RETRANSLOCATION OF BORON IN BROCCOLI AND LUPIN PLANTS DURING REPRODUCTIVE GROWTH

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ABSTRACT

RETRANSLOCATION OF BORON IN BROCCOLI AND LUPIN PLANTS DURING REPRODUCTIVE GROWTH

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This study tested the hypothesis that xylem-borne boron (B) is retranslocated in phloem to reproductive sinks of plants. Intact plants or detached transpiring shoots were supplied simultaneously with enriched ^{10}B, strontium (a xylem marker) and rubidium (a xylem/phloem marker); the distribution of these compounds was determined as a function of time (i.e. up to 12 hours and 4 days for broccoli and lupin plants, respectively). The percent recovery of both ^{10}B and rubidium in broccoli florets and lupin fruits was similar and markedly greater than that for strontium. Furthermore, the xylem-to-phloem transfer was rapid (within 2 h for broccoli and 1 day for lupin, the earliest harvest times), with the extent depending on B status of the plant.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Atomic Absorption spectrometry
B	boron
d	day
DW	Dry weight
EDTA	Ethylenediaminetetracetic Acid
g	gram
h	Hour
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
kg	kilogram
RBS	Root bleeding sap
wk	Week

CHAPTER ONE

INTRODUCTION

Nutrients differ in their mobilities in plants and considerable variability exists between plants species (Pate 1975; Welch, 1986). This differential mobility is related to the relative importance of xylem versus phloem in providing nutrients to developing sinks. The first route involves the primary translocation of minerals by the water stream in xylem whereas the second route is linked to secondary translocation or retranslocation in phloem away from sites of initial deposition. Elements such as K, P, Mg are readily retranslocated, whereas Ca is generally thought to be immobile. In contrast, the retransloction of micronutrients may depend on a number of factors, including stage of growth, plant nutrient status and external supply of nutrient.

Until recently, it was generally believed that the distribution of B, once in the xylem, is related only to the rates of transpiration, and that B is relatively immobile in phloem (Pate, 1975; Raven, 1980; Welch, 1986). For instance, the distribution of B in the shoot organs of broccoli grown with adequate supply of B showed that B concentration is highest in the source leaves and lowest in the young leaves and florets. This typical gradient in the B distribution corresponds to organ age and by implication to transpiration (Shelp *et al.*, 1992b). If the distribution of B is only related to primary translocation in the xylem, then B concentration should always be highest in the source leaves. However, there is growing evidence from different plant species suggesting that B distribution is altered in response to continuous B starvation

or to removal of B after a period of adequate supply (Shelp, 1993). By comparing the relative proportion by weight of elements, Shelp (1987) has estimated the contribution of selected nutrients from phloem to the developing sinks of broccoli grown with adequate B. The phloem stream provides B to an equal or greater extent than it does N, P, K, nutrients which are classified as phloem mobile. Furthermore, the concentration of phloem exudate is similar to that of developing sinks (Shelp, 1993).

One strategy that has been employed to monitor the distribution and retranslocation of B in plants is the use of the mass isotopes ^{10}B and ^{11}B. Movement of foliarly-applied ^{10}B has been demonstrated for clover (Martini and Thellier, 1975) and various fruit trees (Hanson, 1991a,b; Brown *et al.*, 1992; Shu *et al.*, 1993, 1994) and of soil-applied ^{10}B for broccoli and lupin plants (Marentes *et al.*, 1994a,b). Retranslocation of B can also be assessed by comparing the distribution of ^{10}B to the distribution of xylem (e.g. Sr, inulin) and xylem/phloem (e.g. Rb, aminoisobutyric acid) marker compounds (Feller, 1989; Shenck and Feller, 1990; Van Bel, 1984; Da Silva and Shelp,1989).

In this present study, I tested the hypothesis that xylem-borne B is retranslocated in phloem to reproductive sinks of plants. Two studies were conducted: 1) Determination of elemental concentrations, particularly B, of xylem sap and phloem exudate from broccoli and lupin plants during vegetative and reproductive growth; and 2) comparison of the percent distribution of xylem-borne ^{10}B in florets and fruits of broccoli and lupin plants, respectively, with the percent distribution of Sr (xylem marker) and Rb (xylem/phloem marker).

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Warington (1923) provided evidence for broad bean that a continual supply of B is required in small amounts throughout the life cycle to maintain a healthy plant. It is now known that B is essential for normal growth of monocots, dicots, conifers, ferns, several diatom species and nitrogen-fixing cyanobacteria (Hewitt, 1963; Lewis,1980a; Parr and Loughmann, 1983, Lovatt, 1985). In contrast, there is no definitive evidence that B is essential for animals, fungi and most algae.

Researchers have proposed that B is involved in a number of processes including: sugar transport, cell wall synthesis, lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, IAA metabolism, phenol metabolism and membrane structure and function (Dugger, 1983; Parr and Loughmann, 1983; Pilbeam and Kirkby, 1983; Shelp, 1993). Because B deficiency causes a wide variety of anatomical, physiological and biochemical changes, the distinction between primary and secondary effects is difficult. In recent years, research has attempted to identify a primary role during the onset of B deficiency. Shelp (1993) has concluded that evidence favors the involvement of B at the membrane level, which interferes with a number of processes including enzyme function and the transport of ions, metabolites and hormones. However, he cautions that further research is needed on: the possible involvement of B in cell wall synthesis and /or stability; the short-term metabolic

response of nucleic acids, pyrimidines and carbohydrates; and the interactions between B and auxins and between B and calcium.

In Ontario, there are a number of nutritional disorders of horticultural brassicas that are attributed to B deficiency. These include hollow stem of broccoli and cauliflower. The earliest signs of hollow stem in broccoli are small eliptical cracks that develop in the inner stem pith; as the plant matures, these may enlarge and coalesce to form cavities (Shattuck and Shelp, 1985). Also, external signs of stem corkiness and leaf mid-rib cracking, together with brown discoloration and necrosis in the central pith, are evident under greenhouse conditions (Shelp *et al.*, 1992a; Shelp, 1987). Another disorder attributed to B deficiency, brown heart in rutabaga and radish, consists of external signs of skin cracking and internal browning of the root which often appears elongated (Shelp and Shattuck, 1987b). The occurrence of these disorders even when B is in ample supply in the soil suggests that they are physiological in nature and are related to the the mobility of B within the plant. While there is little doubt that the distribution of B in plants is related primarily to its translocation in xylem to sites of greatest water loss (i.e. primary translocation), there is considerable controversy regarding the role that phloem plays in providing B to sites that do not lose water readily (i.e. secondary translocation or retranslocation). The rapid retranslocation of B in plants is of particular interest in this thesis. Therefore, this chapter will discuss the anatomy and structural features of the vascular system of higher plants, long-distance translocation of mineral nutrients, techniques employed in the study of retranslocation, and B distribution and retranslocation.

2.2. Anatomical and Structural Features of the Vascular System of Higher Plants

The vascular system of higher plants consists of xylem, the main function of which is the translocation of water and solutes , and the phloem which supplies the major proportion of the nutrient requirements for actively growing areas. Anatomical and ultrastructural features associated with translocation depict a series of interlocking functional units involving long- and short-distance translocation in the apoplast and symplast. Xylem is a complex tissue which consists of several types of cells, the most important of which are the tracheids, vessel members, fibers and the parenchyma cells which give rise to transfer cells. This tissue is involved in translocation of water and support of the plant (Fahn, 1982). Two basic types of tracheary elements are the tracheids and vessel members, the main difference being that the latter have end walls with perforations which allow the individuals to join end-to-end, thereby forming a vessel. In tracheids, the passage of water from cell to cell occurs mainly through pits in the cell wall (Esau, 1960; Fahn, 1982).

Like the xylem, phloem is a complex tissue composed of several different types of elements. These are sieve elements, companion cells, parenchyma cells and sclereids. Mature sieve elements are the main conduits for the long distance translocation in phloem of angiosperms (Esau, 1960). The phloem invariably accompanies xylem into virtually every branch of the vascular network. Only in the finest ultimate branches of the leaf veins and in specialised structures can vascular traces be found consisting only of xylem or phloem (Pate, 1975), implying that throughout its length, the vascular

tissue provides an extensive and uninterrupted potential for solute exchange between xylem and phloem. The proximity of these two types of conducting elements varies considerably with location in the plant. The more intimate connections between xylem and phloem in the leaf-vein network provide an easier and quantitively more important opportunity for exchange of solutes (direct transfer) than a route via the leaf mesophyll (indirect transfer) (Pate, 1975) (see section 2.4).

2.3. Collection and Composition of Translocation Fluids

The ability to recover xylem sap and phloem exudate of a plant species offers an opportunity to improve our understanding of translocation processes, including xylem-to-phloem transfer (i.e. retranslocation), in higher plants. Xylem sap has been obtained either by low vacuum extraction of tracheal sap from 5-cm segments of upper main stem tissue adjacent to the study leaf or by collection of sap bleeding under root pressure the base of the stem (Pate, 1975, 1984). A limitation of both techniques is contamination by surrounding tissue (Pate, 1984). Also, starvation reactions and changes in water flux following cutting may cause changes in solute composition. These problems can largely be overcome by collecting xylem sap over a short time and by blotting the root stump to remove cut cell contents. Pate and Atkins (1983) showed that the N concentration of RBS of lupin plants is 5-8 times higher than that of tracheal sap. Furthermore, Pate *et al.* (1980), on the basis of water usage and solute accumulation, estimated the N concentration of RBS is 4-29 times higher than that of xylem sap of intact plants. Root bleeding sap has also been collected from detached

root systems which are exposed to enough root pressure to displace the sap; however, this requires the use of a pressure bomb (Jachetta *et al.*, 1986)

In contrast to xylem, phloem is easily damaged and liable to block with p-protein and callose, thereby causing difficulties in its collection. Phloem exudate of high purity has been collected from severed aphid stylets which pierce single sieve elements and have little effect on the translocation process (Pate, 1976). This approach is limited by the feeding specificity of aphids, and the small amounts of sap obtained. Phloem exudate has also been collected from cut inflorescence stalks of *Yucca flaccida* (Van Die and Tammes, 1975) , shallow incisions in the bark of trees (Zimmerman, 1960) or on the stems and petioles of various herbaceous plants (Pate, 1980, 1984, Shelp, 1987)), and cut fruit tips of various legumes (Pate , Sharkey and Lewis , 1974, 1975). In studies of legume-fruit nutrition, such techniques have been applied successfully to *Pisum sativum* L., *Lupinus albus* L., *Spartium junceum* L. and *Jacksonia* spp. (Pate *et al.*, 1974).

For species that do not bleed spontaneously from the phloem, p-protein build up may be prevented by excision of the distal tip of a fruit or the petiole of a translocating leaf in a solution of EDTA, a chelating agent (Fellows *et al.*, 1978). Continuous immersion promotes the leakage of photosynthetically-derived translocate from the pod (Fellows *et al.*, 1978, 1979). During collection, distortion of the phloem composition is likely to occur through contamination from cut surfaces, dilution of contents through osmotic attraction of water to the exudate, and release of nonmotile constituents (callose and p-protein) following turgor release on cutting (Pate, 1975,

1976). A more recent development has been the cryopuncture technique (Pate *et al.*, 1984b) in which the vasculature of the dorsal suture of fruits of cowpea is pierced with a fine needle cooled in liquid nitrogen, thereby inducing the spontaneous release of phloem exudate.

2.4. Long-distance Translocation of Mineral Nutrients in Higher Plants

In plants, the long-distance translocation of mineral nutrients occurs in the vascular system consisting of the xylem and the phloem; water is the translocating agent in these tissues. Upward movement from the roots to the shoots occurs in the non-living cells of xylem and is driven predominantly by a water potential gradient resulting from water loss at the leaf surfaces (transpiration), and to a lesser extent by a hydrostatic pressure (root pressure) resulting from the release of nutrients from the root tissue into the xylem (Marschner, 1986). The concentration of the individual inorganic ions in the xylem depends on their rates of uptake, their concentration in the nutrient medium, and the uptake of water (Mengel and Kirkby, 1987). Solute flow in the xylem is unidirectional, towards the mature leaves that transpire readily, although they are not usually the regions of highest nutrient demand. The mechanisms of solute translocation in xylem is predominantly one of mass flow in the apoplast; however, important interactions between solutes and the cell wall of the vessels surrounding xylem parenchyma cells take place, thereby affecting the concentration and composition of sap along the xylem pathway. Xylem sap contains mainly nitrogenous compounds (e.g. NO_3^-, amides, ureides) and inorganic ions and has an acidic pH (5.5-6.5) (Pate, 1975;

Pate and Atkins, 1983a).

