Redefining Boron Toxicity Symptoms in Some Ornamentals

Patrick H. Brown, Hening Hu and Warren G. Roberts

The phloem mobility of boron (B) in plants varies dramatically between species, variations in phloem B mobility occur as a consequence of the presence of polyols in some species but not others. Since phloem B mobility profoundly affects the expression of B toxicity symptoms it is important to determine the relative phloem mobility in a range of plant species and to determine how this impacts upon the expression of B toxicity. Here, twenty four common ornamental species believed to produce polyols and several that do not produce polyols were selected to test their response to boron (B) toxicity. Species that do not produce polyols exhibited 'classic' B toxicity symptoms that include accumulation of high concentrations of B in, and burning of, the tip and edge of old leaves. In the polyol-producing species these symptoms were absent and B toxicity was expressed as meristematic die-back and an accumulation of B in apical tissues. These symptoms have not historically been associated with B toxicity and hence may have been frequently misdiagnosed.

The typical pattern of B accumulation in plants is for a higher concentration of B to be found in oldest leaves with progressively lower B concentrations in younger tissues and fruits. This distribution of B is typical of an element with limited phloem mobility and results in the development of leaf tip and edge burn of the oldest leaves as a consequence of the high B concentrations in these tissues. Eaton et al. (1941) reported, however, that the leaf symptoms of B toxicity, so characteristic in many plants, are frequently absent in stone fruit trees. In support of these observations, others have also demonstrated that species of *Malus*, *Prunus* and *Pyrus* do not accumulate high levels of B in their leaves, and that B toxicity is expressed as twig die-back and gum exudation in leaf axils and buds (Hansen, 1948; 1955; Woodbridge, 1955; Maas, 1984; Choe et al., 1986; El-Motaium et al., 1994). These 'unusual' symptoms of B toxicity are the result of the high phloem mobility of B in these species (Brown and Hu, 1996), which occurs as a result of the formation and phloem transport of a B-sugar alcohol complex (Brown and Hu, 1996; Hu et al., 1997). Since sugar alcohols such as sorbitol have a very high affinity to bind B (Makkee et al., 1985), and as they represent primary products of photosynthesis we predict that the B-sugar alcohol complex will be readily transported to active sinks such as apical tissues (Brown and Hu, 1996; Hu et al., 1997). As a result, B will be accumulated in the meristematic regions or fruit but not in the mature leaves, of species in which sugar alcohols are resent. In these species, B toxicity will likely appear in apical and neristematic tissues, while the 'typical' symptoms of B toxicity such as leaf tip/edge burn will not be observed.-

Whereas the biochemical basis of B mobiliy in certian species has now been well described, it is not possible from this information alone to predict how B toxicity will be expressed in any particular species. The primary constraint that limits our ability to predict the symptoms of B toxicity is inadequate information on the occurrence of sugar alcohols in plant species and an unfamilarity with the symptoms of B toxicity in species with significant phloem B mobility. The purpose of this experiment was to determine the relationship between sugar alcohol production and to describe the differential expression of B toxicity as it relates to relative phloem mobility of B.

Materials and Methods

Species commonly used in the ornamental industry were selected based upon the known sugar-alcohol production or from a family where some species of that family have been reported to produce sugar alcohols (Bourne, 1958; Plouvier, 1963; Zimmermann and Ziegler, 1975; Wallaart, 1980; Bieleski, 1982). Twenty-two species were purchased from local nurseries in spring 1997. The species used were *Angelica gigas*, *Coprosma kirkii*, *Cotoneaster demmeri* 'Lowfast', *Digitalis purpurea* 'Alba', *Eryngiurn*, *Forsythia* 'lynwood gold', *Fragaria* 'selva', *Galium odoratum*, *Gardenia jasminoides* 'Veitchii', *Liberty mix* (snapdragon), *Ligustrum* 'texanum', *Maytens boaria*, *Olea skylark*, *Osmanthus heterophyllus*, *Potentilla alba* 'snow white', *Punica granatum* 'Nana' dwarf pomegranate, *Pyracantha fortuneana* 'Graberi', *Raphiolepis pinkie* (Dwarf pink india hawtorn), *Spiraea prunifolia*, *Star jasmine*, *Syringa laveuder* forest kresper smith, *Veronic austriaca* teucrium 'Greater lake blue'. The majority of the woody species used in this experiment was one year old seedlings. In addition, a species in which sugar alcohols are absent *Lycopersicon esculentum* (Oertli, 1994) was included as a control.

