This is to certify that the
thesis entitled

Boron and Sour Cherry (*Prunus cerasus*)

presented by

Yufei Xu

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Horticulture

Major Professor's Signature

September 8, 2005

Date

BORON AND SOUR CHERRY (*PRUNUS CERASUS*)

By

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A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

2005

ABSTRACT

BORON AND SOUR CHERRY

By

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In Michigan boron (B) deficiencies in sour cherry have resulted in routine use of B sprays to enhance fruit set and increase fruit yield. However, field observations indicate that high B levels are associated with premature softening, making fruit unacceptable for processing. Our fertilization studies show that fruit B levels are higher but B treatments generally had little or no effect on fruit size, maturity, color, or pull force. However, in some locations, B applications increased the number of soft fruit, especially when harvest was delayed well after the optimum maturity date (as indicated by pull force). The results suggest that B-induced yield increases can be achieved without inducing excessive fruit softening by careful monitoring of fruit maturation and prompt harvest. Determination of leaf and fruit B concentrations show that fruit B, but not leaf B levels are a good indicator of tree B status.

ACKNOWLEDGMENTS

I would like to express my most sincere thanks to my major professor and supervisor, Dr. Wayne Loescher: thank you for your patience, guidance, professionalism, and providing me an opportunity to take my master's study in horticulture as well as an opportunity to explore American culture.

I would also acknowledge great support from Dr. C. Peter Wolk and Dr. Eric Hanson.

The following individuals provided advice and assistance to my cherry project:, Dr. Randy Beaudry, Steve Berkheimer, Matt Blanchard, Bill Chase, Dr. James Flore, Dr. Zhifang Gao, Dr. Sastry Jayanty, Dr. Ning Jiang, Tad Johson, Dr. Alexandra Kravchenko, Dr. Joseph Kuhl, Dr. James Nugent, Dr. Paolo Sabbatini, Dr. Ken Sink, Dr. Guoqing Song, Dario Stefanelli and Dr. Steve Van Nocker.

Last but not least, I would like to thank my parents for supporting me all the time, while I could not do much for them.

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INTRODUCTION

The interest of biologists in B has largely been focused on its role in plants where B was first established as essential in 1923 (Warington, 1923). Evidence that B has a biological role in other organisms was later indicated by the establishment of essentiality of B for diatoms (Smyth and Dugger, 1981) and cyanobacteria (Bonilla et al. 1990; Bonilla et al. 1995). Recently, B was shown to stimulate growth in yeast (Bennett et al. 1999) and to be essential for zebrafish (*Danio rerio*) (Eckhert and Rowe, 1999) and possibly for trout (*Oncorhynchus mykiss*) (Eckhert, 1998; Rowe et al. 1998), frogs (*Xenopus laevis*) (Fort et al. 1998), and mouse (Lanoue et al. 1998). There is also preliminary evidence to suggest that B has at least a beneficial role in humans (Nielsen, 2000).

B and sour cherry

Soft fruit has become an increasingly common problem in the sour cherry industry in Michigan. Affected fruit often rupture during mechanical harvesting or lose their integrity during pitting and processing. This reduces yields of usable pitted cherries, and may render entire lots unsuited for processing. Soft fruit may be rejected by processors or simply not harvested. The economic losses resulting from the soft fruit problem in Michigan, the state that ranks first in the nation in the production of sour cherry, have averaged $6.3 M a year, with more severe losses averaging $14.3 M in 1992, 1995 and 1998.

B has been considered as the cause of the soft fruit problem. Guyer (unpublished) observed a positive correlation between soft fruit and leaf B concentrations. Flore

(unpublished) showed that foliar B sprays increased splitting, one symptom of soft fruit. All this suggested high plant B levels may directly or indirectly contribute to the soft cherry problem. Nonetheless, B use in Michigan cherry orchards has increased over the past decade and B application on sour cherry is now common in industry because growers believe that B application increases fruit set. Studies on sour cherry showed that fruit set and production of sour cherry trees containing leaf B levels of 19 to 25 $\mu g \cdot g^{-1}$ dry weight can often be increased by B applications, although the mechanism by which B influences fruit set is unknown (Hanson, 1991a). However, the increase in fruit set and production is not always observed. In Hanson's experiments (1991b), an increase as much as 100% was found in one trial while no increases were reported in several other trials. Current Michigan recommendations call for B applications when cherry leaf tissue contains less than 30 ppm of B. However, leaf B level may not be a good standard to monitor tree B level. In addition, cherries and most other tree fruit crops are considered sensitive to high soil B levels (Anonymous, 2000).

