Plant and Soil **193:** 49–58, 1997.

Chapter 4

Absorption of boron by plant roots

Hening Hu and Patrick H. Brown
*Department of Pomology, University of California, Davis, CA 95616, USA**

Abstract

Experimental evidence suggests that B uptake is the result of the passive assimilation of undissociated boric acid. Boron uptake by a particular species should, therefore, be primarily determined by the B concentration in the soil solution and the rate of water uptake by the plant. This simple explanation of B uptake, however, does not adequately explain field observations where dramatic differences in B concentrations are observed between species, even when these species are grown under similar environmental conditions. The apparent contradiction between experimental results and in field observations, suggests, that B uptake is determined by factors that are as yet unknown. In the following, we discuss experimental and field observations as they relate to B uptake and discuss the mechanisms that may be involved in determining B uptake in diverse species.

Introduction

Boron is an essential micronutrient for higher plants, and B deficiency results in rapid inhibition of plant growth. The rapid and specific inhibition of plant growth that occurs upon removal of B is a consequence of two important features of B physiology: the specific structural role B plays in the cell wall (Hu and Brown, 1994; Loomis and Durst, 1992; Matoh, Chapter 5) and the limited mobility of B in the majority of species (Brown and Shelp, Chapter 7; Oertli and Richardson, 1970). As a result of its critical role in expanding tissues and its limited mobility, B has to be supplied continually throughout the life of the plant, usually through the root. For this reason, knowledge of the physiology of B absorption is essential.

Boron is absorbed from soil solution by roots mainly as the undissociated boric acid. Theoretical considerations predict that undissociated boric acid should be membrane permeable; in agreement with this, recent experimental results suggest that B absorption is a passive, non-metabolic process. Based solely upon these conclusions, it is predicted that B absorption is primarily determined by B concentration in the uptake medium and the transpiration rate of the plant. However, extensive field observations and experimental results demonstrate that species can differ significantly in their rate of B accumulation even when grown under identical environmental conditions. This contradiction cannot be explained adequately by our current understanding of B absorption mechanisms.

In this chapter we review current information on the mechanism of B absorption by plants and describe the biotic and abiotic factors that influence this process. This information is then discussed in light of observed differences in B absorption amongst cultivars and species.

Mechanisms of boron absorption

Properties of boric acid

Boric acid is a very weak acid in aqueous solution: its activity as an acid appears to be related to OH^- acceptance by the $B(OH)_3$ rather than to H^+ donation according to the following reaction:

$$B(OH)_3 + 2H_2O = B(OH)_4^- + H_3O^+ \ (pK_a 9.25)$$

Thus, in neutral or slightly acid soils, commonly occurring in plant growth environments, B exists mainly as undissociated boric acid (Raven, 1980).

* E-mail: hlhu@ucdavis.edu

Boric acid is soluble in water and has an ether–water partition coefficient of 0.035. Raven (1980) computed the permeability of plant cell membranes to $B(OH)_3$ on the basis of the 'lipid solution' mechanism. Using the linear relationship between $PM^{3/2}$ (P = permeability coefficient, M = mol. wt) and the ether–water partition coefficient, the computed value of P for $B(OH)_3$ is 8×10^{-6} cm s^{-1}. In another approach (Raven, 1980), using the linear relationship between log $PM^{1/2}$ and N (the number of potential H-bonding groups, N = 2 for –OH, and N = 6 for $B(OH)_3$), the estimated P for $B(OH)_3$ is 4×10^{-6} cm s^{-1}. This theoretical calculation led Raven (1980) to suggest that the permeability coefficient is high enough to account for the measured magnitude of boric acid fluxes across many plant cell membranes. The use of active transport of boric acid to maintain B distribution across a membrane away from thermodynamic equilibrium is consequently likely to be energetically expensive. On this basis, Raven (1980) predicted that B absorption by plant roots would be a passive process.

The conclusions made by Raven (1980) though sound, may be over simplified. The 'high' cost of maintaining a non-equilibrium concentration of an element, does not preclude the possibility of active absorption. Protons, for example, have an estimated membrane permeability of 10^{-3} to 10^{-4} cm s^{-1} (Dencher et al., 1986), which is 100 to 1000 times more permeable than boric acid. However, active H^+ transport occurs in all living systems (Briskin and Reynolds-Niesman, 1991).

Additionally, calculations of membrane permeability based upon ether:water partition coefficients are undoubtedly over simplified and may be misleading. For example, urea has a similar molecular wt (60) to boric acid (62) and is also uncharged at neutral pH. Finkelstein (1976) reported P_{urea} in an artificial membrane to be $0.6–4.0\times10^{-6}$ cm s^{-1}, while Lee-Standelmann et al. (1991) calculated P_{urea} in barley (*Hordeum vulgare*) to be $2.3–2.6\times10^{-5}$ cm s^{-1}. Hence, membrane permeability to urea in barley was found to be an order of magnitude greater than in an artificial membrane. Similarly, the permeability of water can vary significantly between membranes of different composition and cannot be adequately predicted from theoretical considerations alone. Jansen and Blume (1995) measured the water permeability of lipid vesicles composed of a variety of different phospholipids with different head groups and fatty acyl chains. Depending on the nature of the head group, on the chemical structure of the side chains and on the type of chain linkage, the permeability of water can differ by up to 3-fold from one kind of lipid vesicles to another.