In contrast, translocation in the phloem is independent of transpiration (Marschner, 1986) with the relative extent being determined by the nutritional requirements of actively growing sink regions such as fruits, meristems, vegetative buds, young leaves and storage organs (Marschner, 1986). Phloem also provides the route for translocation of photoassimilates from mature photosynthetic leaves. Phloem exudate has a high pH (7-8) and high concentration of solids, on average 15-25% dry matter. The main component is usually sucrose, which comprises more than 90% of the solids. In addition, there are fairly high concentrations of organic acids and organically-bound nitrogen, particularly in the form of amino acids and amides (Pate, 1975). Research has generally suggested that NO_3^- is not detectable in phloem exudates and that its presence is an indication of contamination by cell contents; however, there is some good evidence obtained with aphids showing that nitrate is present in phloem (Hayashi and Chino, 1985, 1986). Of the mineral elements, K is usually present in highest concentration, followed by P, Mg and S, with the latter mainly in the reduced form as glutathione, methionine and cysteine (Rennenberg *et al.*, 1979; Bonas *et al.*, 1982); the Ca concentration is always very low.

During translocation, nutrients are transferred between xylem and phloem by extensive exchange processes. In the stem and leaf veins, xylem and phloem are separated by only a few cell layers, and direct exchange of unmetabolised nutrients between these two pathways may occur (Pate, 1975, 1985; Van Bel, 1984; Da Silva and Shelp, 1989). Such exchange may be important in regulating solute distribution in

intact plants. Specialised vascular parenchyma cells known as transfer cells possess extensive wall ingrowths (Gunning *et al.*, 1968; Gunning and Pate, 1969) and are located in the stem and leaf veins of several plant species, suggesting a role in absorption and secretion of plant substances (Pate, 1984). These cells occur not only in the vascular tissues, but also in reproductive organs and other specialised structures such as haustoria, hydathodes, root nodules and nectaries (Gunning and Pate, 1969; Pate and Gunning, 1972). In stems of graminaceous (e.g. cereals) and leguminous species (e.g. cowpea), the nodes are the sites of intensive xylem-to-phloem transfer of mineral nutrients such as potassium (Haeder and Beringer, 1984b; Kuppelwieser and Feller, 1991), and amino compounds such as asparagine (McNeil *et al.*, 1979). The convoluted membranes of transfer cells are probably capable of handling a variety of solutes ranging from inorganic ions to organic solutes (Pate, 1984), with the specificity of solute transfer being determined by carrier proteins (Klotz and Erdei, 1988). The precise localization of transfer processes and the mechanisms involved, as well as regulatory properties, are unknown.

Indirect transfer between xylem and phloem occurs when nutrients are transformed in the leaf and immediately exported in the phloem, or stored and exported at a later stage during development. The latter process is often associated with leaf senescence during the growth of reproductive structures and involves the mobilization of reserve materials such as proteins and carbohydrates. It is less associated with vegetative growth unless the plant has experienced a period of insufficient nutrient supply (Marschner, 1986). During leaf ontogeny, there is a transition in role from primarily

importing to exporting (Pate, and Atkins, 1983b). Although factors which trigger this transition are not fully understood, Turgeon (1984) suggested that the sink phase of leaf development is ended by modification of the transport pathway.

Nutrients differ in their mobilities in phloem and considerable variability in mobility exists between different plant species. Mobility of an element in phloem affects delivery to plant organs with low transpiration rates. Elements such as N, P, K, Mg, Cl that are found in relatively high concentrations in phloem may continue to be retranslocated from mature or old organs to sink regions even when there is no external supply. Therefore, localized deficiency symptoms do not develop until the total nutrient content of the whole plant becomes insufficient. Elements generally considered to be intermediate in phloem mobility include Mn, Zn, Fe and Cu (Zeigler, 1975); their mobility depends on several internal factors such as plant nutrient status and species. Ca and B have generally been considered to be immobile. Interestingly, Penot *et al.* (1976) used ^{45}Ca to trace the long-distance translocation of Ca in higher plants and algae and reported that translocation of Ca via the phloem stream is possible in higher plants. There is also some evidence that B undergoes retranslocation (see section 2.6). However, plant organs can develop properly only if they receive a continuous supply of immobile nutrients from the external medium in the xylem stream. An understanding of nutrient distribution within shoots is necessary to develop diagnostic procedures and to make satisfactory recommedations for treatment of nutrient disorders. The choice of a suitable organ for diagnosis is related to the rapidity and extent of retranslocation of the nutrient within the plant.

2.5. Xylem and Phloem Exchange Processes.

Processes involved in xylem and phloem translocation in legumes have been asssesed by determining C, N and water increments during the growth of plant parts, as well as the C/N weight ratios of xylem and phloem fluids serving specific plant organs (Pate, 1983). These modeling studies indicate the existence of xylem-to-phloem transfer, phloem-to-xylem transfer and xylem-to-xylem transfer (Pate and Atkins, 1983; Pate *et al.*, 1979b ; Layzell *et al.*, 1979, 1981; Pate and Layzell, 1981).

Other studies have more directly examined the contribution of xylem and phloem paths to legume-fruit nutrition by following the short-term distribution and metabolism of radioactive or mass isotopes. The experiments have involved: supply of ^{14}C and ^{15}N compounds as drops onto a foliar or fruit surface previously wetted with a surfactant (Atkins *et al.* 1982, Urquhart and Joy, 1981), or via injection of $^{14}CO_2$ into the gas space of a fruit (Fellows *et al.*, 1979; Pate, 1984); 2); supply of $^{15}N_2$ or $^{15}NO_3^-$ to root and root nodules (Pate, 1984; Pate *et al.*, 1975); 3) supply via the reverse-leaf-flap method (Pate *et al.*, 1980); and 4) supply via the transpiration stream of $^{15}NO_3^-$ or ^{14}C and ^{15}N-labelled ureides or amino compounds (Atkins *et al.*, 1975, 1980a; McNeil *et al.*,1979; Van Bel, 1984; Dickson *et al.*, 1985; Da Silva and Shelp 1989; Shelp *et al.*, 1992). Comparison of the distribution of inulin, a complex carbohydrate which serves as a xylem marker, with the distribution of aminoisobutyric acid, a synthetic amino acid which moves both in xylem and phloem, has been used to estimate xylem-to-phloem transfer (Van Bel, 1984, Da Silva and Shelp, 1989). Da Silva and Shelp (1989) supplied these two compounds to detached transpiring soybean

3 h and found that the developing trifoliolate is a reliable indicator of xylem-to-phloem transfer. The phloem stream provides to the developing trifoliolate up to 4-fold the relative proportion of solute received from the xylem stream; this is markedly reduced by increased light, intensity and consequently water flow, through the xylem. Evidence from heat-girdling experiments was interpreted to suggest that direct xylem-to-phloem transfer accounts for one-half of the aminoisobutyric acid supply to the developing trifoliolate. Such a method was used to demonstrate the xylem-to-phloem transfer of phosphinothricin (Shelp *et al.*,1992) and ureido compounds (Shelp and Da Silva, 1990).

Recently, non radioactive analogs have been used to investigate xylem-to-phloem exchange processes of inorganic ions in phloem and xylem. Rubidium, frequently used as a tracer for K (Haeder and Beringer, 1984a ; Kuppelwieser and Feller, 1990), is highly mobile in phloem. Strontium, which behaves similarly to the macronutrient Ca in higher plants, is not mobile in the phloem (Marschner, 1986) and has been used as a xylem marker compound (Feller, 1989; Kuppelwieser and Feller, 1990; Nelson *et al.*, 1990; Schenk and Feller, 1990). Schenk and Feller (1990) showed that Rb applied to the flag leaf of wheat accumulates mainly in shoot parts above the feeding position (i.e. maturing grains and glumes), whereas Sr accumulates in the fed leaf. Steam girdling the stem below the ear (phloem interruption) does not affect the distribution of Sr, but the retranslocation of Rb is inhibited, resulting in considerable accumulation in the stem. Together, these data indicate that Rb reaches the ear predominantly via the phloem (Feller, 1989).

The existence of xylem-to-xylem transfer has also been reported. The developing inflorescence and young leaves of legumes receive a distinct 'extra' component of transpirationally attracted nitrogen resulting from xylem-to-xylem transfer in the lower regions of the stem (Pate, 1986). Martin (1982) also showed phloem-to-xylem transfer in wheat stems after anthesis; retranslocation from the flag leaf in phloem was followed by a considerable release of P, Mg and N, but not K, into the xylem with the subsequent transport of these mineral elements through xylem to the ears. This particular mechanism may be at least in part responsible for the relatively low content of K in the ears and grains of cereals (Martin ,1982; Haeder and Beringer, 1984b). In another study, Jeschke *et al* (1985,1987) reported that in lupin plants a considerable proportion of ions such as K, Mg and Na, which are supplied in phloem to roots, are recirculated to the shoot.

2.6. Boron Distribution and Retranslocation

Once in the xylem, the translocation of B is related to the loss of water (Bowen,1972; Kohl and Oertli 1961; Michael *et al.* 1969). For instance, the distribution of B in the shoot organs of broccoli grown with a continuous supply of B showed that B concentration is highest in the source leaves and lowest in the young leaves and florets. This typical gradient in the B distribution corresponds to organ age and, by implication, to transpiration (Shelp *et al.*, 1992). Even within a particular leaf, a steep gradient in B concentration (petioles and midribs < middle of lamina, < margins and tips may result from an excessive supply of B (Oertli and Roth, 1969) .

The much higher concentration of B in leaves than in phloem sap (Tammes and Van Die,1966; Shelp, 1987,1988) provides further evidence that B retranslocation in phloem is restricted.

If the distribution of B is only related to primary translocation in the xylem, then B concentration should always be highest in the source leaves. However, when broccoli plants are grown with continuous B deficiency the gradient from old to young tissues disappears or is reversed (Shelp *et al.*, 1992b). Van Goor and Van Lune (1980) observed that apple fruits accumulate B linearly with time even when phloem supplies most of the nutrients. Additional evidence that B retranslocation occurs under conditions of continuous B starvation is derived from ratios of tissue B in young leaves or roots to old leaves of radish (Shelp et al., 1987), rutabaga (Shelp and Shattuck, 1987a) and cauliflower (Shelp and Shattuck, 1987b). Liu *et al.*, (1993) investigated the distribution of B in plants grown at three locations in Ontario that differ in extractable B levels. A decreasing acropetal B gradient is found only in plants supplied with supplemental B. Together, these studies indicate that B is sufficiently retranslocated to meet the demands of developing organs.

Other research has reported that B partitioning within the plant is altered in response to an interrupted B supply. The decreased B contents of mature leaves of grapes (Scott and Schrader, 1947), broccoli (Benson *et al.*, 1961; Shelp, 1988), cotton and turnip (McIlrath, 1965), and unchanging content in fruits of peanut and subterranean clover (Campbell *et al.*, 1975) when the B supply is removed from the nutrient solution suggest that B is retranslocated. Genetic variation in the retranslocation of B is evident

from detailed comparison under controlled environment conditions of two rutabaga cultivars, Laurentian (most widely grown in North America) and Wilhelmsberger (a European cultivar with low susceptibility to B deficiency) (Shelp and Shattuck, 1987a). With sufficient B, the ratio of tissue B in storage roots to old leaves is considerably less than the ratio for N (a phloem- mobile element) and higher than that of calcium (a phloem-immobile element) . Furthermore, removal of B supply when roots are 1.0 to 1.5 cm in diameter increases the B ratio by 110% in 'Laurentian' and 190% in 'Wilhemsberger'. Thus, 'Wilhemsberger' by virtue of its capability to retranslocate B is less suscepible to B deficiency. Another study determined the relative susceptibility of four broccoli cultivars including Commander, which posseses low susceptibility to the hollow stem disorder (Shelp *et al.*, 1992a). In all cultivars, B retranslocation within the shoot is implicated from high tissue B in florets and young leaves, sinks which develop after B supply is removed from the nutrient solution. 'Commander' is the only cultivar that has tissue B in sinks equivalent to those in plants receiving sufficient B continously; this is accompanied by a small but significant decline in tissue B of old leaves. Furthermore in response to continuous B at deficient level, the tissue B of florets from 'Commander' is highest of all cultivars and shows the lowest quantitative decline relative to plants supplied with adequate B. The stability of floret-B concentration supports the view that 'Commander' has a greater retranslocation capacity than the other cultivars examined.