Boron toxicity symptoms in species with phloem B mobility (Ligustrum). Toxicity is localized on young leaves and stems. Stem symptoms include die back of shoot tips and, in certain species (Prunus) the formation of gummy exudates on stems and leaf and bud axils. Young leaves may yellow and exhibit a twisted or deformed appearance. Symptoms generally absent on older tissues of growing plants. In non-growing plants, symptoms can become more widespread. (Fig. 1A)

Boron toxicity symptoms in species with limited boron mobility (Strawberry). Toxicity symptoms consist of marginal leaf burn at edge and tips of oldest leaves. Symptoms become progressively more pronounced with leaf age. In severly affected leaves, chlorosis also occurs at the margins of damaged areas. Young leaves typically show no symptoms. In non-growing plants, symptoms can become more widespread. (Fig. 1B)

Plants were grown in a potting mix (30% humus, 20% peat moss, 30% perlite and 20% sand) in five gallon pots (19L) in full sun under natural conditions. Full strength Hoagland's solution (Hoagland and Arnon, 1950) was applied to the plants once a week beginning on April 11, 1997. When all the plants were vigorously growing, the B treatment was started on May 16, and terminated after five months treatment. Boron was supplied as boric acid with 0.5 (control), 5 and 25 ppm B, respectively applied with each nutrient application. Boron treatments were incorporated in the nutrient solution and applied once a week. In each feeding, one liter of nutrient solution was applied per pot. Each treatment was replicated three times with one plant in each pot, so that each species had nine pots. Irrigation was made every day except the day of the nutrient application and one day after the application. Visible B toxicity symptoms were recorded as they appeared. Photographs were taken on July 8 and Aug. 7, 1997, respectively depending on the appearance of B toxicity symptoms. Samples from the organs with B toxicity as well as non-toxic counter parts were collected on July

25 or Aug. 19, 1997 for B determination. Leaf samples of various age catogaries (from basal to apical) were collected on Sept. 23, 1997 in the 0.5 ppm B treatment for the determination of B concentration along a shoot as an indicator of B mobility (van Goor and van Lune, 1980; Brown and Hu, 1996, 1998). Boron in the samples were determined with inductively coupled plasma-mass spectrometry (ICP-MS) according to Brown and Hu (1994).

Results

There was great diversity in sensitivity to B toxicity among the species. Some species, such as *Coprosma*, *Jasminium*, *Olea*, *Punica* and *Maytens* were very tolerant of B toxicity, and no clear B toxicity symptoms were observed for the duration of the experiment (five months) even though the highest B treatment (25 ppm) is sufficient to kill other species. In contrast, some species such as *Galium odoratum* are so sensitive that within one month of the treatment, clear B toxicity symptom was observed even in the 5 ppm B

treatment. In this species whole plant death occured before the end of the experiment in 25 ppm B treatment.

Boron toxicity symptoms are shown in Fig 1A and 1B. There were two distinct and species specific expressions of B toxicity. 'Classical' B toxicities are exhibited as tip/edge burn in the old leaves, this type of toxicity was observed in *Angelica*, *Eryngiurn*, *Forsythia*, *Fragaria*, *Galium*, *Lycopersicon*, *Potentilla*, *Spiraea*. However, in other species B toxicity was expressed as death of the young growing point in species such as *Cotoneaster*, *Digitalis*, *Gardenia*, *Ligustrum*, *Osmanthus*, *Pyracantha*, *Raphiolepis* and *Veronica*.

