

Chapter 5

General Discussion

5.1 Criteria for determining B mobility

Evidence of B mobility can be found in the distribution of B within leaves, such as in the study on walnut and apple by Brown and Shelp (1997), or within different organs, e.g. the report in apple of Van Goor and Van Lune (1980). The concentration of B in leaves of different age can also give some evidence of B mobility. The occurrence of higher B concentrations in old or mature leaves in comparison to younger leaves is evidence of B immobility while a higher B concentration in younger leaves is an indication of B mobility (Brown and Shelp, 1997). By using this criterion, the preliminary survey for B retranslocation in field-grown crops (Chapter 2) concluded the possibility of B retranslocation taking place in some tropical plants. However, the B concentration gradient was inconclusive in some species. One of the factors that can mask the B gradient is the difference in rate of xylem delivery of B into leaves formed in different seasons due to differential availability of B in soil caused by seasonal variation in soil moisture (Shelp *et al.*, 1998). To overcome difficulties from either alteration in the supply of B to plants or from the dilution effect caused by an increase in leaf biomass after the leaf is fully expanded, an experiment was conducted (Chapter 3) where the B contents of tagged leaves were followed over time to determine if B continued to accumulate or declined

with leaf age. Good evidence for B retranslocation was provided in coffee, guava and jackfruit, which confirmed the tentative findings in Chapter 2.

Marentes *et al.* (1997) suggested that determining the net changes in contents in various plant parts over a time course in plants with adequate nutrient supply in order to study the mobilization of nutrient elements, may provide complicated data due to the simultaneous import and export of nutrients. The occurrence of B retranslocation in both broccoli and lupin was not dependent on the induction of B deficiency, either by growing in media that was continuously B deficient or B being withdrawn from the nutrient supply (Shelp *et al.*, 1995; Marentes *et al.*, 1997). In the current study (Experiment 3.3 of Chapter 3 and Experiment 4.1 of Chapter 4), the B contents were much higher in B-adequate peanut and green gram. This makes it easy to investigate nutrient mobilization after B deficiency is induced in these plants.

It is worth nothing that the detection of B in younger tissues after withdrawal of B does not always signify that retranslocation has taken place because some plants, e.g. sunflower (Matoh and Ochiai, 2005), contain a significant amount of water-soluble B in the stem and roots. This old B may have been taken up earlier and stored in a water-soluble form adjacent to the xylem transport cells and then reloaded into the xylem fluid for long distance transport to young organs. Tracer experiments, using stable isotopes of B (^{10}B and ^{11}B), have proved useful for providing evidence of B mobility in plants since the inductively coupled plasma mass spectrometry (ICP-MS) was introduced (Thellier *et al.*, 1979). Using this technique, retranslocation of B has been reported in a number of species including woody plants and annual field crop species (Hanson, 1991; Brown *et al.*, 1992; Brown and Hu, 1996; Marentes *et al.*, 1997; Lehto *et al.*, 2000; Lehto *et al.*, 2004a; Lehto *et al.*, 2004b). This thesis

verified B retranslocation in peanut by using ^{10}B as a tracer (Experiment 4.2). Both foliar and root-applied tracer experiments provided evidence of B retranslocation within peanut plants regardless of the plant B status. Tracer experiments may not always show the quantitative amount of B retranslocation for particular plants (Lehto *et al.*, 2004b). In peanut (Experiment 2.2 of Chapter 4), the tracer experiment indicated that some B was being retranslocated, but the actual amount was probably small compared to the total B content of the plant. However, even small amounts of B retranslocation may be critical for reproductive development in situations where the external B supply may be interrupted or reduced.

5.2 Variation in B mobility among plant species

The mobility of B in plant shoots can differ from species to species (McIlrath, 1965). Based on the results of this thesis, the variation in B mobility among tropical plant species is summarized in Table 5.1. Most tropical crop species examined in this research did not show any B retranslocation while the occurrence of B retranslocation was confirmed in only a few species. Variation in B mobility among plant species has also been reported in the literature over recent years (Table 5.2) with direct and indirect evidence.