Boron is found at a concentration of 0.3 mM in phloem exudates collected from inflorescence stalks of broccoli plants supplied with an adequate supply of B

(Shelp,1987, 1988), whereas old leaves and florets have B concentrations of 4.6 and 0.4 mM, respectively (Shelp, 1993). The much higher concentration of B in source leaves than in the phloem sap of *Yucca flaccida* (Tammes and Van Die,1966) and similar phloem and inflorescence B concentrations suggest that phloem rather than xylem is the predominant source of B.

By comparing the relative proportion by weight of elements, Shelp (1987) has estimated the contribution of selected nutrients from phloem to the developing sinks of broccoli grown with adequate B. The phloem stream provides B to an equal or greater extent than N, P, and K, nutrients which are generally classified as phloem mobile. Hanson and Breen (1985), using calculated transpiration rates and the B concentration in RBS (0.23 mM), estimated that only 26% of the B entering the prune flower buds is supplied by xylem. While it is generally accepted that the RBS provides a reasonable picture of the relative composition of solutes in the xylem sap of intact transpiring shoots, research has shown that the solute concentration of RBS is an order of magnitude higher than in the xylem sap from intact plants (Pate *et al.*, 1980).

Another strategy that has been employed to monitor the distribution and retranslocation of B in plants is the use of the mass isotopes ^{10}B and ^{11}B. Martini and Thellier (1975) traced the slow movement of foliar-applied ^{10}B from clover leaves using a nuclear reaction to distinguish between B isotopes, and reported that the cell wall contains the highest amounts of ^{10}B, with very little ^{10}B being detected inside parenchyma cells. ^{10}Boron applied to radish and apple fruits (Chamel *et al.*, 1981; Chamel and Andreani, 1985) is distributed to different depths in those tissues, as

determined by spark-source mass spectrometry and laser probe mass spectrometry . Recently, inductively coupled plasma mass spectrometry was used to show that ^{10}B foliar-applied to fruit trees apple, pear (*Pyrus communis* L.), plum (*Prunus domestica* L.) and cherry (*Prunus cerasus* L.) is translocated relatively freely out of the leaves into the bud, wood and bark tissues. The highest concentrations of applied ^{10}B is found in buds, followed by the bark and wood, whereas the total B content of untreated leaves remains unchanged (Hanson, 1991b). These results suggest that foliar-applied B becomes part of a mobile pool.

Recently, Marentes *et al.* (1994a) used ^{10}B to investigate the distribution of xylem-borne B over a 14-d period during the development of the broccoli inflorescence. Regardless of the experimental regime of (i.e. transfer at inflorescence emergence from either luxury or a sufficient B supply to an insufficient one), the floret-B concentrations vary only about 2.5-fold, whereas the B concentrations of mature leaves and B supply vary 25- and 50-fold, respectively. The application of enriched ^{10}B during inflorescence development revealed that the relative contribution to floret B of B acquired either before or after inflorescence emergence depends on stage of leaf growth, plant B status and external B supply. After 14 d, the contribution of B acquired after inflorescence emergence was always higher (66-99 %) than the B acquired before inflorescence emergence. Transient accumulation by and evidence of net efflux from shoot strata during the period of rapid floret growth suggests that stem+petioles and upper canopy serve as temporary reservoirs of B acquired from the root medium after inflorescence emergence. In a follow-up study, Marentes *et al.*

(1994b) investigated the retranslocation of previously-acquired B to developing sinks. Labelling with enriched ^{10}B supplied to the root system was used to quantify the contribution to broccoli florets and lupin fruits of B acquired during a relatively brief period (10 and 15 d for broccoli and lupin, respectively) just before inflorescence emergence. By 15 and 30 d, broccoli florets and lupin fruits respectively, received 9 and 21 % of the B acquired just before inflorescence emergence. The primary sources of this retranslocated B were initially mature leaves and stem+petiole, then recently-matured leaves and finally competing sinks such as young leaves. The retranslocated B satisfied 0.25 and 0.76 % of the B requirements for broccoli florets and lupin fruits during the reproductive period investigated.

2.7. CONCLUDING REMARKS

Retranslocation of B may involve either direct or indirect xylem-to-phloem transfer processes. Given that B acquired after inflorescence emergence in broccoli is transiently accumulated in some plant parts and contributes more B to the growth of reproductive sinks than B acquired before inflorescence emergence, it is tempting to speculate that B undergoes direct xylem-to-phloem transfer without metabolism. This proposal can be investigated by comparing the short-term distribution of ^{10}B with the distribution of xylem (e.g. inulin, strontium) and xylem/phloem (e.g.aminoisobutyric acid, rubidium) marker compounds, together with the use of girdling techniques (Van Bel, 1984; Da Silva and Shelp, 1989, Shelp and Da Silva, 1990).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study One: Determination of Elemental Composition of Xylem Sap and Phloem Exudate from Intact Broccoli and Lupin Plants During Vegetative and Reproductive Growth

Broccoli (*Brassica oleracea* var. *italica* Plenck cv. Commander) plants were grown under greenhouse conditions in the summer months (June- August 1993). Seeds were germinated in seedling trays filled with vermiculite and supplied with one-quarter strength nutrient solution (see below). After 3 wk, the seedlings were transplanted to 6-L pots filled with vermiculite.The average night / day temperature was 25/18°C. Natural lighting was supplemented by high-intensity sodium vapor lamps yielding a quantum flux density at pot level of 60 μmol m^{-2} s^{-1}, resulting in a 16-h day / 8-h night period. The plants were watered daily with 2 L of modified Hoagland solution (pH 6.5) (Hoagland and Arnon, 1950) containing the following macronutrients (mM): Ca, 1.5; K, 6.4; Mg, 0.25; N, 19; (11.5 as NO_3^- and 7.5 as NH_4^+); P, 2.4; S, 0.75. The following micronutrients (μM) were also included: Cl, 2.8; Mn, 1.8; Zn, 0.76; Cu, 0.29; Mo, 0.21; Co, 0.17; Fe, 20 (as Fe-EDTA). In addition, 50 μM B (natural abundance of ^{10}B is 19.098 %, International Union of Pure and Applied Chemistry, 1991) was included ; this supply is adequate for optimal inflorescence yield (Shelp, 1993).

A developmental study of elemental accumulation and water usage by the shoot was

conducted over a 2-wk period beginning at inflorescence emergence. Each day the plants were supplied with nutrient solution; the excess was allowed to drain from the pot. The pots were then enclosed in plastic bags exposing the shoot only. The combined weight of the pot and plant was recorded prior to and after watering, with the daily losses indicating water usage by the shoot.

Plants were arranged in a complete randomized block design with six plants each being harvested at 0, 1 and 2 wk after inflorescence emergence (beginning 7 wk after sowing). They were fertilized 2 h before collection of the translocation fluids. At 11:00 h, shallow incisions were made in the stems of the attached inflorescence and the phloem exudate collected for approximately 0.5 h with an automatic pipetter equipped with plastic tips (Shelp, 1987). A second round of incisions was made to collect additional phloem exudate . Then the shoot was removed, and the stem base remaining attached to the root blotted. The root bleeding sap (RBS) was collected with a pipetter with plastic tubes for 1 h from the root stump after the first drops were discarded. The fluids were held on ice during the collection period and later stored at -20 °C. Both phloem exudate and RBS were centrifuged in a microfuge to remove any particulate matter before elemental analysis. The volumes of phloem exudate and RBS collected from each replicate were, respectively, 50-100 µl and 1000 µl; the phloem exudate from all replicates was pooled to provide adequate volumes for analysis.

During the same summer months , lupin (*Lupinus albus* L. cv. Ultra) seeds , inoculated with *Rhizobium lupini* (Lipha Tech Inc., Milwaukee, USA), were germinated in vermiculite and supplied with N-free Hoagland-type nutrient solution

containing the following macronutrients (mM): Ca, 3.0; K, 3.2; Mg, 0.49; P, 0.50; S, 0.75. The following micronutrients (μM) were also included : Cl, 2.8; Mn, 0.36; Zn, 0.15; Cu, 0.08; Mo, 0.04; Co, 0.05 and Fe, 39. In addition, 30 μM B (natural abundance) was included; this supply is considered adequate for optimal fruit yields (Hocking and Pate, 1978). Three weeks later, the seedlings were individually transplanted to 2-L pots containing vermiculite and the concentrations of K and Ca in the nutrient solution doubled.

A 6 -wk developmental study of element accumulation and water usage by the shoot began 2 wk after transplanting, and included both vegetative and reproductive phases. Flowering occurred about 4-5 wk after seed germination. Plants were arranged in a complete randomized block design, with six plants each being harvested at 2-8 wk after transplanting. They were fertilized 2 h before collection of the translocation fluids. At 11:00 h, phloem exudate was collected as droplets from either shallow incisions in the stem, two nodes below the base of the primary stem, or from excised fruit tips (Pate, 1974) for approximately 15 min using an automatic pipetter equipped with plastic tips; fruit tips were excised twice in order to obtain enough exudate for analysis. Then the shoot was removed, and the RBS was collected for 1 h from the cut, blotted root stumps by placing a 2-cm section of tygon tubing over the stem base; the first drops of sap were discarded. The fluids were held on ice during the collection period and later stored at -20 °C. Both phloem exudate and RBS were centrifuged in a microfuge to remove any particulate matter before analysis. The volumes of phloem exudate and RBS collected from each replicate were, respectively, 100 μl and 200-500

µl; the phloem exudate from all replicates was pooled to provide adequate volumes for elemental analysis.

Shoots from both broccoli and lupin plants were oven dried to a constant dry weight at 60°C and subsequently digested overnight with 70 % HNO_3 (v/v) in closed vessels at 110°C as described by Topper and Kotuby-Amacher (1990). The digest was further diluted (1:20) with nanopure water. Root bleeding saps and phloem exudate were also diluted with nanopure water (1:10). Elemental composition of the shoot digest and translocation fluids was determined using inductively coupled plasma -atomic emission spectrometry (ICP-AES) as described by Spiers *et al.* (1990).

Information about water usage and element accumulation by the shoot allowed calculation of the concentration of particular elements required in xylem sap of intact plants. Comparison of the composition of xylem sap, rather than RBS, to that of phloem exudate should more appropriately indicate the extent of xylem-to-phloem transfer in the plant. The composition of intact xylem sap should also indicate the concentration requirements for "artificial" xylem sap used to feed detached transpiring shoots (section 3.2).

3.2. Study Two: Comparison of the Distributions of Xylem-borne ^{10}Boron, Rubidium (a Potassium Analog Which Served as a Marker for Movement in Both Xylem and Phloem) and Strontium (a Calcium Analog Which Served as a Marker for Movement in Xylem) in Broccoli and Lupin Plants.

Broccoli plants were grown in the greenhouse during the months of October-

November 1993 under similar environmental conditions and with nutrient solution as described for study one. In the first experiment ("attached plants"), when the inflorescence was about 10 cm in diameter, the two lowest leaves were removed. A day later, the plant was supplied with nutrient solution containing 0.5 mM $SrCl_2$, 0.5 mM RbCl and 50 µM B (95.5 % ^{10}B- enriched Cambridge Isotope Laboratories, Woburn, Mass) for up to 12 h, then divided into: leaves 3-4, leaves 5-8, leaves 9-14, leaves 15-22, stem+petioles (including midribs) and florets. Plants were arranged in a complete randomized block design with three replicates/ harvest time.

In the second experiment, the four lowest leaves were removed and the shoot was detached under water just above the root region and transfered to a tube containing "artificial" xylem sap composed of 50 µM $SrCl_2$, 50 µM RbCl, 5 µM B (95.5 % ^{10}B-enriched), 1 mM glutamine [major amino acid of xylem (Shelp, 1987)], and 1 mM malic acid [major organic acid of xylem (Shelp, unpublished)], adjusted to pH 5.5 with 1 M KOH. Care was taken to ensure that a water bubble clung to the cut base of the shoot to prevent the occurrence of embolisms in the xylem stream. These cut transpiring shoots were supplied for up to 8 h (plants that showed signs of wilting were discarded), then divided into five strata; leaves 5-8, leaves 9-14, leaves 15-22, stem+petioles (including midribs) and florets. Plants were arranged in a completely randomized block design (four replicates / harvest time). In both experiments, the dry weights of the plant strata were determined and digested as described previously (section 3.1).