Table 1 shows the B concentration in leaves expressing moderate toxic symptoms and control leaves of the same age from plants receiving no supplemental B . The tissues in which B toxicity was first observed differed significantly between species and could be classified as either occuring in the oldest leaves or in the youngest. In no instance were symptoms first expressed in leaves of intermediate age. There was also a great diversity in B accumulation amongst species and the occurrence of highest B concentrations occured in leaves expressing greates toxicity. In some species B toxicity was exhibited at relatively low leaf B concentrations (315 _g B·g^{-1} dry weight B in *Spiraea prunifolia)*, while in others very high concentration of B were present in leaves exhibiting toxicity, such as 2184 _g B·g^{-1} dry weight in *Lycopersicon esculentum*. In the majority of species, B toxicity symptoms were observed in tissues with a B concentration ranging from 600 to 1600 _g·g^{-1} dry weight. Irrespective of the age of tissue in which B toxicity symptoms typically occured, the concnetrations associated with symptom expression were relatively uniform. In species with no B toxicity symptoms, the B concentrations ranged from 100 to 400 _g·g^{-1} dry weight after three to four months of 0.5 ppm B treatment.

Table 2 shows the B concentration in the leaves along a single shoot. An increasing concentration from young to old leaves is typical of a phloem immobile element whilst the opposite ios tru of a phloem mobile element. In several species the highest B concentration occurred in the basal leaves, while lowest B concentration occurred on the apical leaves. Those species include *Angelica*, *Eryngiurn*, *Fragaria*, *Lycopersicon*, *Potentilla*, *Coprosma*, and *Jasminium*. In contrast several species have the opposite concentration gradient with the lowest B concentration occuring in the basal leaves, while the highest B concentration occurred in the apical leaves. These species include *Cotoneaster*, *Digitalis*, *Gardenia*, *Ligustrum*, *Pyracantha*, *Raphiolepis*,*Veronica, Spiraea, Syringa, Maytens, Olea*, *Osmanthus*, *Petroselinum*, and *Punica*.

Discussion

Oertli (1960) suggested that B toxicity symptoms reflect the patterns of B accumulation and that the occurrence of B toxicity symptoms in old leaves and specifically in the marginsand tips of these leaves corresponded to the site of maximal B accumulation. The data presented here supports the conclusion that toxicity is related to local B concentration but in contrast to Oertli (1960) we have demonstrated that this can occur either in young or old leaves and that this is species dependent. There does not appear to be any clear difference in relative tolerance of young or old leaves to B accumulation since toxicity symptoms were expressed at similar B concentrations in either tissues. The appearance of B toxicity symptoms is therefore determined solely by the patterns of B distribution in the plant and will be exhibited as leaf tip/edge burn in old leaves of species in which B is phloem immobile, and as leaf burn and stem death in young meristem tissues of species in which B is phloem mobile.

Differences in relative B mobility can be infered from data in table 1 and Table 2. The occurrence of B toxicity in old leaves is indicative of phloem mobility (Table 1) and is verified by the patterns of B accumulation in Table 2. In almost every case, B accumulation patterns match very well with the leaf types accumulating B. In other words, if the concentration gradient indicates that highest B concentration is found in the oldest leaves in a species (B is immobile), then old leaf tip/edge burn will occur when this species is subject to high B treatments. These species include *Angelica*, *Eryngiurn*, *Fragaria*, *Lycopersicon* and *Potentilla*. On the other hand, if the concentration gradient indicates that highest B accumulation occurs in the apical leaves (B is phloem mobile), then young meristematic damage or tip die-back will be found when B toxicity symptom develops. These species include *Cotoneaster*, *Digitalis*, *Gardenia*, *Ligustrum*, Pyracantha, *Raphiolepis* and *Veronica*.

There were few species which did not strictly fit this general classification. *Forsythia* and *Antirrhinum* both exhibited old leaf tip burn and would be expected to have a decreased acropetal concentration gradient (i.e. lowest B concentration in the youngest leaves)

Species	Toxic leaf	Control leaf	Leaf type
Angelica gigas	1252 ± 41	209 ± 82	old
Eryngiurn sp.	885 ± 151	195 ± 50	old
Fragaria 'selva'	1676 ± 165	487 ± 104	old
Lycopersicon esculentum	2184 ± 532	752 ± 124	old
Potentilla alba	971 ± 46	186 ± 35	old
Cotoneaster demmeri	637 ± 80	110 ± 25	young expanding
Digitalis purpurea	1686 ± 368	374 ± 27	young expanding
Gardenia jasminoides	745 ± 74	169 ± 32	young expanding
Ligustrum 'texanum'	924 ± 17	293 ± 41	young expanding
Pyracantha fortuneana	736 ± 480	315 ± 87	young expanding
Raphiolepis pinkie	1566 ± 64	392 ± 62	young expanding
Veronica austriaca	370 ± 38	115 ± 11	young expanding
Forsythia 'lynwood gold'	1465 ± 112	100 ± 14	old
Liberty mix	1458 ± 154	126 ± 45	old
Osmanthus heterophyllus	964 ± 99	227 ± 13	young expanding (and old)
Spiraea prunifolia	315 ± 5	95 ± 13	young expanding (and old)
Syringa laveuder	594 ± 153	35 ± 1	young expanding