One mechanism of B retranslocation in plants was proposed to be associated with sugar alcohols or polyols, such as sorbitol in almond, apple and nectarine (Brown and Hu, 1996), olive (Perica *et al.*, 2001) and some species of deciduous forest trees (Lehto *et al.*, 2004b). Sugar alcohols can form stable complexes with B and this formation may facilitate B mobilization by preventing its complexation to insoluble compounds or may alter the membrane permeability of B (Brown and Hu,

1996; Hu *et al.*, 1997). Variation in B mobility among plant species may result from the occurrence of different polyols or their absence in each plant species. Moing (2000) summarized the distribution of sugar alcohols in some orders of higher plants, such as, galactitol in the Celastrales, Laurales and Saxifragales; galactitol and mannitol in the Scrophulariales (family Oleaceae); mannitol in the Euphorbiales, Myrtales, Oleales, Santalales and Umbellales (family Apiaceae); and sorbitol in the Plantaginales (family Plantaginaceae) and Rosales (family Rosaceae). Sugar alcohol distribution also varies among plant orders or families. Plouvier (1963, cited by Loescher and Everard, 1996) suggested that sorbitol was found in the subfamilies Pomoideae (e.g. apple and pear) and Prunoideae (e.g. cherry, peach and plum) of the family Rosaceae but was absent in the Rosoideae (e.g. rose). According to this review, some plants in the present study are members of groups which contain genera with sugar alcohols, including, cassava (order Euphorbiales) and guava (order Myrtales). However, Lehto *et al.* (2004b) found that the presence of polyols was not related to B mobility in some deciduous tree species. Stangoulis *et al.* (2001) also reported that there were no borate-complexing substances in phloem fluids of canola which is able to translocate B between leaves. Therefore, sugar alcohols are not always a prerequisite for B transport in plants (Matoh and Ochiai, 2005). More recently, a B transporter protein for xylem loading was identified in higher plants by Takano *et al.* (2002). Since then it has been proposed that this B transporter may be involved in the distribution and retranslocation of B in plants (Matoh and Ochiai, 2005).

Table 5.1 Variation of B mobility among plant species based on results of this thesis.

Phloem B mobility	Plant species	Evidence
Immobile	Cashew (<i>Anacardium occidentale</i>) Mango (<i>Mangifera indica</i>) Custard apple (<i>Annona squamosa</i>) Cassava (<i>Manihot esculenta</i>) Indian walnut (<i>Samanea saman</i>) Cork wood tree (<i>Sesbania grandiflora</i>) Passion fruit (<i>Passiflora edulis</i>) Lime (<i>Citrus aurantifolia</i>) Lychee (<i>Lychi chinensis</i>)	Boron concentration gradient between old and young leaves followed the same trend as Ca (phloem immobile element) (Chapter 2)
Immobile	Lab lab (<i>Dolichos lablab</i>) Soybean (<i>Glycine max</i>) Garden pea (<i>Pisum sativum</i>) Yam bean (<i>Pachyrizus tuberosus</i>) Rice (<i>Oriza sativa</i>) Wheat (<i>Triticum aestivum</i> cv. Bonza) Barley (<i>Hordeum vulgare</i>)	Boron content in old leaves increased over time after withdrawal of B (Experiment 3.2 of Chapter 3)
Mobile	Durian (<i>Durio zibethinus</i>) Mangosteen (<i>Garcinia mangostana</i>) Tamarind (<i>Tamarindus indica</i>) Guava (<i>Psidium guajava</i>) Teak (<i>Tectona grandis</i>)	Boron concentration gradient between old and young leaves followed the same trend as K (phloem mobile element) (Chapter 2)
Mobile	Coffee (<i>Coffea arabica</i>) Guava (<i>Psidium guajava</i>) Jackfruit (<i>Artocarpus heterophyllus</i>)	- Boron content of foliar-treated leaves declined over time - Boron content in young leaves increased over time compared to untreated trees (Experiment 3.1 of Chapter 3)

Table 5.1 (Continued)

Phloem B mobility	Plant species	Evidences
Mobile	Black gram (<i>Vigna mungo</i>) Wheat (<i>Triticum aestivum</i> cv. Fang 60) Maize (<i>Zea mays</i>)	Boron content in older leaves was reduced after withdrawal of B (Experiment 3.2 of Chapter 3)
Mobile	Green gram (<i>Vigna radiata</i>) Peanut (<i>Arachis hypogaea</i>)	The higher B content in young tissues (YFEL, reproductive organs) developed after withdrawal of B compared with B-deficient plants (Experiment 3.3 of Chapter 3)
Mobile	Peanut (<i>Arachis hypogaea</i>)	<u>^{10}B applied to leaves</u> - Foliar-treated ^{10}B moved out from treated leaves to other parts <u>^{10}B applied to roots</u> - ^{10}B content and abundance decreased in older parts and there was ^{10}B in new growth (Experiment 4.2 of Chapter 4)
Mobile	Peanut (<i>Arachis hypogaea</i> cv. Tainan 9 and TAG 24)	- Boron content in foliar-treated leaves increased at the end of foliar treatment - Boron content in new organs was higher than in untreated plants (Experiment 4.3 of Chapter 4)

Table 5.2 Variation of B mobility among plant species from the literatures.