Effectively nodulated lupin plants (one-four replicates / harvest time), arranged in

a complete randomized block design, were grown in the greenhouse during the autumn (Sept- Nov 1993). Environmental and nutrient conditions were similar to those in study one. The plants were supplied with adequate (3 μM) or luxury (30 μM) B (natural abundance) in the nutrient solution. The plants were used when the fruits on the primary inflorescence had attained nearly full size (approximately 7 cm in length). Because some of the leaves on the primary stem had abscissed, one day prior to the supply of ^{10}B and marker solutes, all but the five uppermost leaves were removed from primary stem to ensure uniformity among the plants. The plants were fed for up to 4 d with nutrient solution containing 0.5 mM $SrCl_2$, 0.5 mM RbCl and the same B concentration (3 or 30 μM, 95.5 % ^{10}B- enriched) supplied during growth. Root bleeding sap was collected from the blotted root stumps and phloem exudate from the fruits as described above (section 3.1) and stored at -20°C. The harvested plants were divided into primary stem+petioles, primary leaves, fruits on primary inflorescence, secondary stem+petioles, secondary leaves, and secondary inflorescence The dry weights of these strata were determined, then the samples were digested.

The tissue digests were diluted 1:1, 1:5 or 1:100 with nanopure water for the measurements of rubidium and potassium, and 1:100 for the measurements of strontium, calcium and magnesium using atomic absorption spectrometry (AA) and ICP-AES, respectively; these instruments were housed in the Department of Land Resource Sciences at the University of Guelph. For B and ^{10}B analyses, an aliquot of each plant digest was diluted to 1 % HNO_3, spiked with beryllium (Esar, ICP standard solution, 1000 ppm Be, Specpure) as an internal standard, then measured

using a PlasmaQuad 2+/Turbo inductively-coupled plasma mass spectrometer (Marentes *et al.* 1994a). Aliquots of RBS and phloem fluids were respectively, diluted 1:50 and 1:200 with 50 ppb Be in 1% HNO_3 acid and analysed for total B and ^{10}B (VG Elemental, Winsford, Chechire, UK) at the Human Nutrition Research Centre, University Station, Grand Forks, North Dakota in collaboration with Dr Richard Vanderpool.

Statistical Analysis

The percent distribution data were arcsin transformed and subjected to statistical analysis using the general linear models procedures of Statistical Analysis of System (SAS). Analysis of variance, and separation of means and significance ($p \leq 0.05$) were determined, respectively, by a one way ANOVA and Student-Newman-Keuls method ($p \leq 0.05$).

CHAPTER FOUR

RESULTS

4.1. Elemental Composition of Translocation Fluids from Intact Plants of Broccoli and Lupin

Preliminary experiments were conducted to determine the elemental composition of translocation fluids from intact broccoli and lupin plants. Plants were grown with adequate B and the weekly elemental accumulation and water usage by the shoot determined. From this information, I calculated the concentration of particular elements in the xylem sap of intact plants. For instance, from data on shoot B accumulation and water usage by broccoli plants (Table 1), the calculated B concentration of xylem sap from an intact plant during inflorescence development was 2.9-3.5 μM, a value only 8 % of the B concentration of RBS (approximately 40 μM) (Tables 2 and 3). In contrast, the B concentration of phloem exudate from attached inflorescence stalks was about 0.4 mM, which is 113-147 times the B concentration of xylem sap (Tables 2 and 3). During the vegetative and reproductive development of lupin plants, the xylem-sap B ranged from 2.5-5.6 μM (Table 4), whereas the B concentration of RBS ranged from 28-80 μM (Fig 1), resulting in RBS: xylem sap concentration ratios of 10-15 (Fig 2).The B concentration of phloem exudate collected from stem incisions and fruit tips ranged from 0.2-0.5 mM (Fig 1), values 50-87 times higher than those found in xylem (Fig 2).

Table 1. *Calculation of xylem-sap B in intact plants from dry weight and boron accumulation, and water usage by broccoli shoots during inflorescence development.*

B concentration in xylem sap was calculated as (B accumulated wk^{-1}) ÷ (water usage wk^{-1}). Data represent the mean ±SE (n=5).

Time After Inflorescence Emergence	Dry Weight	B Content	Water Usage	Rate of B Accumulation	Xylem-sap B
wk	*g*	*μmol*	*mL* wk^{-1}	*μmol* wk^{-1}	*μM*
0	72±2	47±4			
1	108±3	79±7	9210±128	32±8	3.5±0.5
2	110±5	105±5	9097±140	26±4	2.9±0.2

Table 2. *Elemental composition of the translocation fluids of broccoli plants during inflorescence development.*

Root bleeding sap (RBS) and xylem sap data represent the mean±SE (n=5); phloem exudate from five plants was pooled for analysis.

Time After Inflorescence Emergence	Translocation Fluid	K	P	Mg	Ca	Zn	Fe	Mn	Mo	B
wk				*mM*					*μM*	
0	RBS	6.9±1.2	2.3±0.2	1.3±0.1	0.8±0.1	6.1±0.8	4.6±1.2	6.0±0.3	10.4±1.6	39.8±4.5
1	RBS	17.0±1.7	2.6±0.3	2.4±0.3	1.6±0.2	4.9±0.8	4.3±1.1	5.8±0.9	11.5±5.8	43.5±6.7
	Xylem sap	1.5±0.0	0.3±0.0	0.2±0.0	0.1±0.0	0.6±0.2	0.8±0.3	0.5±0.1	0.80±0.3	3.5±0.5
	Phloem exudate	74.9	23.2	10.7	1.2	108.7	125.1	39.8	23.5	395.4
2	RBS	23.2±2.1	3.5±0.6	3.3±0.7	2.2±0.7	5.8±0.6	8.4±1.1	10.2±3.3	11.2±2.0	37.8±4.1
	Xylem sap	2.0±0.1	0.3±0.0	0.2±0.0	0.2±0.0	0.6±0.2	0.7±0.1	0.8±0.3	1.2±0.1	2.9±0.2
	Phloem exudate	90.8	22.6	11.1	1.7	113.1	104.0	34.5	20.6	425.9

Table 3. *Concentration ratios of elements between translocation fluids of broccoli during inflorescence development.*
Ratios were calculated from data in Table 2.

Time After Inflorescence Emergence	Ratio	K	P	Mg	Ca	Zn	Fe	Mn	B
wk									
1	RBS:xylem	11.4±1.3	8.4±1.2	13.1±1.9	16.0±1.6	8.2±2.3	5.4±1.2	12.8±1.6	12.3±1.3
	Phloem:xylem	49.9	77.3	53.5	12.0	181.1	156.7	79.6	113.0
2	RBS:xylem	11.5±0.9	11.8±2.5	17.5±3.2	11.7±1.5	9.7±2.4	8.0±1.8	12.0±1.6	13.0±1.5
	Phloem:xylem	45.4	75.3	55.5	8.5	188.3	148.7	43.1	146.9

Table 4. *Calculation of xylem-sap B in intact plants from dry weight and boron accumulation, and water usage by lupin shoots during vegetative and reproductive development.*

B concentration in xylem sap was calculated as (B accumulated wk^{-1}) ÷ (water usage wk^{-1}). Data represent the mean ±SE (n=5).

Time After Transplanting	Dry Weight	B Content	Water Usage	Rate of B Accumulation	Xylem-sap B
wk	*g*	*μmol*	*L* wk^{-1}	*μmol* wk^{-1}	*μM*
2	0.5±0.1	0.3±0.0			
3	1.1±0.1	0.8±0.1	0.2±0.0	0.5±0.1	2.5±0.4
4	1.9±0.2	1.8±0.0	0.3±0.0	1.0±0.0	3.3±0.3
5	3.5±0.3	4.0±1.1	0.6±0.0	2.2±1.0	3.7±1.8
6	6.2±0.7	7.7±1.4	1.0±0.1	3.7±1.4	3.7±1.2
7	11.0±0.2	14.6±1.5	1.3±0.1	6.9±1.3	5.3±0.4
8	22.1±1.0	22.0±3.2	1.6±0.3	8.9±2.9	5.6±1.5

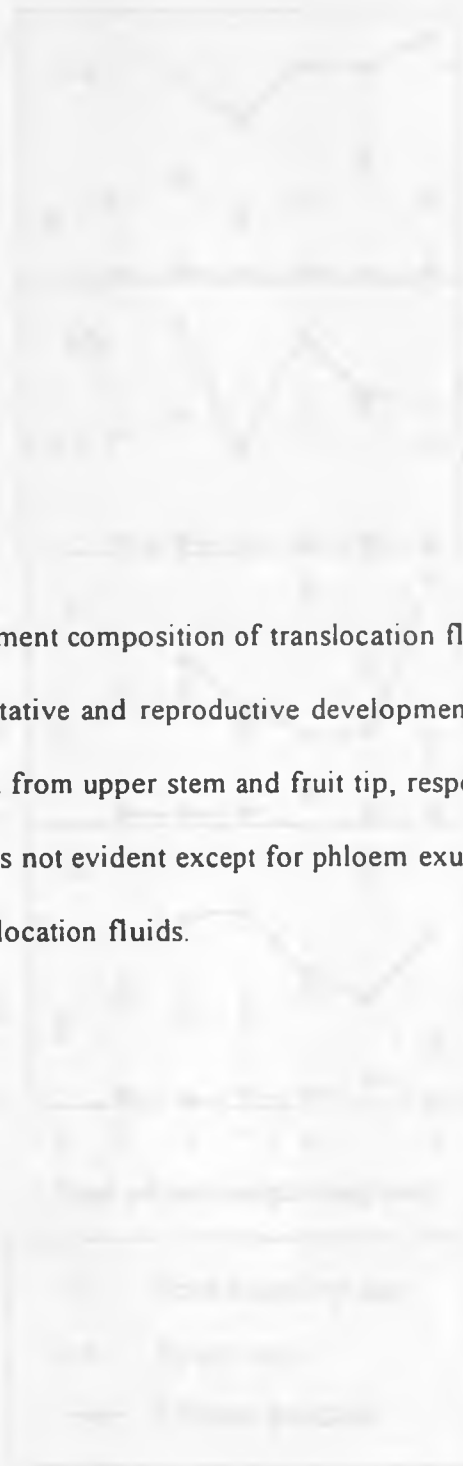


FIGURE 1A. Macroelement composition of translocation fluids from lupin plants during a 6- wk period of vegetative and reproductive development. Phloem exudate for weeks 4-7 and 8 was collected from upper stem and fruit tip, respectively. Data represent mean ± SE (n=3); where SE is not evident except for phloem exudate, it is within the symbols. Key indicates the translocation fluids.

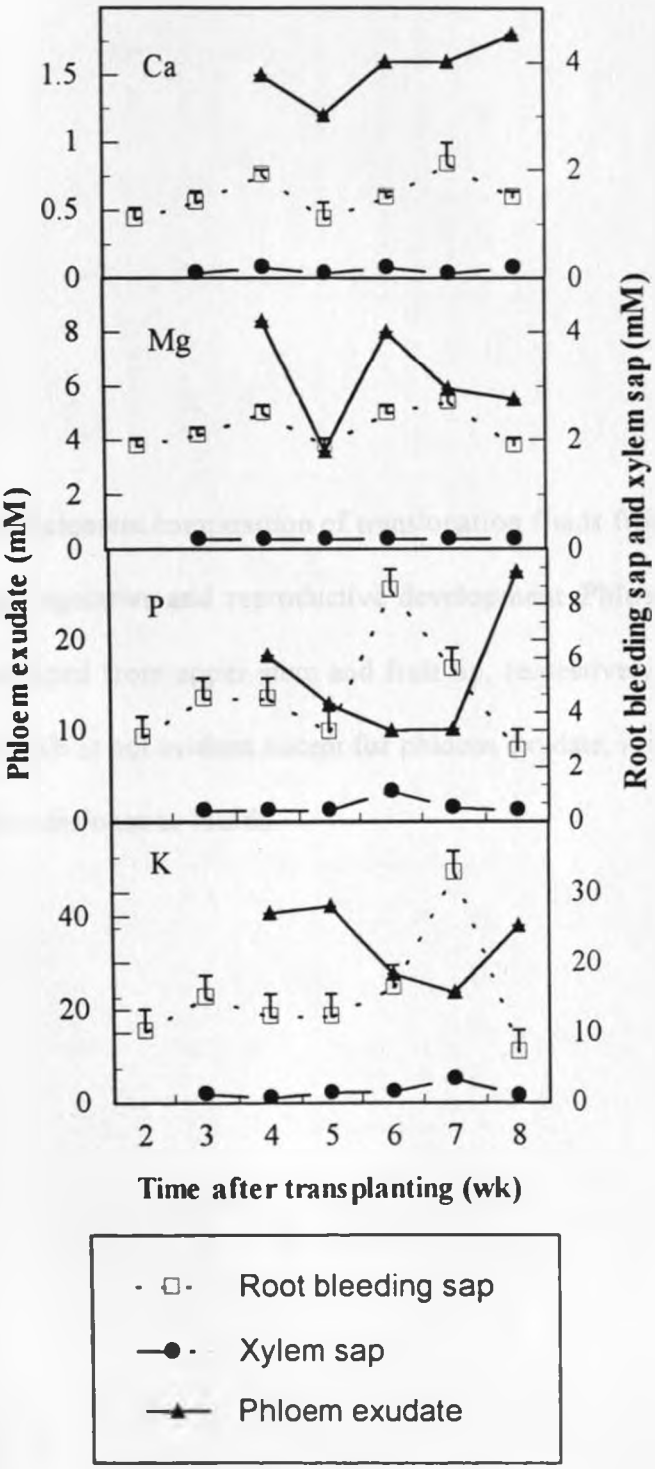

Ca
Mg
P
K
Phloem exudate (mM)
Root bleeding sap and xylem sap (mM)
Time after transplanting (wk)
Root bleeding sap
Xylem sap
Phloem exudate

FIGURE 1B. Microelement composition of translocation fluids from lupin plants during a 6- wk period of vegetative and reproductive development. Phloem exudate for weeks 4-7 and 8 was collected from upper stem and fruit tip, respectively.Data represent mean ± SE (n=3); where SE is not evident except for phloem exudate, it is within the symbols. Key indicates the translocation fluids.