Leaf B concentration (_g·g^{-1} dry wt) in the B toxicity and control leaves. Samples were collected on July 25 or Aug. 19, 1997 dependent on the time of toxicity symptom occurrence. Boron concentration was analyzed by ICP-MS. (Table 1)

according to our predication. However, they showed no gradient (Table 2). This may have occured since at the time of sampling, all the leaves were fully mature an no young tissues were present.

In addition to the species described here the symptoms of B toxicity have been described in many other species. Whereas the majority of plant species show 'typical' symptoms of B toxicity (leaf tip and edge burn in old leaves) several species do not. Stem die-back as a result of B toxicity has been reported in *Prunus* species by Eaton et al. (1941), Hansen (1948, 1955), Woodbridge (1955), Dye et al. (1983) and El-Motaium et al. (1994). Die-back of apical or lateral shoots and cracked fruit has also been found in pear (*Pyrus serotina*) by Choe et al. (1986). Both *Prunus* and *Pyrus* transport sorbitol (Loescher, 1987) and hence a high B accumulation in the meristem or fruit can be expected in these species due to high mobility of B (Brown and Hu, 1996, Brown and Shelp, 1997). The B toxicity induced die-back in *Ligustrum 'texanum'* described in this experiment has also been reported in the same ge-

nus but different species (*Ligustrum japonicum*) by Francois and Clark (1979). *Ligustrum* transports mannitol in its phloem (Zimmermann and Ziegler, 1975) and hence B would be expected to be highly mobile (Brown and Hu, 1996). Francois (1988) also found B toxicity in young growing leaves in celery (*Apium graveoleus*), but not in old leaves. This is consistent with the results presented here since mannitol (a sugar alcohol with B complexing properties) is the primary photosynthetic product and the major form of translocated carbon in celery (Davis et al., 1988), as a result, B is highly mobile in celery (Hu et al., 1997).

Some of the species utilized here did not exhibit B toxicity, however the tissue in which toxicity would ultimately occur can be predicted from information of B distribution since the B accumulation pattern reflects B toxicity patterns. *Coprosma kirkii* and *Jasminum* will show old leaf tip/edge burn, while *Petroselinum crispum* and *Punica granatum* will show stem die-back. In *Maytens boaria* and *Olea skylark* stem die-back is likely to occur given the higher B concentration in api-

	Leaf Position		
Species	Basal	Middle	Apical
Angelica gigas	178 ± 41	134 ± 14	78 ± 5
Eryngiurn sp.	250 ± 37	95 ± 8	64 ± 1
Fragaria 'selva'	512 ± 11	176 ± 18	68 ± 5
Lycopersicon esculentum	721 ± 7	318 ± 24	94 ± 0
Potentilla alba	176 ± 4	128 ± 4	115 ± 32
Cotoneaster demmeri	157 ± 15	190 ± 3	439 ± 121
Digitalis purpurea	146 ± 10	335 ± 42	688 ± 58
Gardenia jasminoides	73 ± 1	89 ± 7	311 ± 22
Ligustrum 'texanum'	103 ± 5	153 ± 40	380 ± 13
Pyracantha fortuneana	50 ± 5	66 ± 7	268 ± 71
Raphiolepis pinkie	85 ± 0	171 ± 71	425 ± 24
Veronica austriaca	59 ± 1	61 ± 0	199 ± 60
Forsythia 'lynwood gold'	437 ± 44	387 ± 53	401 ± 66
Liberty mix	347 ± 212	312 ± 106	390 ± 21
Osmanthus heterophyllus	169 ± 31	204 ± 18	464 ± 67
Spiraea prunifolia	58 ± 0	51 ± 3	339 ±70
Syringa laveuder	40 ± 3	42 ± 3	60 ± 6
Coprosma kirkii	489 ± 177	238 ± 1	66 ± 6
Maytens boaria	95 ± 12	101 ± 10	115 ± 4
Olea skylark	42 ± 8	51 ± 15	56 ± 10
Petroselinum crispum	141 ± 9	387 ± 170	473 ± 105
Punica granatum	38 ± 2	40 ± 6	155 ± 46
Star jasmine	665 ± 58	253 ± 80	183 ± 11