Phloem B mobility	Plant species	Evidence
Immobile	Tomato (<i>Lycopersicon esculentum</i>)	<p><u>Excessive B supply</u></p> <ul style="list-style-type: none"> – The first symptom of B toxicity appeared at margin and tip of leaf <p><u>Deficient B supply</u></p> <ul style="list-style-type: none"> – Boron deficiency symptoms developed in younger and immature leaves (Oertli, 1993)
Immobile	Squash (<i>Cucurbita</i> sp.)	<p>Boron deficiency symptoms occurred rapidly after withdrawal of B (Hu and Brown, 1994)</p>
Immobile	Fig (<i>Ficus carica</i>) Pistachio (<i>Pistacia vera</i>) Walnut (<i>Juglans regia</i>)	<p><u>Foliar-applied enriched ^{10}B</u></p> <ul style="list-style-type: none"> - No significant translocation of foliar-applied ^{10}B - Boron concentration in older leaves was higher than in young leaves and the lowest B concentration was in fruit tissue (Brown and Hu, 1996)
Immobile	<i>Alnus glutinosa</i>	<p><u>Application of ^{10}B to mature leaves</u></p> <ul style="list-style-type: none"> – ^{10}B abundance did not increase in new leaves (Lehto <i>et al.</i>, 2004b)

Table 5.2 (Continued)

Phloem B mobility	Plant species	Evidences
Mobile	Peanut (<i>Arachis hypogaea</i>) Subterranean clover (<i>Trifolium subterraneum</i>)	Unchanging B concentration in fruits when B was supplied to the roots but omitted from the fruiting medium (Campbell <i>et al.</i> , 1975)
Mobile	Apple (<i>Malus domestica</i>) Pear (<i>Pyrus communis</i>) Plum (<i>Prunus domestica</i>) Cherry (<i>Prunus cerasus</i>)	- After foliar B spray, B content in treated leaves similar to non-treated leaves and the highest B concentration found in buds - Polyol-B complexes in phloem sap (Hanson, 1991)
Mobile	Olive (<i>Olea europaea</i>)	Boron concentration increased in leaf blades, petioles, bark of bearing shoot, flowers and fruits after application of B to leaves at anthesis (Delgado <i>et al.</i> , 1994)
Mobile	Almond (<i>Prunus amygdalus</i> syn. <i>P. Dulcis</i>) Apple (<i>Malus domestica</i>) Nectarine (<i>Prunus persica</i> var. <i>nectarina</i>)	<u>Foliar-applied enriched ^{10}B</u> - ^{10}B transported from treated leaves to adjacent fruit - Slight difference between old and young leaves - Fruit tissue had greater B concentrations than leaves (Brown and Hu, 1996)

Table 5.2 (Continued)

Phloem B mobility	Plant species	Evidences
Mobile	Celery (<i>Apium graveolens</i>) Peach (<i>Prunus persica</i>)	Polyol-B complexes in phloem sap (Hu <i>et al.</i> , 1997)
Mobile	Broccoli (<i>Brassica oleracea</i>) Lupin (<i>Lupinus albus</i>)	<u>Tracer-¹⁰B to root system</u> - ¹⁰ B enriched recovery in broccoli florets and lupin fruits - The higher B concentration in phloem exudates than in xylem sap (Marentes <i>et al.</i> , 1997)
Mobile	Scot pine (<i>Pinus sylvestris</i>) Norway spruce (<i>Picea abies</i>)	<u>Applying ¹⁰B to shoots</u> - Increased ¹⁰ B amount and proportion of ¹⁰ B in root (Lehto <i>et al.</i> , 2000)
Mobile	<i>Alnus incana</i> , <i>Betula pubescens</i> , <i>Fraxinus excelsior</i> , <i>Larix sibirica</i> , <i>Prunus padus</i> , <i>Sorbus aucuparia</i> , <i>Ulmus glabra</i>	<u>¹⁰B application to mature leaves</u> - ¹⁰ B translocated into new leaves (Lehto <i>et al.</i> , 2004b)

Boron retranslocation was found to differ among plant cultivars, for example in rutabaga (Shelp and Shattuck 1987a), broccoli (Shelp *et al.*, 1992), wheat (Subedi *et al.*, 2001) and canola (Stangoulis, 1998). The retranslocation of B may relate to the tolerance to B deficiency in wheat (Subedi *et al.*, 2001). In the present study there was a difference between wheat cultivars, B was retranslocated in Fang 60 (B-efficient) while it was not retranslocated in Bonza (B-inefficient). However,

Stangoulis *et al.* (2001) reported that variation in B retranslocation in canola did not relate to their B-efficiency rating. They applied ^{10}B to mature leaves of canola and observed the retranslocation of B to younger leaves of one efficient cultivar (Huashuang-2) and absence of retranslocation in the other two cultivars [Dunkeld (efficient), Barossa (inefficient)]. Accordingly, Brown and Hu (1998) explained that differences in sensitivity to B deficiency may be observed between cultivars in some species which may be involved in the differences of B mobility among cultivars.