In summary, it is clear that calculations of B fluxes based solely on theoretical lipid permeability are inadequate to predict B absorption by plants. Genotypes within a species could differ several fold in B permeability (see "the paradox of boron uptake"), which may have a physiologically important effect on B uptake by roots.

Role of boron complex formation in boron absorption

Another important property of boric acid that will influence B absorption is its ability to form *cis*-diol complexes with a variety of organic molecules. For example, in celery (*Apium graveolens*), B present in the cell sap and phloem exudates occurs almost entirely as the B–mannitol complex. No free boric acid was detected. The reaction of boric acid with mannitol is described in the following reaction (Hu et al., 1997):

$$H_3BO_3 + \underset{\text{mannitol}}{2C_6O_6H_{14}} = \underset{[\text{mannitol}-\text{B}-\text{mannitol}]^-}{[(C_6O_6H_{12})B(C_6O_6H_{12})]^-} + H_3O^+ + 2H_2O$$

The equilibrium constant for the reaction is 10^{-4}. The formation of borate–diol complexes has a much lower pK_a than that of borate. The pK_a of B–mannitol complex (about 5.2) is four units lower than that of free boric acid (9.25) (Raven, 1980), strongly suggesting that at normal B supply free boric acid concentrations inside the cytoplasm of celery (pH about 6.2–6.5) would approach zero. This situation is true for celery, in which mannitol is present at millimolar concentrations, and would also be true for other species where B-chelating diols are present at millimolar or greater concentration.

There are a great number of biological compounds that can form complexes with B, both in the cytoplasm and in the cell wall. Compounds capable of complexing with boric acid include sugars, their derivatives (sugar alcohols), phenols, organic acids, and some polymers (Boeseken, 1949; Raven, 1980). Common examples are sorbitol, mannitol, glycerol, ribose, apiose, nicotinamide-adenine-dinucleotide (NAD) and fructose (Loomis and Durst, 1992; Makkee et al., 1985). Low molecular weight B-complexes with mannitol, sorbitol and fructose have been recently isolated and characterised from plant tissue (Hu et al., 1997;

Penn et al., 1997). In addition, B complexes with rhamnogalacturonan-II in the cell wall have been characterised (Ishii and Matsunaga, 1996; Kobayashi et al., 1996; O'Neill et al., 1996).

The presence of significant amounts of B-complexing compounds in plant cells would have a significant effect on B uptake if uptake is solely determined by the free boric acid concentration gradient across the membrane. Raven (1980) suggested that the total B content of the tissue can greatly exceed the free boric acid concentration in the bathing solution due to the formation of B complexes, so that the demonstration of a net B influx into a tissue, in which the total internal B concentration exceeds that of external boric acid concentration is not adequate to show that active transport is occurring.

The uncharged nature of boric acid, the relatively high (though poorly defined) membrane permeability of boric acid, combined with its propensity to form complexes both in the cell wall and in the cytoplasm greatly complicates the study of B absorption. Classic analysis of elemental uptake based upon determination of transmembrane electrochemical gradients, analysis of absorption kinetics and measurement of electrical changes upon ion uptake do not readily apply to studies of B. Nevertheless, important insights into the mechanism of B absorption can be gained from a review of existing experimental data.

Is boron absorption active or passive?

Over the last 30 years this question has been addressed by many research groups with varied and conflicting results. The reason is partly due to the characteristic permeability of boric acid across cell membranes and the significant effects of *cis*-diol complex formation which adds considerable complexity to the interpretation of uptake experiments. Several of the studies of B absorption were conducted using unrealistically high B concentrations with highly variable absorption or desorption periods, and in only rare cases has the compartmentation of B within the root been adequately considered.

Bingham et al. (1970) conducted one of the first detailed studies of B absorption using excised roots of barley. With a B concentration of 0.93 m*M* and a 4 h absorption period, a strong pH dependency in the alkaline range for B absorption was found. Analysis of the pH effect on the proportion of $B(OH)_3$ and $B(OH)_4{}^-$ in the absorption solution reveals that B absorption by barley roots was primarily contingent upon the $B(OH)_3$ concentration regardless of pH. In a time study from 1 to 24 h, they did not find B accumulation within the root, nor did they find B accumulation against a concentration gradient in solution B concentration ranges from 0.0185 to 7.39 m*M*. Within 3 to 4 h of transfer to $CaSO_4$ solution, approximately 95% of the B, initially absorbed by the root tissue was lost to the $CaSO_4$ solution. At pH 6.0, changes in substrate temperature (from 10 to 30 °C), salt composition (Ca or K salt) or addition of KCN and 2,4-dinitrophenol (DNP) failed to exert any influence on B absorption. These results led Bingham et al. (1970) to conclude that B absorption is a physical, non-metabolic process acting in response to B concentration gradient and that undissociated boric acid is favoured for absorption.

Wilders and Neales (1971) used sliced disks from carrot (*Daucus carota*) and red beet (*Beta vulgaris*) storage roots suspended in 0.1 m *M* boric acid solutions for absorption or desorption studies. They reported that disks of carrot and red beet absorb B to an equilibrium internal concentration which is greater than that of the external solution and that this accumulation of B in the tissues is inhibited by anoxia, by DNP and also by low temperature, suggesting active uptake. On the other hand, B content of storage tissues, after a period of absorption in 0.1 m *M* boric acid solution, was rapidly and almost completely desorbed into a B free solution, suggesting a passive, diffusion type process. Therefore, Wilders and Neales (1971) concluded that there are two components of B uptake: a passive diffusion of $B(OH)_3$ and an active transport for $B(OH)_4^-$ ion.