Leaf B concentration ($\mu g \cdot g^{-1}$ dry wt) in the leaves along a shoot. Samples were collected on Sept 23, 1997 and B concentration was analyzed by ICP-MS. (Table 2)

cal than basal leaves. However, leaves on these speceis are long lived and growth occurs in cycles through the year making visual determination of leaf age difficult.

The identification by Brown and coworkers (see Brown and Shelp, 1997) that phloem B mobility and tissue B distribution is a consequence of the formation and phloem mobility of a sugar alcohol-B complex provides an explanation for the symptom expression observed here. In species used here or reprted elsewhere, for which B complexing sugar alcohols have been experimentally determined, B is clearly phloem mobile and toxicity symptoms occur in young tissues, these speceis include   . We predict basedupon these results that the presence of B complexing sugar alcohols in a species will result in apical B accumulation and expres-

sion of B toxicity in young tissues of that species. In species for which sugar alcohols have not been found in any member of that familly B is immobile and symptoms appear in old leaves (Lycospersicon), this will be true for all speceis in which B-complexing sugar alcohols are not present.

For species and familles in which sugar alcohol content has not been measured it is impossible to determine B transport and toxicity patterns. Hence, several of the species utilized here which were chosen on the basis that some members of that same plant familly were known to produce B complexing sugar alcohols though this has often been a purely qualitative determination. Of the speceis in this group several (e.g. *Gardenia jasminoides, Ligustrum* 'texanum') were clearly

B transporters and exhibited apical B toxicity while others (e.g. *Angelica gigas, Eryngium*) were not and exhibited B toxicity in old leaves. On the basis of this work and the results reported by others we conclude that B is phloem mobile and hence B toxicity will be expressed in young tissues of species within the Rosaceae, Oleaceae, Rubiaceae, Scrophulariaceae, Apiaceae, and Celestraceae. Not all species within these famillies necessarily exhibit B mobility and the consequent B toxicity symptoms, for example, Fragaria and Potentilla (Rosaceae), Eryngium and Angelica (Apiaceae) do not exhibit B mobility while other members of the same family do (eg Pyracantha, Cotoneaster (Rosaceae), Petrosilium (Apiaceae).

The results presented here illustrate that patterns of B accumualtion and B toxicity symptoms differ dramatically between speceis and that the well described symptoms of 'typical' B toxicity are not observed for many species. The patterns of B toxicity in species with phloem B mobility would not be recognized by many horticulturalists and could easily be mistaken for other diseases and physiological problems. This report provides new insight into the determination of B toxicity in diverse species and should help identify problems in production and home horticultural practice.

Literature Cited

Bieleski R.L. 1982. Sugar alcohols. In: F. Loewus, W. Tanner (eds.). P 158-192. *Encyclopedia of Plant Physiology, New Series.* V 13A, Srpinger-Verlag, Berlin.

Boeseken J. 1949. The use of boric acid for the determination of the configuration of carbohydrates. p. 189-210. In: W.W. Pigman, M.L. Wolfrom (eds.). *Advances in carbohydrate chemistry* V 4. Academic Press, New York.

Bourne E.J. 1958. The polyhydric alcohols. Acyclic polyhydric alcohols. P 345-361. *Encycl. Plant Physiol.* VI. Springer-Verlag, New York.

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

Brown, P.H. and Hu H. 1996. Phloem mobility of boron is species dependent: Evidence for phloem mobility is sorbitol-rich species. *Ann. Bot.* 77: 497-505.

Brown, P.H. and Hu H. 1998. Phloem boron mobility in diverse plant species. *Bot. Acta.* (In press).

Brown P.H. and Shelp B.J. 1997. Boron mobility in plants. *Plant and Soil.* 193: 85-101.