Besides sour cherry, B applications have had variable effects on fruit quality in many species, including a reduction in the firmness of prunes (*Prunus domestica*) (Wojcik, 1999), lowbush blueberries (*Vaccinium angustifolium*) (Chen et al. 1998), and an increase in the tendency of apples to develop internal breakdown and watercore (Bramlage et al. 1962; Martin et al. 1976).

These observations are difficult to reconcile with the recent evidence for borate in cross-linking cell wall constituents (Matoh et al. 1993). It is hypothesized that these covalently

cross-linked borate ester linkages affect the assembly or maintain the structure of cell walls (Fleischer et al. 1999). Although this theory is consistent with the gross anatomical changes in the walls of B-deficient cells (Spurr, 1957), whether B esters are essential to the structure integrity of all plant cell walls is unknown.

Another confounding factor is that the mobility of B in the phloem varies greatly among species. Species exhibiting high mobility of B, including sour cherry (Hanson, 1991a), utilize sorbitol as a primary transport carbohydrate. Not only sorbitol, but other sugar alcohols such as mannitol (Loescher and Everard, 2000) were proved to form complexes with B, suggesting a way to facilitate transport of B in the phloem (Bellaloui et al. 1999, Brown and Hu, 1996). When B supplies are abundant, polyol translocating species with consequent high B mobility may accumulate high levels of B in sinks such as fruits (Bellaloui et al. 1999). Alternatively, high B levels may facilitate transport of polyols, contributing to fruit osmotic potential (and internal water potential) and thus a susceptibility to splitting, a problem related to the soft fruit.

In this project, we use sour cherry fruit as a model system to study the involvement of B in fruit quality and maturation. Our hypothesis was that B levels affected soft fruit. We tested the hypothesis by measuring several fruit quality parameters.

LITERATURE REVIEW

B and the Plant Cell Wall

The role of B in plant cell walls has recently been reviewed extensively by O'Neill et al. (2004). Part of this section is a synopsis of that review. B's role has long been believed to be related to the plant cell wall. An early symptom of B deficiency in flowering plants is the formation of primary walls that have abnormal morphology and mechanical properties (Dell et al. 1997). Further evidence comes from a study of species variability in B requirement, when B content was shown to be positively correlated with cell wall pectin (Hu et al. 1996). For example, in the *Poaceae*, whose primary walls contain quantitatively small amounts of pectin, B requirements are much lower than the dicotyledons and nongraminaceous monocotyledons. All this has suggested a relationship between B and primary wall pectic polysaccharides (Hu et al. 1994; Matoh et al. 1996).

The chemical structure of primary cell wall pectins

Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic acid (Gal*p*A) residues. The major pectic polysaccharides isolated and structurally characterized from the primary cell walls of gymnosperms and angiosperms are homogalacturonan (HG), rhamnogalacturonan I (RG-I), and the substituted galacturonan (SG) including rhamnogalacturonan II (RG-II) (Ridley et al. 2001). Homogalacturonan (HG) is mostly a linear chain of 1,4-linked α-D-galactopyranosyluronic acid (Gal*p*A) residues in which some of the carboxyl groups are methyl esterified, and a few are partially O-acetylated at C-3 or C-2. Rhamnogalacturonan-I (RG-I) is a family of pectic polysaccharides that contain a

backbone of the repeating disaccharide [→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→]. Substituted galacturonans (SG) are a diverse group of polysaccharides that contain a backbone of linear 1,4-linked α-D-Gal*p*A residues (O'Neill et al. 1990). Rhamnogalacturonan II (RG-II) belongs to SG and it is found in all higher plant primary walls analyzed to date (O'Neill et al. 1990). More detail will be provided in the section entitled 'RG-II'.