5.3 Implications of B mobility for diagnosis of the B status of plants and for fertilizer management

The B status of a plant can be examined by leaf diagnosis which depends on knowledge of the distribution of B in the species of interest (Brown and Hu, 1996). In species with high phloem B mobility, analysis of B concentration in the first mature leaf or leaf pair might be satisfactory for considering the B status of the plant. By contrast, it would be more reliable to consider the concentration in young or immature organs for phloem immobile species (Huang *et al.*, 1996; Brown and Shelp, 1997).

Fertilizer management is also affected by the pattern of B mobility in plants. Foliar application is effective for species in which B is phloem-mobile at any time (Brown and Hu, 1996). For example, foliar B can be translocated to flower buds and enhance flower bud B concentrations and fruit set of almond, cherry and prune, phloem-B mobile species, in the year following application of B (Hanson, 1991). By contrast, foliar B application to phloem-B immobile species, e.g. pistachio, fig and walnut (Brown *et al.*, 1994; Brown and Hu, 1996), was effective only when applied directly to the requiring tissues. The very high B-fixing capacity in cells in these

species (Brown and Hu, 1996) makes foliar-applied and naturally obtained B unavailable for re-translocation from older to younger, growing tissues. In the detailed study in peanut (Chapter 4) it was found that tracer B (^{10}B) applied to roots during early vegetative growth or foliar application (Experiment 4.2, Chapter 4) could be retranslocated to new growth developed later, especially the reproductive organs. Furthermore, in Experiment 4.1 of Chapter 4, peanut plants grown with adequate B supply and then subjected to withdrawal of B (BA/BD) had reduced percentage of hollow heart seed compared to peanut grown with B-deficient supply throughout (BD/BD).

Peanut yield has often been found to respond to foliar B application. For example, on a low B Sansai soil in Chiang Mai Valley, peanut kernel yield was increased 37% by a foliar B application at the rate of 0.3 kg B/ha and 59% at 0.6 kg B/ha (Hobbs, 1974). Based on such empirical results, foliar application of B has long been recommended as superior to broadcast application in spite of cautions about B immobility (Marten and Westermann, 1991). The recommendation in the USA is to apply foliar B to peanut during early bloom (Hill and Morrill, 1974). Results from the present study provide definitive evidence to support these field-based recommendations, and also the suggestion made by Campbell *et al.* (1975) that B can be retranslocated from older to younger tissues in peanut.

5.4 General conclusions

Evidence of B retranslocation and its variation among tropical crop species was found in this thesis based on various criteria. A short study in field-grown plants by using B concentration in different leaf ages compared with concentration of a phloem mobile element (K) and a phloem immobile element (Ca) suggested the possibility of B retranslocation in some species of tropical trees. Controlled experiments with both foliar application and withdrawal treatments provided more reliable evidence of the possibility of B retranslocation in tropical trees and field crop species by using the changes of B content in treated and non-treated leaves (foliar experiment) or the B content in tagged leaves before and after withdrawal of B. These findings can be used to further refine the diagnosis of B deficiency in the plant species examined and fertilizer management in crop production. The simple procedures used in the early phase of the research were effective in identifying a short-list of species with possible phloem B mobility. However, the use of ^{10}B tracing allows the confirmation of B retranslocation in species of interest, such as in peanut in this thesis.

5.5 Implications for further research

Further research on B mobility is necessary, especially for economically and ecologically important tropical crop species. Since genotypic variation in B mobility has been demonstrated, it should be further study on genotypic variations within species is also required because B mobility may be associated with their sensitivity to B deficiency (Brown and Hu, 1998).

Although it has been suggested that sugar alcohols are associated with the retranslocation of B within plants, in some species, for example canola (Stangoulis *et al.*, 2001), rice (Bellaloui *et al.*, 2003) and some forest trees (Lehto *et al.*, 2004b), there appears to be no relationship between B mobility and the occurrence of sugar alcohols. Recently, Matoh and Ochiai (2005) suggested that a B transporter may be implicated in B retranslocation in plants. Therefore, the mechanisms of B mobility in tropical crop species should be further examined by exploring the presence of B-polyol complexes and B transporters.