Wilders and Neales (1971) based their conclusion of active uptake on 1) the observation that internal B concentrations were greater than the external B concentrations as a result of the transport of the $B(OH)_4^-$ ion; and 2) that uptake was inhibited by anoxia and DNP. Their conclusion of active absorption based upon the occurrence of higher internal B concentrations is not, however, supported by their data. The passive membrane permeability of $B(OH)_3$ (suggested by the authors, and proposed by Raven, 1980) predicts that B can diffuse into both internal and external solution. In other words, the internal B concentration would be equilibrated with the external B concentration. From the desorption data of Wilders and Neales (1971), we can calculate that $B(OH)_3$ passively crosses the membrane at a rate of at least 0.1 μmol h^{-1} g^{-1} fresh wt, while the B accumulation rate was more than 0.1 μmol h^{-1} g^{-1} fresh wt. This means that the cells had to pump B internally at a rate of at least 0.2 μmol h^{-1} g^{-1} fresh wt to obtain a net accumulation of 0.1 μmol h^{-1} g^{-1}

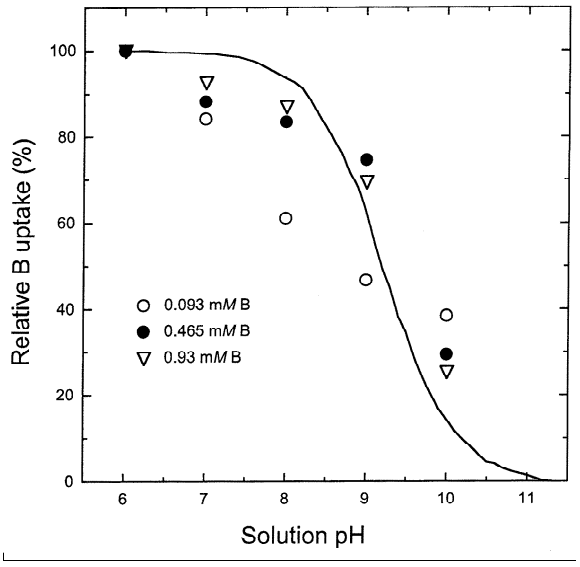

Figure 1. Relative uptake of boron as a function of solution pH. Uptake at pH 6 = 100% at each supply concentration. Solid line: percentage of undissociated H_3BO_3. Adapted from Oertli and Grgurevic (1975).

fresh wt. It is extremely doubtful, that given the limited surface area provided by the storage root disks used, that $B(OH)_4^-$ could be pumped into the cells at a rate of 0.2 μmol h^{-1} g^{-1} fresh wt. This is particularly improbable given the external $B(OH)_4^-$ concentration of 32 n*M* (0.03% of 0.1 m*M* boric acid at pH 5.7) utilised by the authors. It seems clear that Wilders and Neales (1971) conclusion of active uptake was the result of insufficient tissue rinsing and may also have been influenced by the relatively high concentrations of B-binding ligands in carrot (mannitol) and fructose in sugar beet (*Beta vulgaris*).

Oertli and Grgurevic (1975) used excised root sections of 6 day old barley seedlings and studied the pH effect on B absorption. Changes in pH from 6 to 10 and B concentration from 0.093 to 0.93 m *M* suggested that B uptake and the final B contents increased nearly linearly with increasing B supply. The relative B uptake (uptake at pH 6 = 100%) at all supply levels decreased with increasing pH similar to the decrease of the fraction of undissociated $B(OH)_3$ (Figure 1). At pH 6, final B concentrations in roots and treatment solutions were similar. Based on these results, they concluded that B in root tissues and external solution tends to approach a diffusion equilibrium which is governed by the proportion of $B(OH)_3$ vs $B(OH)_4^-$ in the system.

The work of Bingham et al. (1970) and Oertli and Grgurevic (1975) was criticised by Bowen and Nissen (1976), because the barley roots used were only rinsed for 30 s or 1 min after B absorption. As a result, much B remaining in the water free space and free space in the cell wall may have been wrongly considered to be absorbed B. However, it is not a simple matter to determine what is an appropriate time for B desorption. For example, if active uptake is the primary mode of B accumulation, then the desorption period should be long enough to remove all the non-specifically bound B from the free wall space, e.g. 30 min (Bowen and Nissen, 1976). On the other hand, if passive B uptake predominates, then the desorption should be short enough to avoid passive desorption of B across the membrane, while still removing the majority of the apoplastic B. This is clearly an unattainable task for a highly permeable compound such as boric acid. Unfortunately, the significance of the desorption period has not been adequately considered in most studies of B absorption.