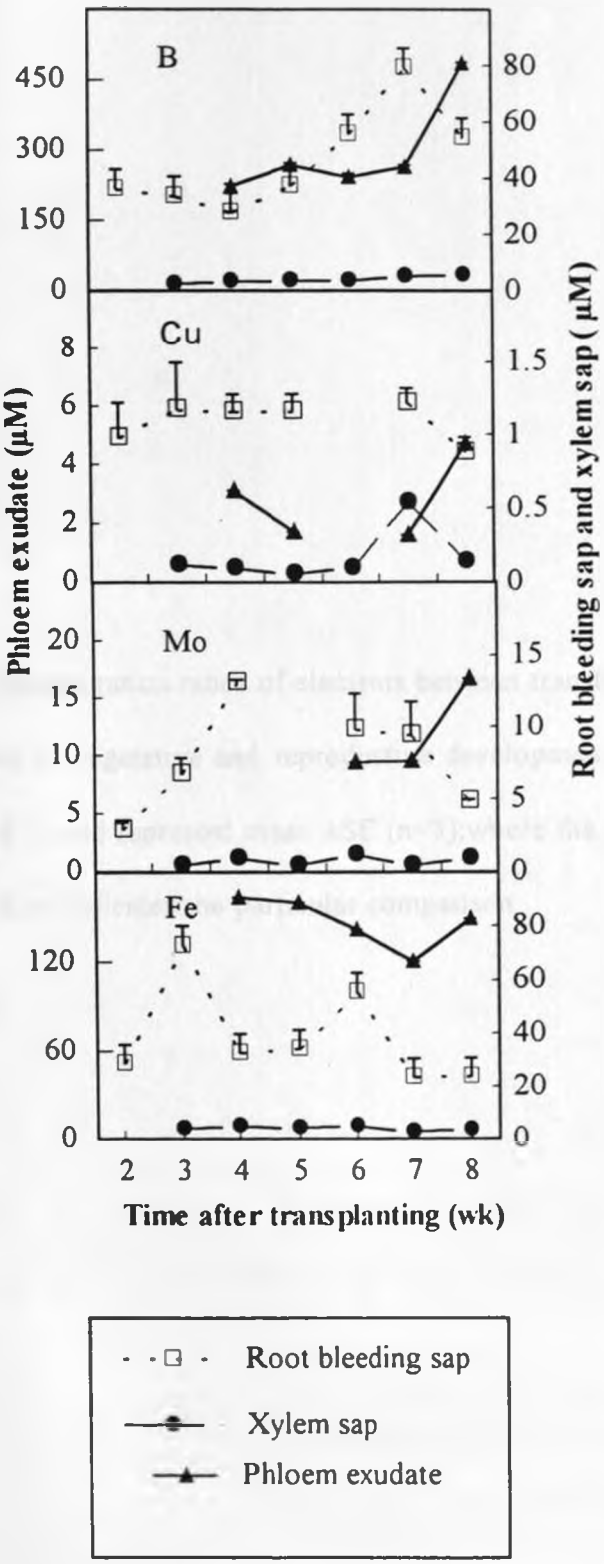
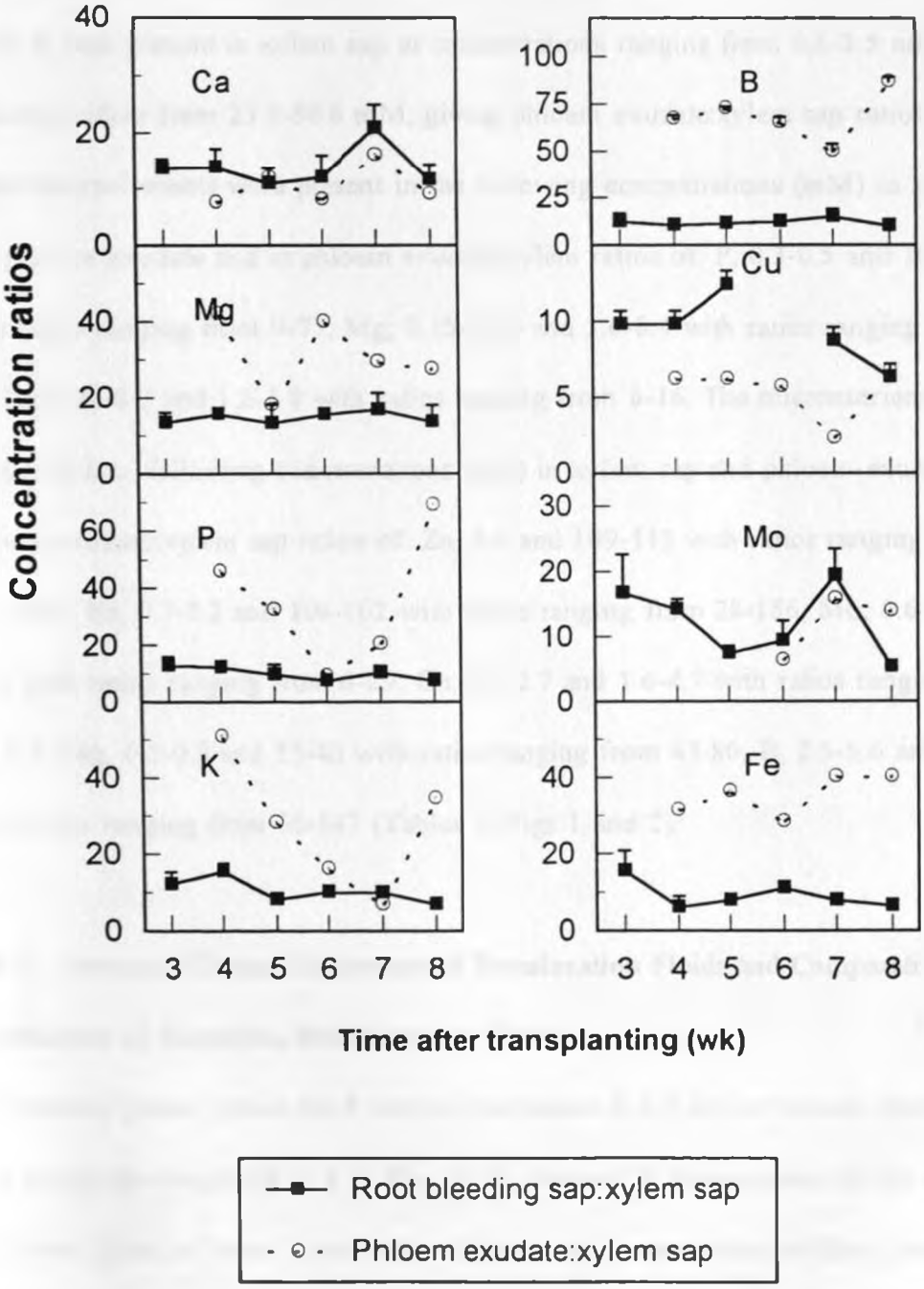

B
Cu
Mo
Fe
Phloem exudate (μM)
Root bleeding sap and xylem sap (μM)
Time after transplanting (wk)
Root bleeding sap
Xylem sap
Phloem exudate

FIGURE 2. Concentration ratios of elements between translocation fluids of lupin during a 5-wk period of vegetative and reproductive development. Data were calculated from Figures 1 and 2, and represent mean ±SE (n=3);where the SE is not shown, it is within the symbol. Key indicates the particular comparison.



The xylem saps from both broccoli and lupin plants also contained other elements. These were generally present in decreasing concentration as K > P > Mg > Ca > B > Fe > Zn = Mo = Cu = Mn (Table 2, Figs 1 and 2). In phloem exudates, they were generally present in decreasing order as K > P > Mg > Ca > B > Fe = Zn > Mo = Cu = Mn. K was present in xylem sap at concentrations ranging from 0.8-3.5 mM and in phloem exudate from 23.7-90.8 mM, giving phloem exudate:xylem sap ratios of 7-51. Other macroelements were present in the following concentrations (mM) in xylem sap and phloem exudate and at phloem exudate:xylem ratios of: P, 0.3-0.5 and 10.0-27.5 with ratios ranging from 9-77; Mg, 0.15-0.20 and 3.6-8.4 with ratios ranging from 18-56; Ca, 0.1- 0.2 and 1.2-1.8 with ratios ranging from 8-16. The micronutrients were present in the following concentrations (μM) in xylem sap and phloem exudate and at phloem exudate:xylem sap ratios of: Zn, 0.6 and 109-113 with ratios ranging from 181-188 ; Fe, 0.7-5.2 and 104-162 with ratios ranging from 28-156; Mo, 0.6-1.2 and 9-24 with ratios ranging from 6-29; Cu, 0.3-2.7 and 1.6-4.7 with ratios ranging from 2.3-6.7; Mn, 0.5-0.8 and 35-40 with ratios ranging from 43-80; B, 2.5-5.6 and 222-485 with ratios ranging from 50-147 (Tables 2, Figs 1 and 2).

4.2. Broccoli: ^{10}Boron Enrichment of Translocation Fluids and Comparative Shoot Distribution of Strontium, Rubidium and ^{10}Boron

Broccoli plants, grown for 8 wks with adequate B (50 μM at natural abundance), had a total dry weight of 51.1 g. The Ca, K, Mg and B concentration of the various strata are given in Table 5; the ratios of elemental concentrations in floret:leaves 3-4

were 0.41 for Ca, 0.61 for K, 0.39 for Mg and 0.34 for B. These reproductive plants were fed simultaneously with Sr, Rb and enriched ^{10}B (50 μM) in the nutrient solution for a 12-h time course.

In RBS and phloem exudate, the concentration of total B remained steady over the entire period (about 21.5 and 271 μM, respectively Table 6). The concentration of ^{10}B increased over the time course by approximately 70%; however, the ratio of ^{10}B in phloem exudate:RBS (11) was similar to that for total B. Prior to feeding, plants contained 12, 6 and 41 μmol of Sr, Rb and ^{10}B, respectively (Table 7); these elements increased by about 6-, 17- and 1-fold over the 12-h time course. The accumulation of these compounds was not linear with time. Thus, the background Sr and Rb contents could be ignored, whereas the relatively high ^{10}B background could not as it would contribute to estimates of B partitioning during the time courseof the experiment. In the plant, approximately 58-69 % of the Sr, 50-75 of the Rb and 17-44 of the ^{10}B was recovered in the stem+petioles. Because of the different retention of these elements by the stem+petioles, it seemed inappropriate to describe the distribution of ^{10}B in the florets on a whole plant basis or in terms of the Sr and Rb content of the florets. Therefore, after correction for the mean ^{10}B content at zero time [this assumes that the

Table 5 . *Dry weights and calcium, potassium, magnesium and B concentrations of strata from* "intact " *broccoli plants grown with adequate B.*

Data represent the mean ±SE (n=15).

