some B is sequestered within the mycelium with complex fungal carbohydrates.

Plants are thought to take up B from the soil mostly as undissociated boric acid (Hu and Brown 1997). This is also the most likely uptake form in the present experiment, as the boric acid was applied directly on the external mycelium. Apart from uptake as boric acid, uptake from soil as B-mannitol or other complexes is also conceivable, especially in the case of mycorrhizas. However, the pH in upper soil layers in birch forests is commonly about 4–5, whilst the stability of the B-mannitol complex is increased at higher pH values, up to pH 8.0 (Power and Woods 1997). Therefore the B-mannitol complex as such may be less important in boreal forests, as the pH in coniferous forests tends to be even lower than under birch. Nevertheless, soil organic fractions contain a wide range of different compounds with *cis*-diol groups, which have been only partially characterised.

Earlier, it was often assumed that B is taken up by plants only passively with water and that the driving force for within-plant transport was the transpiration stream (reviewed by Hu and Brown 1997). Recent studies support, on the one hand, passive uptake, and evidence of channel mediated transport of boric acid has also been presented (Dordas and Brown 2000, 2001; Dordas et al. 2000). On the other hand, evidence of active B uptake in plants has been provided by Dannel et al. (1998), who demonstrated that sunflower root cell sap was rich in B compared to the culture solution, when B was applied at a very low rate. Pfeffer et al. (1999) further showed that the B concentrating mechanism was suppressed by metabolic inhibitors and low root zone temperature. It cannot be concluded from our results whether the uptake by the fungus and translocation of B from the fungus to plant is active or passive, but this will be a subject for further studies.

Paxillus involutus sporocarps had higher B concentrations than most other ectomycorrhizal fungal species in our survey (T. Lehto and A. Lavola, unpublished data). This was considered as an indication of B translocation in the mycelia of this fungus. Moreover, differences between fungal species in this respect may indicate differences in the ability of mycorrhizal symbionts either to supply B to the host plant, or to immobilise B within the mycelium. It has now been shown that one fungus takes up B, and further studies will show if this is a widespread function among mycorrhizal species.

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