Bowen and Nissen (1977) used excised roots from 5 to 7 day old barley seedlings in their short term (hours) B absorption studies. Within the concentration range used, 0.2 – 5 m*M*, they did not find B accumulation against a concentration gradient after 5 h in the absorption solution. However, when they studied B absorption as a function of the external B concentration over the range of 0.05 – 13.3 m*M*, and plotted the data as a double reciprocal plot, they observed that the data could be described by a series of straight lines (phases) separated by sharp transitions or 'jumps', which occurred at B concentrations of 0.16, 0.20, 0.47, 0.55 and 8 m*M*, respectively. Based on these results, they concluded that B absorption in barley is an active transport process since it is compatible with the concept of multiphasic absorption mechanisms outlined by Nissen (1974). To further support their argument, Bowen and Nissen (1977) reported that B uptake from 1 m*M* B solution was strongly dependent upon temperature over 3 – 47 °C range, and also that B uptake from 1 m*M* B solution was inhibited by metabolic inhibitors, including 0.05 m*M* DNP, 0.05 m*M* NaN_3, 1 m*M* NaCN, 5 m*M* arsenate and 0.05 m*M* dicoumarol.

A major concern with Bowen and Nissen's (1977) study is that the B concentrations used were much higher (0.05 – 13.3 m*M*) than is physiologically relevant for barley. It has been reported that barley plants have a low B requirement (Hu et al., 1996; Loomis and Durst, 1992), while sensitive barley cultivars develop B toxicity symptoms after 11 days growth at solution B concentrations of 0.2 m*M*, and moderately resistant cultivars developed toxicity 17 days after exposure to 0.5 m*M* B (Nable, 1988). Bowen and Nissen (1977)

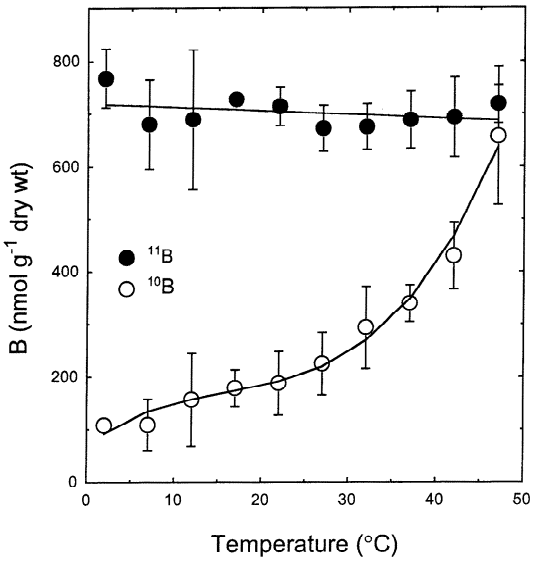

Figure 2. Influence of temperature on the uptake of ^{10}B into ^{11}B precultured intact tobacco cells. Cells were placed in 0.1 m*M* ^{10}B uptake medium for 30 min at various temperatures then transferred to B free desorption solution for 30 min at 5 °C. Values are means ± SE. Adapted from Brown and Hu (1994).

observed transitions in B uptake at concentrations of 0.47, 0.55 and 8 m*M*, which are well in excess of the concentrations that are required for optimal barley growth. Therefore, it is extremely unlikely that the uptake kinetics described by Bowen and Nissen (1977) are of any physiological relevance. The transitions observed by Bowen and Nissen (1977) may be better explained by the saturation of the B-binding capacity of various cellular compartments.

Thellier et al. (1979) conducted a $B(OH)_3$ flux study using isotopes of ^{10}B and ^{11}B in duckweed (*Lemna minor*). After a period in ^{10}B uptake solutions, plants were placed in either ^{11}B boric acid or pure water to determine efflux kinetics. The results suggested that four compartments existed within the cell, i.e. free space, cytoplasm, vacuole and the cell wall. Each of these compartments contained B concentrations much higher than that of the external solution (0.16 m*M*). They did not interpret the occurrence of higher internal than external B concentrations as evidence of active absorption of B, but attributed this to the formation of borate mono- or di-ester within the various compartments and subsequent exchange of B isotopes.

Using stable B isotopes and inductively coupled plasma-mass spectrometry, Brown and Hu (1994) reported that B absorption is linear over a wide range of B concentrations (0–0.2 m*M* or 0.2–10 m*M*) in cultured tobacco (*Nicotiana tabacum*) cells and in the roots of sunflower (*Helianthus annuus*) and squash (*Cucurbita pepo*). There was no indication of saturation kinetics and B absorption was not inhibited by DNP or KCN. Although B absorption did respond to temperature in cultured tobacco cells, absorption was not inhibited by very low (2 °C) temperature, and no inhibition of uptake was reached at temperatures as high as 47 °C (Figure 2). The temperature effect was not typical of temperature dependent kinetics, and the Q_{10} was less than 2. On the basis of these results it was concluded that shifts in B absorption with temperature were associated with changes in membrane conformation (and hence permeability) and the effects of temperature on the relative activity of B molecules in solution.

In these experiments, ^{11}B incorporated during pretreatment growth was not displaced by ^{10}B in the uptake solution even in freeze-thaw killed cells, suggesting that B can be incorporated into some non-exchangeable complexes within the cell (Brown and Hu, 1994). In desorption studies with cultured tobacco cells, after a 30 min uptake period in either 0.1 or 10 m*M* ^{10}B solution, it was found that B desorption was almost complete within 1 min of transfer to B free solution. This rapid desorption of B probably represents B within apoplastic spaces. Further loss of recently acquired ^{10}B was very limited. Based on these findings, Brown and Hu (1994) concluded that B absorption is a non-metabolic process, which is controlled, in large, by the formation of non-exchangeable B-complexes in the cytoplasm and cell wall.