Stratum	Dry weight	Calcium	Potassium	Magnesium	Boron
	g		*mol (kg DW)* $^{-1}$		*mmol (kg DW)* $^{-1}$
Leaves 3-4	1.8±0.1	0.82±0.03	1.35±0.04	0.59±0.04	12.8±4.2
Leaves 5-8	6.4±0.2	0.82±0.02	1.27±0.05	0.49±0.04	12.1±4.3
Leaves 9-14	9.5±0.4	0.58±0.01	1.09±0.21	0.38±0.04	11.1±3.4
Leaves 15-22	4.3±0.3	0.36±0.05	0.96±0.09	0.32±0.04	7.0±1.6
Stem+Petioles	25.8±0.5	0.33±0.02	2.45±0.13	0.39±0.02	4.2±0.6
Florets	3.3±0.1	0.34±0.02	0.82±0.08	0.23±0.02	4.4±1.4
Total	51.1				

Table 6. *Total B and ^{10}B concentrations of root bleeding sap and phloem exudate from broccoli plants fed simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B (50 μM) for a 12-h period during inflorescence development.*

Data represent mean ±SE (n=3); phloem exudate was pooled for analysis.

Feeding	Root Bleeding Sap		Phloem Exudate	
Time	Total B	^{10}B	Total	^{10}B
h		*μM*		
0	23.6±0.6	4.9±0.1	269	57
3	22.1±4.6	7.9±1.9	306	78
6	18.9±2.6	6.7±1.0	260	76
9	22.1±0.8	8.3±0.4	239	79
12	20.7±1.9	8.2±0.9	279	97

Table 7. *Strontium, rubidium and ^{10}B contents of "intact" broccoli shoots fed simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B (0.5 mM) for a 12-h period during inflorescence development.*

Data represent mean ±SE (n=3). ND, indicates not detectable.

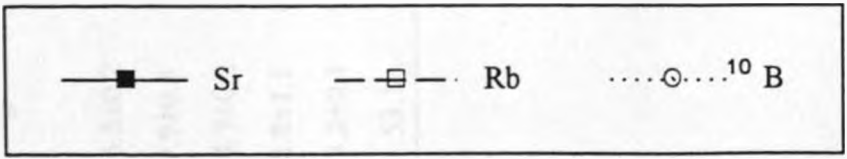
Fed Element	Stratum	Time (h)				
		0	3	6	9	12
				µmol		
Strontium	Stem+Petioles	8.3±0.3	36.7±2.7	44.1±8.3	48.0±1.9	56.5±9.7
	Leaves 3-22	3.7±0.0	25.8±3.0	27.7±1.9	28.5±1.6	32.3±1.2
	Florets	ND	ND	0.1±0.0	0.11±0.0	0.2±0.1
	Total	12.0±0.3	62.5±1.3	71.9±6.4	76.6±1.0	89.0±9.3
Rubidium	Stem+Petioles	3.2±0.1	39.5±2.1	43.2±5.3	56.3±3.5	85.2±1.9
	Leaves 3-22	2.7±0.2	37.2±0.7	36.3±1.4	33.8±1.7	27.1±0.8
	Florets	0.4±0.1	1.6±0.1	1.7±0.3	2.1±0.1	2.6±0.3
	Total	6.3±0.1	78.3±2.3	81.2±6.8	92.2±2.1	114.9±2.8
^{10}Boron	Stem+Petioles	12.1±1.0	17.4±1.1	22.0±0.6	14.3±1.3	22.4±2.6
	Leaves 3-22	28.2±0.1	49.6±1.5	61.8±5.8	62.3±1.6	60.2±1.6
	Florets	0.6±0.1	1.3±0.2	3.0±0.1	3.1±0.2	4.6±0.9
	Total	40.9±1.1	68.3±0.9	86.8±5.6	79.7±5.8	87.2±2.0

background ^{10}B was not readily retranslocated during the short-time course (Marentes *et al.*, 1994b)], contents in florets of the three fed compounds at 3-12 h were expressed as a percent of the total recovered in the inflorescences and foliage (Da Silva and Shelp, 1989). There was an approximately linear accumulation of Sr up to 0.7 % over the time course (Fig 3). The increase in Rb was much more rapid than for Sr, reaching 4.4 % within 3 h, and 8.7 % after 12 h. The percent ^{10}B in florets also increased in a fashion similar to Rb; it was significantly different from that for Sr, but not Rb.

A second experiment with broccoli used transpiring shoots. The dry weight accumulation and the Ca, K, Mg and B concentration of the various strata of these plants were comparable to the experiment described above (Table 8). The plants were supplied with 50 μM $SrCl_2$, 50 μM RbCl and 5 μM ^{10}B in the nutrient solution. These fed compounds increased by 67-, 133- and 1-fold over the 8-h time course; once again, the rate of accumulation was not linear with time (Table 9). As with "attached plants", retention of Sr (58-72%), Rb (51-92 %) and ^{10}B (23-32 %) in stem+petioles was different. Therefore, the mean background ^{10}B content was subtracted from the ^{10}B contents in the remainder of the time course, and the distribution of ^{10}B in florets expressed as a percent of the total recovered in the inflorescence and foliage. There was an approximately linear accumulation of Sr up to 2.5 % over the 8-h time course (Fig 4). The increase in Rb was much more rapid than for Sr, reaching 10.3 % within 2 h, and 11.8 % after 12 h. The percent ^{10}B in florets also increased in a fashion similar to Rb reaching 16.7 % within 2 h; it was significantly different from that for

Sr, but not Rb

FIGURE 3. Percent distribution of strontium, rubidium and ^{10}B in the florets of intact broccoli shoots supplied simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B (50 μM) over a 12-hour time course. Plants were grown with natural abundance B at the adequate level (50 μM). Data represent mean ±SE (n=3); where the SE is not evident (3-12 h) it is within the symbol. Key indicates the fed elements. Means followed by the same letter are not significantly different.


Sr
Rb
10 B

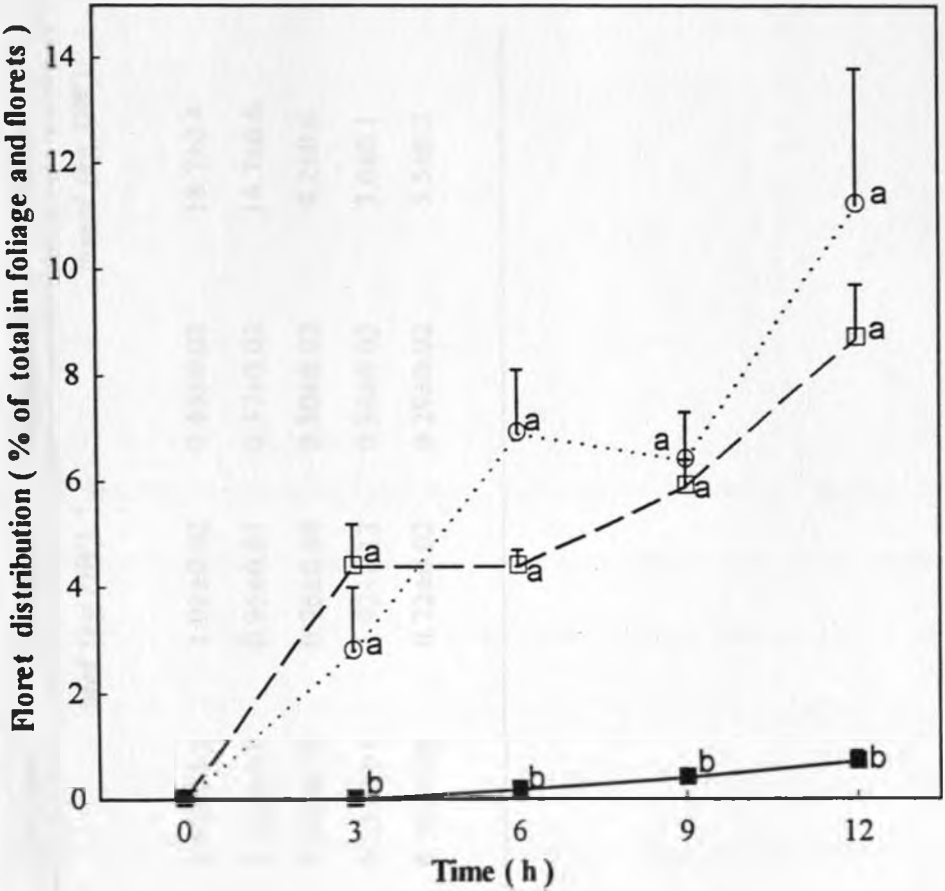

Floret distribution (% of total in foliage and florets)
14
12
10
8
6
4
2
0
0
3
6
9
12
Time (h)
a
a
a
a
a
a
a
a
b
b
b
b

Table 8. *Dry weights and calcium, potassium, magnesium and B concetrations of strata from "detached" broccoli plants grown with adequate B.*
Data represent the mean ±SE (n=15).

Stratum	Dry Weight	Calcium	Potassium	Magnesium	Boron
	g		*mol (kg DW)* $^{-1}$		*mmol (kg DW)* $^{-1}$
Leaves 5-8	6.5±0.2	0.57±0.03	1.01±0.02	0.45±0.03	18.7±0.4
Leaves 9-14	9.9±0.5	0.42±0.01	0.95±0.01	0.37±0.02	16.7±0.6
Leaves 15-22	4.7±0.2	0.30±0.03	0.76±0.04	0.30±0.02	9.2±0.6
Stem+Petioles	28.8±1.1	0.22±0.01	1.92±0.13	0.36±0.02	3.6±0.1
Florets	3.2±0.1	0.20±0.00	0.72±0.02	0.29±0.02	5.5±0.2
Total	53.1				

FIGURE 4. Percent distribution of strontium, rubidium and ^{10}B in the florets of detached broccoli shoots supplied simultaneously via the transpiration stream with strontium (50 μM), rubidium (50 μM) and enriched ^{10}B (5 μM) over a 8-hour time course. Plants were grown with natural abundance B at the adequate level (50 μM). Data represent mean ±SE (n=3); where the SE is not evident (2-8 hours) it is within the symbol. Key indicates the fed elements. Means followed by the same letter are not significantly different.

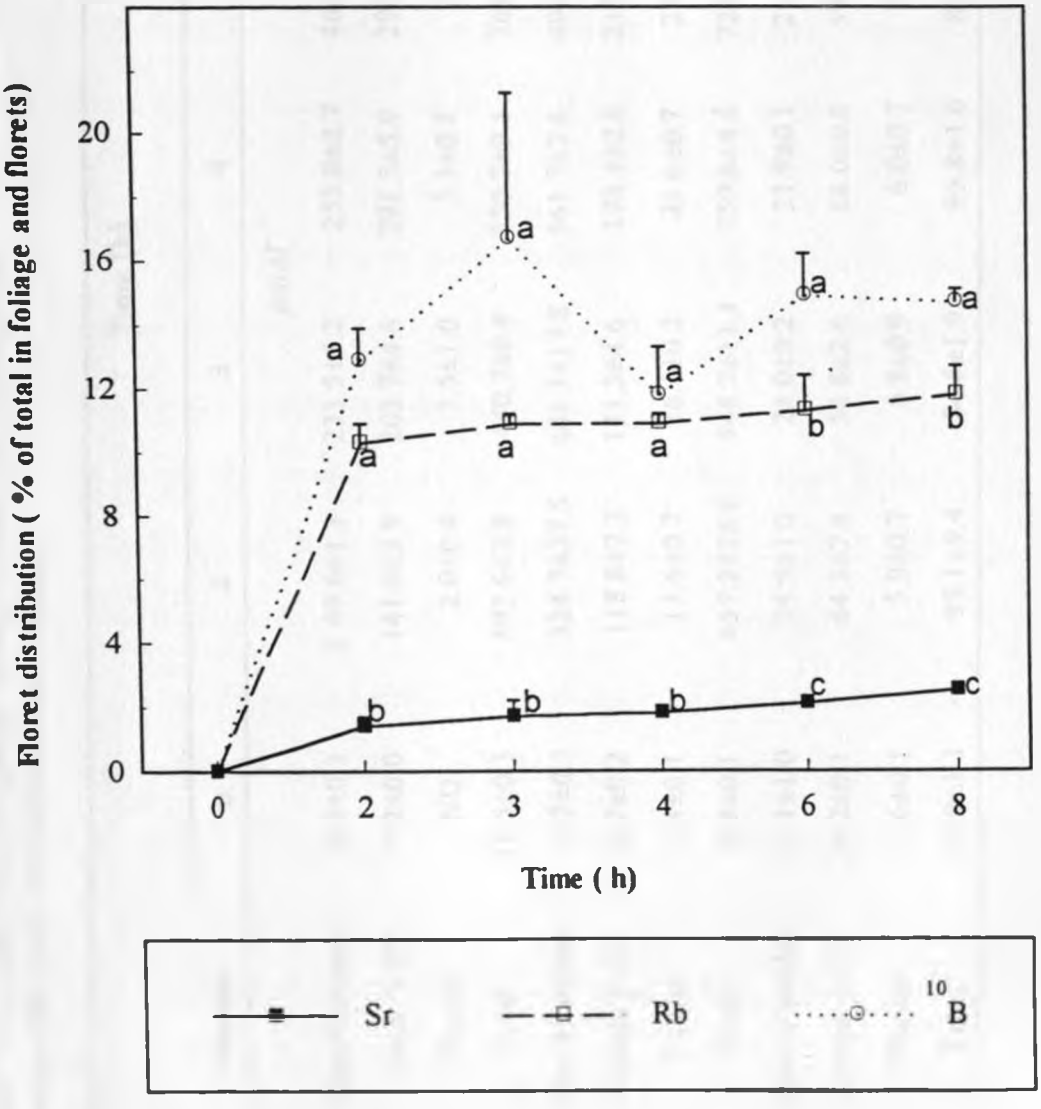

Floret distribution (% of total in foliage and florets)
20
16
12
8
4
0
0
2
3
4
6
8
Time (h)
Sr
Rb
10 B

Table 9. *Strontium, rubidium and ^{10}B contents of "detached" broccoli shoots fed simultaneously with strontium (50 μM), rubidium (50 μM) and enriched ^{10}B (5 μM) for a 8-h period during inflorescence development.*

Data represent mean ±SE (n=3). ND indicates not detected.