These three polysaccharides are covalently linked to one another to form a pectic macromolecule. A covalent link between RG-II and HG is highly likely because they both have backbones composed of 1,4-linked α–D-GalA resides. Additional evidence that RG-II is covalently linked to HG to form a high-molecular-weight (>100kDa) complex was obtained by characterizing the material that aqueous buffers solubilized from sugar beet (*Beta vulgaris* L.) (Ishii et al. 2001), *Chenopodium album* (Fleischer et al. 1999), and *Arabidopsis* cell walls (Reuhs et al. 2003). Further covalent and non-covalent cross-linking of some glycosyl residues in this macromolecule forms a three-dimensional pectic network. For example, Ca^{2+} forms ionic cross-links between some of the carboxylates of the Gal*p*A residues in HG. A recently discovered covalent HG cross-link involves B. The first B-polysaccharide complex was isolated and characterized from radish roots by Matoh and his colleagues in 1993. It was proved to be a B-rhamnogalacturonan-II complex (Matoh et al. 1993). In 1996, the same complex was also found in sugar beet pulp by the same research group (Ishii and Matoh, 1996). Moreover, its chemical structure was characterized to be a cross-linked borate-diol ester (Kobayashi et al. 1996). This covalent cross-linking of RG-II and the Ca^{2+}-dependent ionic cross-linking of HG

combine to form a stable three-dimensional pectic network *in muro*. There are in primary cell walls two other networks, the load-bearing cellulose microfibrils and the structural glycoproteins. The interactions within and between these networks give the wall its mechanical strength.

RG-II

RG-II was first identified in 1978 as a structurally complex yet quantitatively minor polysaccharide that is solubilized by endopolygalacturonase (EPG) treatment of suspension-cultured sycamore cell walls (Darvill et al. 1978). RG-II belongs to a group of pectic polysaccharides referred to as substituted galacturonans. A common feature of this group is that these polysaccharides all have a backbone composed of linear 1,4-linked α–D- Gal*p*A residues.

A localization study showed that RG-II is distributed throughout the primary wall and that regions of the wall that are close to the plasma membrane may be somewhat enriched with RG-II, while little if any RG-II is detected in the middle lamella (Matoh et al. 1998). RG-II is ubiquitously found in the cell walls of all gymnosperms and angiosperms. It has been isolated from cell walls of a variety of plants including suspension-cultured sycamore cells (O`Neill et al. 1996), etiolated pea stems (O`Neill et al. 1996), sugar beet (*Beta vulgaris*) pulp (Ishii et al. 1996), apple fruit (Doco et al. 1997), carrot tuber (Doco et al. 1997), tomato fruit (Doco et al. 1997), bamboo (*Phyllostachys edulis*) shoot (Kaneko et al. 1997), ginseng (*Panax ginseng*) leaf (Shin et al. 1997), radish and rice roots (Matoh et al. 1998), cultured tobacco cells (Matoh et al. 1998), red clover root

nodules (Matoh et al. 1998), red pine (*Pinus densiflora*) (Shimokawa et al. 1999), suspension-cultured *Chenopodium album* cells (Fleischer et al. 1999), grape berry (Vidal et al. 2001), pumpkin leaf (Ishii et al. 2001), red beet (*Beta vulgaris* L var *conditiva*) (Strasser et al. 2001), and lily pollen (Holdaway-Clarke et al. 2003).

RG-II accounts for between 1% and 4% of the pectin-rich primary walls of dicots, non-graminaceous monocots, and gymnosperms, but less than 0.1% of the pectin-poor primary walls of the *Poaceae* (Matoh et al. 1996). This is consistent with the observation that *Poaceae* crops require relatively low B.