In summary, an unequivocal conclusion about B uptake mechanism can not be made at this time. The reason is partly a result of the diverse plant materials and B concentrations used by different authors, the failure to distinguish B compartmentation and the failure to adequately assess B complex formation. It is clear, however, that there is little or no sound evidence supporting active B absorption. Evidence to support a non-metabolic process for B absorption, is somewhat more convincing though far from conclusive. We propose that B absorption can be best explained as a passive diffusion of free boric acid into the cell, followed by a rapid formation of B-complexes within the cytoplasm and the cell wall. The decline in free boric acid within the cell by the formation of B-complexes allows further absorption of B from the external solution and results in tissue B concentration that can greatly exceed the free boric acid concentration in the uptake solution.

Factors affecting boron absorption

Boron absorption by plant roots is affected by various environmental factors, both in the soil and non-soil environment. Important factors influencing B adsorption from solution include the initial B content of the soil, the pH of the soil, the type of exchangeable ions present in the soil solution, the amounts and types of minerals in the soil, the soil organic matter content, the wetting and drying cycles, and the water to soil ratio (Goldberg, Chapter 3; Keren et al., 1985). All these factors affect B absorption by plants. In coarse textured, low organic matter, and high pH soils, low B availability to the plants would be expected.

Among the soil factors, pH is most important in affecting B absorption by plants (Gupta, 1979). In general, plant B absorption is decreased with the increase in soil pH because of two reasons. Firstly, below pH 7, $B(OH)_3$ predominates and since the affinity of soil clay for this species is relatively low, the amount of B adsorption by soil is small. As the pH increases, $B(OH)_4^-$ concentration relative to $B(OH)_3$ increases, and as a consequence of the relatively strong affinity of $B(OH)_4^-$ for clay minerals, the amount of adsorbed B increases (Keren and Bingham, 1985). In other words, with the increase in soil pH, B availability to the root decreases. For more information about reactions of B with soils, the reader is referred to Chapter 3 in this volume. Secondly, Oertli and Grgurevic (1975) found that the absorption of B by solution grown barley roots decreased with the increase of solution pH, this corresponded to the decrease in the fraction of undissociated $B(OH)_3$, demonstrating that $B(OH)_3$ is the form of B available to plants.

In non-soil environments, the transpiration rate seems to be the most important factor governing B absorption. As a result, relative humidity, temperature and light intensity will all alter B absorption. For example, Bowen (1972) made a comprehensive study about the environment factors on B absorption and found that in sugarcane (*Saccharum officinarum*) seedlings, increasing light intensity increased B absorption, while decreasing relative humidity increased B absorption. Similarly, an increase in air temperature increased total B absorption even though the relative humidity was held constant. When other environment conditions were constant, an increase in root temperature increased B absorption.

Similar information was reported by Oertli (1994) in barley seedlings. He found that increasing duration and intensity of illumination significantly increased water consumption as well as B uptake, and that the uptake of water and B was reduced 50% at high humidity in comparison to low humidity. However, the B to water uptake ratio remained constant (about 3.4 μg B mL^{-1} water at 0.93 m*M* external B) irrespective of humidity or temperature treatment. Temperature increased water and B absorption significantly at constant relative humidity (as a consequence of differences in vapour pressure deficits). For example, at an external B concentration of 0.05 m*M*, B absorption was about 0.2 μg per barley seedling at a temperature of 10 °C. When temperature increased to 27 °C, B absorption was about 0.65 μg per barley seedling. However, the ratio of B absorption to water uptake remained constant at 0.39 μg B mL^{-1} water. On the other hand, Oertli (1994) found that the ratio of B absorption to water uptake did not correspond to the external B concentration. For example, at external B concentration of 0.05, 0.23 and 0.93 m*M*, the B to water uptake ratio was 0.39, 1.07 and 3.46 μg B mL^{-1} water, respectively.

Forno et al. (1979) reported that two cassava (*Manihot* sp.) cultivars growing in nutrient solution developed B deficient symptoms when growing at root temperature of 18 °C; their shoot B concentration ranged from 9.7 to 11.9 mg B kg^{-1} dry wt. However, when these plants were grown at 23 °C root temperature, they developed much less severe B deficiency symptoms and symptoms appeared only towards the end of the experiment. These plants accumulated 17 – 19 mg B kg^{-1} dry wt in the shoot. Plants were symptom free at root temperatures of 28 – 33 °C and they accumulated 42 – 55 mg B kg^{-1} dry wt in the shoot.

An exception to the temperature effect on B absorption described above was reported by Nable et al. (1990). These authors reported that nutrient solution temperature had little effect on tissue B concentration within cultivars of barley. For example, at 15 μM B solution, B absorption in 'Sahara 3771' was the same (20 mg B kg^{-1} dry wt) at 5 and 25 °C. At 1 m*M* B in solution, an increase in temperature from 5 to 25 °C resulted in an increase of B absorption from 80 to 90 mg B kg^{-1}. However, different genotypes exhibited differences in B accumulation.