Fed Element	Stratum	Time (h) 0	2	3	4	6	8
				μmol			
Strontium	Stem+Petioles	8.3±0.3	2 49.6±1.3	233.5±5.2	253.8±2.7	460.3±2.2	452.5±0.3
	Leaves 5-22	3.2±0.0	141.0±3.9	203.7±4.6	291.5±5.9	296.4±1.2	318.0±5.6
	Florets	ND	2.0±0.4	3.5±1.0	5.3±0.8	6.4±0.6	8.2±0.7
	Total	11.5±0.3	392.6±3.8	440.7±0.4	550.7±9.5	763.1±3.4	778.7±6.6
Rubidium	Stem+Petioles	3.2±0.1	524.7±27.5	401.3±17.8	561.7±7.8	490.2±0.7	526.0±27.4
	Leaves 5-22	2.7±0.2	118.8±2.3	131.3±4.6	176.4±2.6	211.9±1.4	281.9±15.8
	Florets	0.4±0.1	13.6±0.7	16.1±0.2	21.6±0.7	27.0±3.0	37.7±1.3
	Total	6.3±0.1	657.2±26.0	548.7±13.1	759.6±4.4	729.1±3.8	845.6±15.6
^{10}Boron	Stem+Petioles	12.1±1.0	24.9±1.0	24.0±0.2	21.9±0.1	21.8±0.9	24.3±2.7
	Leaves 5-22	28.2±0.1	64.3±7.8	54.8±2.6	68.0±0.8	59.7±1.8	62.2±1.6
	Florets	0.6±0.1	5.9±0.7	5.8±0.9	6.0±0.7	6.1±0.4	6.4±0.1
	Total	40.9±1.1	95.1±9.4	84.6±1.9	95.8±1.6	87.7±2.5	92.9±2.0

4.3. Lupin: ^{10}Boron Enrichment of Translocation Fluids and Comparative Shoot Distribution of Strontium, Rubidium and ^{10}Boron

Lupin plants grown with adequate B (30 μM at natural abundance) had a total dry weight of 7.3 g. The Ca, K, Mg and B concentration of the various strata are given in table 10. The ratios of elemental concentrations in fruits:primary leaves were 0.51 for Ca, 0.61 for K, 0.44 for Mg and 3.2 for B (Table 10). These plants were fed simultaneously with Sr, Rb and enriched ^{10}B (30 μM) in the nutrient solution for a 4-d time course.

In RBS and phloem exudate, the concentration of total B remained steady over the entire period (about 74 and 292 μM, respectively) (Table 11). The concentration of ^{10}B in RBS and phloem exudate increased over the time course by approximately 27 % and 52 %, respectively; however, the ratio of ^{10}B in phloem exudate:RBS (3) was similar to that for total B. Prior to feeding, plants contained 0.32, 0.32 and 2.5 μmol of Sr, Rb and ^{10}B, respectively (Table 12); these elements increased by about 8-, 24- and 1-fold over the 4-d time course. The accumulation of these elements was not linear with time. Once again, the background Sr and Rb contents could be ignored, whereas the relatively high ^{10}B background could not . In the plant, approximately 34-60 % of the Sr, 53-78 of the Rb and 19-31 of the ^{10}B was recovered in the stem+petioles. Therefore, after correction for the mean ^{10}B content at zero time, the contents in fruits (primary inflorescence) of the three fed elements at 1-4 d were expressed as a percent of the total recovered in the inflorescences and foliage. There was an approximately linear accumulation of Sr up to 8.7 % within 3 d period (Fig 5).

Table 10. *Dry weights and calcium, potassium, magnesium and B concentrations of strata from lupin plants grown with adequate B (30 µm).*
Data represent the mean ±SE (n=14).

Stratum	Dry Weight	Calcium	Potassium	Magnesium	Boron
	g		*mol (kg DW)* $^{-1}$		*mmol (kg DW)* $^{-1}$
Primary Stem+Petioles	1.1±0.1	0.15±0.01	0.95±0.04	0.10±0.01	0.9±0.0
Primary Leaves	0.3±0.0	0.29±0.00	0.70±0.03	0.47±0.07	0.5±0.1
Primary Inflorescence (Fruits)	1.0±0.4	0.15±0.01	0.43±0.07	0.21±0.02	1.6±0.2
Secondary Stem+Petioles	1.3±0.0	0.19±0.01	1.78±0.07	0.18±0.02	1.7±0.2
Secondary Leaves	3.3±0.1	0.41±0.03	1.17±0.03	0.35±0.05	4.1±0.1
Secondary Inflorescence	0.3±0.1	0.15±0.01	0.62±0.03	0.21±0.02	0.4±0.0
Total	7.3				

Table 11. *Total B and ^{10}B concentrations of root bleeding sap and phloem exudate from lupin plants grown with adequate B (30 μM at natural abundance), then fed simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B for a 4-day period during reproductive development.*

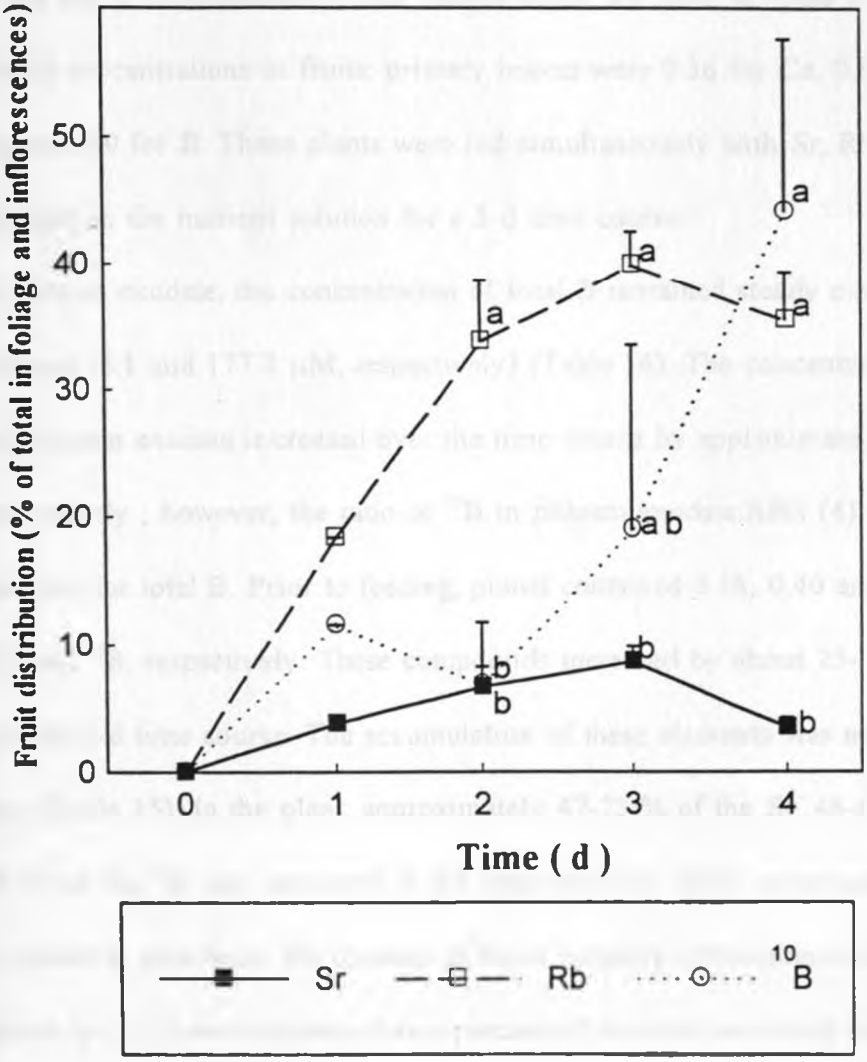
Data represent mean ±SE (n=3); phloem exudate was collected from fruit tips.

	Root Bleeding Sap		Phloem Exudate	
Time	Total B	^{10}B	Total B	^{10}B
Day		μM		
0	67.5±4.5	21.8±1.5	241±11	52.5±2.7
1	69.9±7.1	21.9±1.5	283±17	65.8±2.5
2	83.2±5.3	28.3±1.7	338±19	79.3±3.1
3	74.5±4.6	26.4±1.6	321±29	83.8±5.9
4	75.0±3.3	27.6±1.6	279±24	79.8±6.2

Table 12. *Strontium, rubidium and ^{10}B contents of lupin shoots grown with adequate B (30 μM), then fed simultaneously with strontium (0.5 mM), rubidium (0.5mM) and enriched ^{10}B for a 4-d period during reproductive development.*
Data represent mean± SE (n=1-4).

Fed Element	Stratum	Time (days) 0	1	2	3	4
				μmol		
Strontium	Stem+Petioles	0.16±0.01	1.02	1.13±0.19	0.55±0.08	1.24±0.04
	Leaves+Secondary Inflorescence	0.12±0.02	1.02	0.70±0.03	1.00±0.13	1.11±0.02
	Primary Inflorescence (Fruits)	0.04±0.01	0.04	0.05±0.01	0.09±0.01	0.08±0.04
	Total	0.32±0.01	2.08	1.89±0.21	1.64±0.06	2.42±0.06
Rubidium	Stem+Petioles	0.17±0.01	1.18	1.95±0.04	3.82±0.80	5.55±0.26
	Leaves+Secondary Inflorescence	0.11±0.01	0.65	0.88±0.21	0.65±0.05	1.30±0.09
	Primary Infloresence (Fruits)	0.05±0.0	0.15	0.44±0.01	0.42±0.02	0.69±0.07
	Total	0.32±0.01	1.97	3.27±0.27	4.90±0.84	7.54±0.36
^{10}Boron	Stem+Petioles	0.76±0.15	0.89	1.02±0.07	0.71±0.07	0.73±0.11
	Leaves+Secondary Inflorescence	1.74±0.12	2.27	2.06±0.16	2.70±0.17	1.91±0.18
	Primary Inflorescence (Fruits)	0.25±0.04	0.32	0.27±0.05	0.45±0.20	0.59±0.37
	Total	2.75±0.09	3.48	3.34±0.09	3.81±0.29	3.23±0.09

FIGURE 5 . Percent distribution of strontium, rubidium and ^{10}B in the fruits of intact lupin shoots supplied simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B (30 μM) over a 4-day time course. Plants were grown with natural abundance B at an adequate level (30 μM). Data represent mean ± SE (n=1-4); where SE is not evident (1-4 days) it is within the symbols. Key indicates the fed elements. Means followed by the same letter are not significantly different.