Choe, J.S., Lee J.C., Kim S.B. and Moon J.Y. 1986. Studies on the cause of shoot die-back in pear trees (Pyrus serotina Rehder). J. Korean Soc. *Hort. Sci.* 27: 149-156.

Davis, J.M., Fellman J.K. and Loescher W.H. 1988. Biosynthesis of sucrose and mannitol as a function of leaf age in celery (Apium graveolens L.). *Plant Physiol.* 86: 129-133.

Dye, M.H., Buchanan L., Dorofaeff F.D. and Beecroft. 1983. Die-back of apricot trees following soil application of boron. *New Zealand J. Exp. Agri.* 11: 331-342.

Eaton, F.M, McCallum R.D. and Mayhugh M.S. 1941. Quality of irrigation waters of the Hollister area of California. *Tech Bull. No.746. United States Department of Agriculture.* Washington, D.C.

El-Motaium, R., Hu H. and Brown P.H. 1994. The relative tolerance of six *Prunus* rootstocks to boron and salinity. *J. Amer. Soc. Hort. Sci.* 119: 1169-1175.

Francois, L.E. 1988. Yield and quality responses of celery and crisphead lettuce to excess boron. *J. Amer. Soc. Hort. Sci.* 113: 538-542.

Francois, L.E. and Calrk R.A. 1979. Boron tolerance of twenty-five ornamental shrub species. J. *Amer. Soc. Hort. Sci.* 104: 319-322.

Hansen, C.J. 1948. Influence of rootstock on injury from excess boron in Frence (Agen) prune and President plum. *Proc. Amer. Soc. Hort. Sci.* 51: 239-244.

Hansen, C.J. 1955. Influence of rootstock on injury from excess boron in Nonpareil almond and Elberta peach. *Proc. Amer. Soc. Hort. Sci.* 65: 128-132.

Hoagland, D.R. And Arnon D.J. 1050. The water-culture method for growing plants without soil. *Circ. Calif. Agr. Expt. Sta. 347.*

Hu, H. Penn S.G., Lebrilla C.B. And Brown P.H. 1997. Isolation and characterization of soluble B-complexes in higher plants. *Plant Physiol.* 113: 649-655.

Loescher W.H. 1987. Physiology and metabolism of sugar alcohols in higher plants. *Physiol. Plant.* 70: 553-557.

Maas E.V. 1984. Salt tolerance of plants. P 57-75. In: Christie B.R. (Ed.) *Handbook of plant science in agriculture.* V 2. CRC Press, Bota Raton.

Makkee, M., Kieboom A..P.G. and van Bekkum H. 1985. Studies on borate esters III. Borate esters of

D-mannitol, D-glucitol, D-fructose and D-glucose in water. *Rec des Tra. Chim. Pays-Bas.* 104: 230-235.

Oertli. J.J. 1960. The distribution of normal and toxic amount of boron in leaves of rough lemon. *Agron. J.* 52: 530-532.

Oertli, J.J. 1994. Non-homogeneity of boron distribution in plants and consequence for foliar diagnosis. *Commu. Soil Sci. Plant Anal.* 25: 1133-1147.

Plouvier B. 1963. Distribution of aliphatic polyols and cyclitols. p 313-336. In: T. Swain (ed.). *Chemical plant taxonomy.* Academic Press. New York.

Wallaart R.A.M. 1980. Distribution of sorbitol in Rosaceae. *Phytochemistry.* 19: 2603-2610.

Woodbridge C.G. 1955. The boron requirements of stone fruit trees. *Can. J Agri. Sci.* 35: 282-286.

Van Goor, B.J. And van Lune P. 1980. Redistribution of potassium, boron, magnesium and calcium in apple trees determined by an indirect method. *Physiol. Plant.* 48: 21-26.

Zimmermann, M.H. and Ziegler H. 1975. List of sugars and sugar alcohols in sieve-tube exudates. P 480-503. In: Zimmermann M.H. And Milburn J.A. (Eds.). *Encyclopedia of plant physiology.* V1. Transport in plants. I. Phloem transport. Springer-Verlag, New York.

Patrick Brown is Associate Professor and Hening Hu is Research Fellow, Department of Pomology, UC Davis. Warren Roberts is Superintendent and former Acting Director, UC Davis Arboretum.