The reported environmental effects on B absorption can largely be explained by changes in transpiration (Raven, 1980). Reduced humidity increases transpiration, resulting in an increase in B absorption. Increased temperature creates water vapour deficits, which will also increase transpiration, resulting in an increase in B uptake. Illumination has the same effect as increased temperature or reduced relative humidity, and as a

result, transpiration increases and B uptake increases. However, the observed increases in B uptake with the increase in root temperature can not be explained by transpiration effects. No simple relationship exists between transpiration rates and whole plant B uptake. For example, in wheat (*Triticum aestivum*) cultivars, water use efficiency ranged from 3.1 to 4.0 g dry wt kg^{-1} water. The less than 30% difference in transpiration rate within cultivars of one species can not account for the 400–700% difference in B uptake (Nable, 1988). Further, genotype differences in resistance to B toxicity and B absorption are known to occur in roots assayed without the shoot attached and B uptake in these roots has been frequently observed demonstrating that transpiration is not essential for uptake (Huang and Graham, 1990).

The paradox of boron uptake

The preponderance of available experimental evidence suggests that B uptake is a passive process, the rate of which is influenced by passive diffusion of B through the membrane, the formation of B complexes within the cell and plant water fluxes. If these assumptions are true then one would predict that B uptake would be very closely correlated with water use and that species grown in like environments supplied with non-limiting water would have very similar B uptake rates. Nevertheless, there is clear evidence that B uptake differs dramatically between species and even within genotypes of a single species. For example, Nable (1988) reported that in solution culture experiments, the B concentration or total B content in all organs of five barley and six wheat cultivars differ dramatically. The cultivars resistant to B toxicity always accumulated less B than the sensitive cultivars. When grown in 5 m*M* B solution, barley cultivars 'Sahara 3763' and 'Schooner' accumulated 112 and 710 mg B kg^{-1} dry wt in the youngest expanded leaf blade, respectively, a more than 6-fold difference in B accumulation between genotypes. These differences in B uptake cannot be explained through differences in water use, which has less than 1-fold difference among the cultivars. This apparent contradiction between passive B uptake and significant differences among the genotypes is difficult to reconcile but is of fundamental importance to studies of B nutrition. Several mechanisms have been postulated to explain this apparent paradox (Nable, 1988; Nable et al., 1990).

Firstly, B uptake may be partially under metabolic control. For example, Bowen and Nissen (1977) reported that active uptake accounts for less than 10% of the total B accumulated by barley roots. Alternatively, there may be an active B exclusion mechanism. In this regard, the extent of active uptake may be different from species to species or from genotype to genotype. This mechanism may explain the differences in B absorption among different species (genotypes) and it could also account for the apparently conflicting results obtained when metabolic inhibitors are used. However, in view of the discussion in "Mechanisms of boron absorption", we conclude there is little support for this concept.

Alternatively, one plausible explanation is that the exudation of B complexing agents into the rhizosphere restricts B uptake from the soil. The extent to which B-binding exudates are produced may be species dependent. Organic compounds containing *cis*-diols, as well as some amino acids or some organic acids can complex with B. Because the resultant B-complex is generally an anion, it would have a greatly reduced membrane permeability in comparison to uncharged boric acid, and hence absorption would be reduced. Many compounds present in root exudates, particularly the pectin-rich polysaccharides that are abundant in plant mucilage, would be capable of forming stable B complexes. These complexes could realistically reduce B uptake. However, one might expect that these exudates would be saturated by B at high external B supply and once saturated, uptake should show no difference among genotypes. Even at the very high B application rates (6.4 m*M*) used by Nable et al. (1990), this did not appear to be the case. Also, one would expect that experiments conducted in solution culture (in which rhizosphere exudates would be diluted in the culture medium) would result in diminished genotype differences. In the relatively long term studies of Nable et al. (1990) (see also Nable, 1988; Paull et al., 1988) this did not appear to be true. On the other hand, genotype differences in long term B uptake studies have not been confirmed in short term solution culture experiments (Garnett et al., 1993). Nevertheless, this hypothesis cannot be entirely excluded without more specific information on the amount and localisation of these putative B-binding exudates.

Thirdly, root B-adsorption capacity may differ significantly and hence may affect B uptake. Tanaka (1967) observed a 3- to 10-fold difference in the adsorption capacity of roots between dicots and graminaceous monocots with a much smaller difference

within the dicots or within monocots. Yamauchi (1971) demonstrated a highly significant correlation between 'protopectin' (old terminology for water insoluble parent pectic substance) and 0.5 *N* HCl soluble but water insoluble B in 33 species across 13 families. Hu et al. (1996) reported that among the fourteen species studied, there is significant correlation between species B requirement and cell wall pectin. In general, more B is required by dicots than graminaceous monocots presumably because of the higher pectin content in the dicots. Recently, Matoh et al. (1996) demonstrated a high correlation between cell wall B and 2-keto-3-deoxy sugars in the high molecular weight fragments of cell walls. In combination, these results suggest that differences in the cell wall B requirement exist among species and that these differences might affect B uptake. This observation is not adequate, however, to explain the observed differences in B uptake and is not consistent with results from uptake experiments. For example, Hu et al. (1996) report a less than 2-fold difference in cell wall B binding capacity between various monocot species even though differences in B uptake can be as great as 4- to 7-fold between these same species (Nable, 1988). Further, if the cell wall B-binding capacity is the key determinant of the observed differences in B uptake between species, then once the cell wall binding capacity is saturated, uptake should be the same for all species. There is no indication that this is the case.