Fruit distribution (% of total in foliage and inflorescences)
50
40
30
20
10
0
0
1
2
3
4
Time (d)
a
a
a
a b
b
b
b
b
Sr
Rb
10 B

The increase in Rb was much more rapid than for Sr, reaching 18 % within 1 d, and a maximum of 40 % after 3 d. Initially, there was a lag in percent ^{10}B in fruits, but after 2 d this was followed by a more rapid increase up to 44 % at 4 d. A second experiment with plants grown with inadequate B (3 μM) had a total dry weight of 7.8 g. The Ca, K, Mg and B concentration of the various strata are given in table 13. The ratios of elemental concentrations in fruits: primary leaves were 0.36 for Ca, 0.67 for K, 0.71 for Mg and 5.0 for B. These plants were fed simultaneously with Sr, Rb and enriched ^{10}B (3 μM) in the nutrient solution for a 3-d time course.

In RBS and phloem exudate, the concentration of total B remained steady over the entire period (about 38.1 and 177.2 μM, respectively) (Table 14). The concentration of ^{10}B in RBS and phloem exudate increased over the time course by approximately 50 % and 107 %, respectively ; however, the ratio of ^{10}B in phloem exudate:RBS (4) was about similar to that for total B. Prior to feeding, plants contained 0.38, 0.40 and 3.2 μmol of Sr, Rb and ^{10}B, respectively. These compounds increased by about 25-, 71- and 1-fold over the 3-d time course. The accumulation of these elements was not linear with time (Table 15). In the plant, approximately 47-73 % of the Sr, 48-68 of the Rb and 24-56 of the ^{10}B was recovered in the stem+petioles. After correction for the mean ^{10}B content at zero time, the contents in fruits (primary inflorescence) of the three fed elements at 1-3 d were expressed as a percent of the total recovered in the inflorescences and foliage. There was an approximately linear accumulation of Sr up to 6 % within 3 d period (Fig 6). The increase in Rb was much more rapid than for Sr, reaching 17 % within 1 d, and of 34 % after 3 d. The percent ^{10}B in fruits also

increased rapidly up to 32 % ,but remained relatively constant thereafter.

Table 13. *Dry weights and calcium, potassium, magnesium and B concentrations of strata from lupin plants grown with deficient B (3 μM).*

Data represent the mean ±SE (n=12).

Stratum	Dry Weight	Calcium	Potassium	Magnesium	Boron
	g	*mol (kg DW)* $^{-1}$			*mmol (kg DW)* $^{-1}$
Primary Stem+Petioles	1.8±0.2	0.08±0.00	1.00±0.05	0.06±0.001	1.2±0.0
Primary Leaves	0.3±0.1	0.33±0.01	0.87±0.04	0.21±0.01	0.3±0.1
Primary Inflorescence (Fruits)	1.1±0.6	0.12±0.00	0.58±0.03	0.15±0.01	1.5±0.7
Secondary Stem+Petioles	1.4±0.3	0.16±0.01	1.76±0.05	0.12±0.01	2.3±0.2
Secondary Leaves	2.9±0.9	0.27±0.02	0.98±0.04	0.26±0.02	4.1±0.5
Secondary Inflorescence	0.3±0.1	0.13±0.01	0.75±0.03	0.19±0.02	0.2±0.0
Total	7.8				

Table 14. *Total B and ^{10}B concentrations of root bleeding sap and phloem exudate from lupin plants grown with deficient B (3 μM at natural abundance), then fed simultaneously with Sr (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B for a 3-day period during reproductive development.*

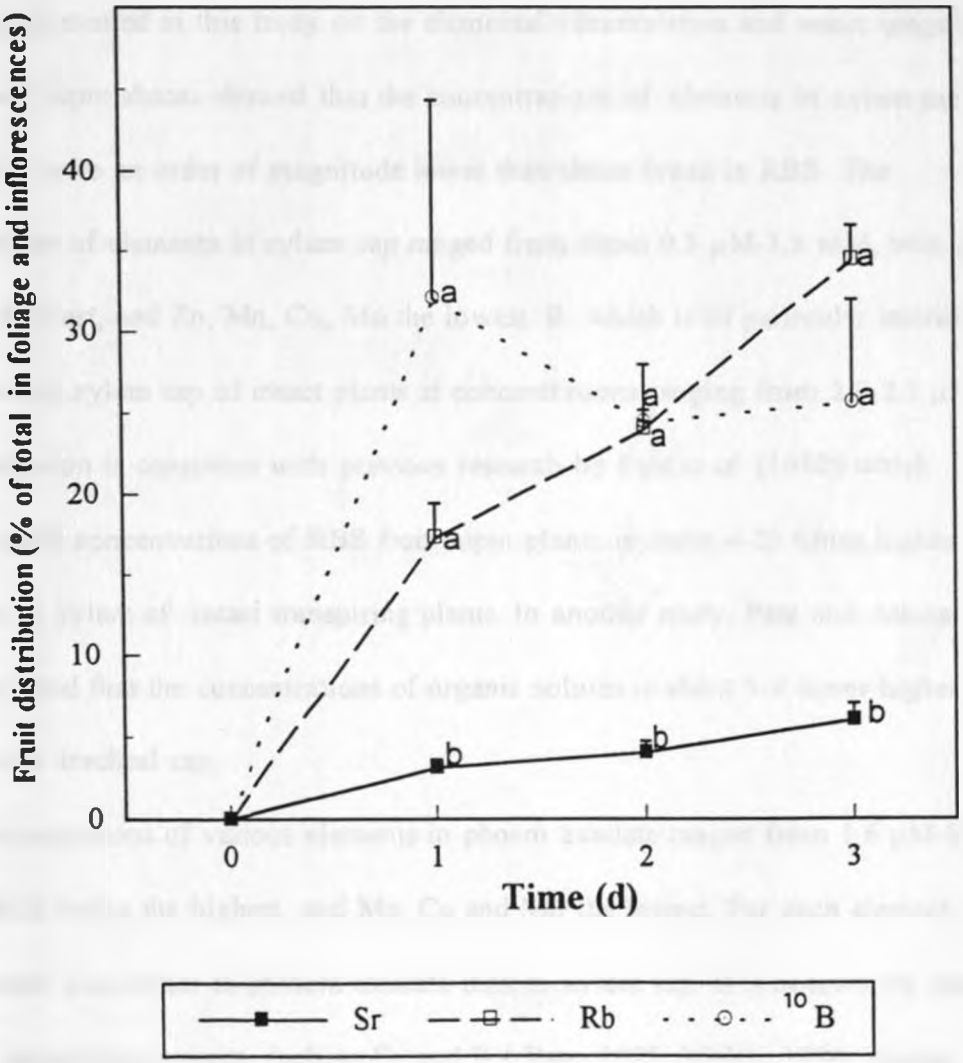
Data represent mean ±SE (n=3); phloem exudate was collected from fruit tips.

	Root Bleeding Sap		Phloem Exudate	
Time	Total B	^{10}B	Total B	^{10}B
d		μM		
0	31.7±0.6	9.9±0.1	104±26	28.2±6.0
1	33.5±1.5	10.5±0.4	204±3	49.6±0.4
2	39.3±2.0	14.3±1.3	169±2	45.0±1.8
3	43.5±3.7	15.0±1.0	210±10	58.2±1.9

Table 15. *Strontium, rubidium and ^{10}B contents of lupin shoots grown with deficient B (3 μM), then fed simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B for a 3-d period during reproductive development.*
Data represent mean ±SE (n=3).

Fed Element	Stratum	Time (days) 0	1	2	3
		μmol			
Strontium	Stem+Petioles	0.18±0.02	1.41±0.06	5.95±0.22	6.86±0.22
	Leaves+Secondary Inflorescence	0.16±0.04	0.99±0.06	2.42±0.07	2.44±0.22
	Primary Inflorescence (Fruits)	0.03±0.00	0.03±0.01	0.10±0.02	0.15±0.03
	Total	0.38±0.04	2.93±0.11	8.47±0.36	9.45±0.45
Rubidium	Stem+Petioles	0.19±0.04	2.74±0.09	9.99±0.84	19.1±2.37
	Leaves+Secondary Inflorescence	0.17±0.05	1.42±0.10	5.02±0.55	6.05±0.81
	Primary Inflorescence(Fruits)	0.04±0.01	0.29±0.02	1.59±0.20	3.12±0.22
	Total	0.40±0.09	4.45±0.15	16.6±1.23	28.3±2.08
^{10}Boron	Stem+Petioles	1.78±0.83	0.84±0.08	1.70±0.02	1.06±0.03
	Leaves+Secondary Inflorescence	0.96±0.13	1.35±0.12	2.29±0.07	2.67±0.40
	Primary Inflorescence (Fruits)	0.06±0.02	0.21±0.05	0.49±0.03	0.74±0.27
	Total	3.24±1.5	2.40±0.06	4.48±0.13	4.47±0.64

FIGURE 6. Percent distribution of strontium, rubidium and ^{10}B in the fruits of intact lupin shoots supplied simultaneously with strontium (0.5 mM), rubidium (0.5 mM) and enriched ^{10}B (3 μM) over a 3-day time course. Plants were grown with B at a deficient level (3 μM). Data represent mean ±SE (n=3);where the SE is not evident (1-3 d) it is within the symbol. Key indicates the fed elements. Means followed by the same letter are not significantly different.


Fruit distribution (% of total in foliage and inflorescences)
40
30
20
10
0
0
1
2
3
Time (d)
a
a
a
a
a
a
b
b
b
b
Sr
Rb
10 B

CHAPTER FIVE

DISCUSSION

The data presented in this study on the elemental accumulation and water usage by broccoli and lupin shoots showed that the concentrations of elements in xylem sap of intact plants were an order of magnitude lower than those found in RBS. The concentrations of elements in xylem sap ranged from about 0.3 μM-3.5 mM, with K being the highest, and Zn, Mo, Cu, Mn the lowest. B, which is of particular interest, was present in xylem sap of intact plants at concentrations ranging from 2.9-3.5 μM. This information is consistent with previous research by Pate *et al.* (1980) which showed that N concentrations of RBS from lupin plants is about 4-29 times higher than those in xylem of intact transpiring plants. In another study, Pate and Atkins (1983) reported that the concentrations of organic solutes is about 5-8 times higher in RBS than in tracheal sap.

The concentrations of various elements in phoem exudate ranged from 1.6 μM-90.8 mM, with K being the highest, and Mo, Cu and Mn the lowest. For each element, the concentration was higher in phloem exudate than in xylem sap. It is noteworthy that so-called immobile elements, such as Ca and B (Pate, 1975; Welch, 1986; Raven, 1980) also showed this trend, which is consistent with the hypothesis that they are at least to some degree, phloem mobile. Earlier, Penot *et al.* (1976) reported the phloem movement of ^{45}Ca applied to the leaf of various higher plants. Marentes and Shelp (1994a) have also provided support for the retranslocation of B to phloem-fed tissues

of broccoli and lupin plants, but the rapidity of the xylem-to-phloem processes is not known.

In this study, B retranslocation was assessed by comparing the percent distribution of xylem-borne ^{10}B that was recovered in the florets and fruits of broccoli and lupin plants, respectively, to that of Sr (xylem marker) and Rb (xylem / phloem marker). Regardless whether these compounds were fed to intact or detached transpiring shoots, a much greater percent Rb than Sr was recovered in broccoli florets within 2-3 h and lupin fruits within 1 d indicating that these developing sinks were primarily fed with nutrients via the phloem. Similar studies with wheat plants showed that Rb is rapidly retranslocated to the ear through the phloem, whereas Sr accumulates in the flag leaf, the region of application (Kuppelwieser and Feller, 1990). Other studies with tomato and soybean plants have used inulin (xylem marker) and aminoisobutyric acid (xylem/ phloem marker) to determine rapid xylem-to-phloem transfer processes that are sensitive to heat girdling and light intensity (Van Bel, 1984; Da Silva and Shelp, 1989; Shelp and Da Silva, 1990).

The percent ^{10}B recovered in broccoli florets was similar to rubidium, indicating therefore that xylem-to-phloem transfer of ^{10}B occurred rapidly in plants supplied with adequate B supply. However, in lupin plants ^{10}B was more rapidly transfered from xylem to phloem in plants that were supplied with deficient B. Previously, Marentes *et al.*, (1994a) also reported that B retranslocation in broccoli is dependent upon the B status of the plants and the B supply. Given the rapidity of ^{10}B retranslocation observed in the present study, it is tempting to propose that direct xylem-to-phloem

transfer is involved. The use of girdling techniques to interrupt phloem may help to distinguish between direct and indirect xylem-to-phloem transfer.

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