Fourthly, physical barriers related to root cell wall structure may determine B uptake rates. Some evidence in support of this was provided by Jenkin et al. (1993) who investigated B uptake in protoplasts of two barley varieties with stable B isotope techniques. The two barley varieties used were known to have significant differences in B accumulation on a whole plant and long term basis, with the B toxicity resistant cultivar 'Sahara 3771' accumulating much less B than the sensitive cultivar 'Stirling'. However, in the absence of the cell wall, the two varieties did not show any significant difference in B accumulation (Jenkin et al., 1993). There are three possible explanations for this result: (1), the difference in B accumulation between these two cultivars is determined by the cell wall and, once the cell wall is removed, the cultivar difference disappears; (2), since the membrane integrity was not assessed in the protoplasts used by Jenkin et al. (1993), genotype difference might be overshadowed by membrane leakiness as a consequence of the protoplast isolation technique; or (3), the duration of the uptake experiment was too short to determine species differences. Evidence to support this is provided in the work of Garnett et al. (1993) who reported that in short term B uptake studies (4 h), the wheat cultivar 'Halberd' (resistant to B toxicity) did not differ from 'W1*MMC' (sensitive to B toxicity) in B accumulation. In longer term studies (days) these two varieties did show significant difference in B accumulation. The results of Jenkin et al. (1993) may therefore reflect short term effects and may not be relevant to long term B accumulation. At present, insufficient information is available to determine if there is any relationship between root cell wall structure and B uptake.

If B uptake is a purely passive process, then the differences in B accumulation may be due to differences in membrane permeability to B. Membrane permeability to urea, water and protons may all vary significantly depending upon membrane composition (see "Properties of boric acid"). Boric acid has a lipid solubility of the same order as urea and hence small differences in membrane composition (head groups, fatty acyl chains, etc.) between genotypes may affect B uptake. If passive membrane permeation is the primary means of B uptake, then B uptake would be linear with increasing concentration, and would not be influenced by metabolic inhibitors. This basically agrees with existing information (see "Is boron adsorption active or passive?". If species and genotypes had different membrane permeability coefficients for boric acid, they would also exhibit a linear response to increasing B supply. However, the slope of this response line may differ and the resulting B absorption rate would differ. The genotype comparisons of Nable et al. (1990) can be suitably explained in this manner.

Similarly, small differences in the relative permeability of membranes to B relative to water would alter the ratio of B accumulated to water transpired, though the total amount of water transpired would still directly affect B uptake. This would adequately explain the observed relationship between water uptake and B accumulation while accommodating the observed genotype differences. It is also possible that differences in membrane permeability to B may not be sufficient to influence whole plant B uptake during short term uptake studies, these differences may only become apparent after longer B uptake periods.

Though differences in membrane permeability could theoretically result in the observed differences in B uptake between species (or genotypes), it should be noted that this has not been experimentally verified. Further, we are unaware of any evidence of genotypic differences in membrane composition resulting in

the differential membrane permeability of any solute. Finally, experimental evidence suggests that genotypic differences in B uptake are associated with relatively few genes (Nable et al., 1990 and references therein), it would be intriguing to determine if these genes code for subtle differences in membrane composition.

Further research to determine the mechanism of genotype and species differences in B absorption is clearly required.

Conclusions and future directions

Boron uptake in higher plants is probably a passive process acting in response to external boric acid concentration, membrane permeability, internal complex formation and transpiration rates. Species differences in B uptake rate cannot be adequately explained on the basis of current knowledge but may result from differences in membrane permeability, B-complex formation inside or outside the root or some as yet unidentified mechanism.

Future studies on B absorption should include consideration of both short and long term uptake periods, must be conducted at realistic B concentrations and should always consider the impact of B-complex formation in experimental design. Experimental verification of the theoretical membrane permeability coefficient proposed by Raven (1980) remains a priority and may ultimately allow permeability to be compared between species. The recent identification of closely related genotypes differing significantly in B absorption rates has provided invaluable experimental material. These genotypes hold great potential for providing the physiological and genetic information necessary to determine the mechanism of B uptake in plants.

References

Bingham F T, Elseewi A and Oertli J J 1970 Characteristics of boron absorption by excised barley roots. Soil Sci. Soc. Am. Proc. 34, 613–617.

Boeseken J 1949 The use of boric acid for the determination of the configuration of carbohydrates. *In* Advances in carbohydrate chemistry V4. Eds. W W Pigman and M L Wolfrom, pp 189–210. Academic Press, New York.

Bowen J E 1972 Effect of environmental factors on water utilisation and boron accumulation and translocation in sugarcane. Plant Cell Physiol. 13, 703–714.

Bowen J E and Nissen P 1976 Boron uptake by excised barley roots I. Uptake into the free space. Plant Physiol. 57, 353–357.

Bowen J E and Nissen P 1977 Boron uptake by excised barley roots II. Characteristics and kinetics of active uptake. Physiol. Plant. 41, 109–115.

Briskin D P and Reynolds-Niesman I 1991 Determination of H^+/ATP stoichiometry for the plasma membrane H^+-ATPase from red beet (*Beta vulgaris* L.) storage tissue. Plant Physiol. 95, 242–250.

Brown P H, and Hu H 1994 Boron uptake by sunflower, squash and cultured tobacco cells. Physiol. Plant. 91, 435–441.

Dencher N A, Burghaus P A and Grzesiek S 1986 Determination of the net proton-hydroxide ion permeability across vesicular lipid bilayers and membrane proteins by optical probes. Methods in Enzymology 127, 746–760.

Finkelstein A 1976 Water and nonelectrolyte permeability of lipid bilayer membranes. J. Gen. Physiol. 68, 127–135.

Forno D A, Asher C J and Edwards D G 1979 Boron nutrition of cassava, and boron × temperature interaction. Field Crops Res. 2, 265–279.

Garnett T P, Tester M A and Nable R O 1993 The control of boron accumulation by two genotypes of wheat. *In* Plant nutrition – from genetic engineering to field practice. Ed. N J Barrow. pp 397–400. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Gupta U C 1979 Boron nutrition of crops. Adv. Agron. 31, 273–307.

Hu H and Brown P H 1994 Localisation of boron in cell walls of squash and tobacco and its association with pectin. Evidence for a structural role of boron in the cell wall. Plant Physiol. 105, 681–689.

Hu H, Brown P H and Labavitch J M 1996 Species variability in boron requirement is correlated with cell wall pectin. J. Exp. Bot. 47, 227–232.

Hu H, Penn S G, Lebrilla C B and Brown P H 1997 Isolation and characterisation of soluble B-complexes in higher plants. The mechanism of phloem mobility of boron. Plant Physiol. 113, 649–655.

Huang C and Graham R D 1990 Resistance of wheat genotypes to boron toxicity is expressed at cellular level. Plant and Soil 126, 295–300.

Ishii T and Matsunaga T 1996 Isolation and characterisation of a boron-rhamnogalacturonan-II complex from cell walls of sugar beet pulp. Carbohydr. Res. 284, 1–9.

Jansen M and Blume A 1995 A comparative study of diffusive and osmotic water permeation across bilayers composed of phospholipids with different head groups and fatty acyl chains. Biophysical J. 68, 997–1008.

Jenkin M, Hu H, Brown P, Graham R, Lance R and Sparrow D 1993 Investigations of boron uptake at the cellular level. *In* Plant nutrition – from genetic engineering to field practice. Ed. N J Barrow. pp 157–160. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Keren R and Bingham F T 1985 Boron in water, soils, and plants. *In* Advances in soil science VI. Ed. B A Stewart. pp 230–276. Springer-Verlag, New York.

Keren R, Bingham F T and Rhoades J D 1985 Effect of clay content in soil on boron uptake and yield of wheat. Soil Sci. Soc. Am. J. 49, 1466–1470.

Kobayashi M, Matoh T and Azuma J-I 1996 Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol. 110, 1017–1020.

Lee-Standelmann O Y, Bushnell W R, Curran C M and Standelmann E J 1991 Permeability to non-electrolytes as an indicator of change in membrane structure in *Hordeum vulgare* cells infected by *Erysiphe graminis* f. sp. *Hordei*. Physiol. Mol. Plant Pathol. 38, 163–178.

Loomis W D and Durst R W 1992 Chemistry and biology of boron. Biofactors 3, 229–239.

Makkee M, Kieboom A P G and van Bekkum H 1985 Studies on borate esters III. Borate esters of D-mannitol, D-glucitol, D-fructose and D-glucose in water. Recl. Trav. Chim. Pays-Bas 104, 230–235.

Matoh T, Kawaguchi S and Kobayashi M 1996 Ubiquity of a borate-rhamnogalacturonan II complex in the cell walls of higher plants. Plant Cell Physiol. 37, 636–640.

Nable R O 1988 Effects of B toxicity amongst several barley wheat cultivars: a preliminary examination of the resistance mechanism. Plant and Soil 112, 45–52.

Nable R O, Lance R C M and Cartwright B 1990 Uptake of boron and silicon by barley genotypes with differing susceptibilities to boron toxicity. Ann. Bot. 66, 83–90.

Nissen P 1974 Uptake mechanisms: inorganic and organic. Ann. Rev. Plant Physiol. 25, 53–79.

Oertli J J 1994 Non-homogeneity of boron distribution in plants and consequences for foliar diagnosis. Comm. Soil Sci. Plant Anal. 25, 1133–1147.

Oertli J J and Grgurevic 1975 Effect of pH on the absorption of boron by excised barley roots. Agron J. 67, 278–280.

Oertli J J and Richardson W F 1970 The mechanism of boron immobility in plants. Physiol. Plant. 23, 108–116.

O'Neill M A, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill A G and Albersheim P 1996 Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cells, forms a dimer that is covalently cross-linked by a borate ester. J. Biol. Chem. 271, 22923–22930.

Paull J G, Cartwright B and Rathjen A J 1988 Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica 39, 137–144.

Penn S G, Hu H, Brown P H and Lebrilla C B 1997 Direct analysis of sugar alcohol borate complexes in plant extracts, by matrix assisted laser desorption fourier transform mass spectrometry (MALDI FTMS). Anal. Chem. (*In press*).

Raven J A 1980 Short- and long-distance transport of boric acid in plants. New Phytol. 84, 231–249.

Tanaka H 1967 Boron adsorption by plant roots. Plant and Soil 27, 300–302.

Thellier M, Duval Y and Demarty M 1979 Borate exchanges of *Lemna minor* L. as studied with the help of the enriched stable isotopes and of a (n,α) nuclear reaction. Plant Physiol. 63, 283–288.

Wilders R A and Neales T F 1971 The absorption of boron by disks of plant storage tissues. Aust. J. Biol. Sci. 24, 873–884.

Yamauchi M 1971 The role of B in higher plants. Relationship between B and Ca on the pectin substances in plants. J. Sci. Soil Manure 42, 207–213.