RG-II accounts for between 0.2% and 2% of the walls of pteridophytes and lycophytes, which is of a similar order of magnitude to the amounts of RG-II present in the primary walls of angiosperms and gymnosperms. The amounts of borate cross-linked RG-II present in the sporophyte primary walls of members of the most primitive extant vascular plant groups (Lycopsida, Filicopsida, Equisetopsida, and Psilopsida) are comparable with the amounts of RG-II in the primary walls of angiosperms. By contrast, the gametophyte generation of members of the avascular bryophytes (Bryopsida, Hepaticopsida, and Anthocerotopsida) have primary walls that contain small amounts (approximately 1% of the amounts of RG-II present in angiosperm walls) of an RG-II-like polysaccharide. There are data indicating that the amount of RG-II incorporated into the walls of plants increased during the evolution of vascular plants from their bryophyte-like ancestors (Matsunaga et al., 2004). Thus, the acquisition of a B-dependent growth habit, may be correlated with the ability of vascular plants to maintain upright growth and to form

lignified secondary walls. The glycosyl sequence of RG-II remains essentially unchanged in all spore- and seed-bearing tracheophytes that have been examined to date. The conserved structures of pteridophyte, lycophyte, and angiosperm RG-IIs suggest that the genes and proteins responsible for the biosynthesis of this polysaccharide appeared early in land plant evolution and that RG-II has a fundamental role in wall structure (Matsunaga et al., 2004).

So far, nothing is known about RG-II metabolism during fruit ripening. However, the primary cell walls of the suspension-cultured cells from kiwifruit (*Actinidia deliciosa*) contained twice the amount of RG-II found in the cell walls of the same intact fruit, while the composition of RG-II glycosyl residues were very similar in both cultured cells and the intact kiwifruit (Fischer, 1996).

Borate cross-linking of RG-II

B has the ability to form monoesters and diesters with compounds containing cis-hydroxyl groups, resulting in enhanced acidity and a negatively charged complex (Lewis, 1980; Loomis and Durst, 1991). A polysaccharide such as rhamnogalacturonan II has such propertites. ^{11}B-NMR spectroscopic analysis of a B-polysaccharide complex extracted and purified from radish (cv. Aokubi-daikon) roots demonstrated that most of the wall-bound B is present as a tetravalent 1:2 borate-diol ester (Matoh et al. 1993). Later, Kobayashi`s group showed that the removal of B from the complex reduced the molecular weight by one-half without causing a significant increase in the number of reducing end groups (Kobayashi et al. 1996). Their results indicated that B, as boric acid,

links two rhamnogalacturonan II chains together to form the B-polysaccharide complex. This provides the structural basis for the relationship between B and primary wall pectins. Subsequent studies confirmed that borate cross-links two chains of RG-II to form a dimer in the primary walls of angiosperms, gymnosperms (Shimokawa et al. 1999), lycophytes, and pteridophytes (Matsunaga et al. 2004).

Borate can cross-link molecules because it contains two pairs of hydroxyl moieties that can form reversible diester bonds with molecules containing cis-diols in a favorable conformation. Borate esters are believed to form with apiosyl residues of RG-II since apiose is the only component of RG-II which has the β-D-erythrofuranose configuration ready to form an ester. One common belief is that two molecules of RG-II are cross-linked by a single borate diester. One alternative model is that RG-II dimer contains two B atoms. If such a dimer exists, it would contain one borate diester cross-linking the apiosyl residue of each side chain A of RG-II and a second that cross-links the apiosyl residue of each side chain B of RG-II. However, the existence of such a model is only partially proven by ^{13}C NMR spectroscopic analyses and still awaits further proof (Ishii and Ono, 1999). In that model, the borate diol diesters of methyl beta-D-apiofuranoside are present as two diastereomers in approximately equal molar ratios.

Studies with mutant plants further confirmed the relationship between B and RG-II. Experiments on *Arabidopsis mur1* mutant and tobacco *nolac-H18* mutant (Iwai et al. 2002) showed that a seemingly small change in the structure of RG-II could dramatically reduced its ability to form a borate cross-linked dimer and that these structural changes

adversely affected plant growth and development. *Arabidopsis* plants carrying the *mur1* mutation are dwarfed and have brittle stems. This results from the fact that about 50% of the RG-II in the rosette leaves of mur1 plants is cross-linked by borate, while at least 95% of the RG-II is cross-linked in wild-type plants. This suggested that the dwarf phenotype and brittle tissue of *mur1* plants was a consequence of altered RG-II structure and therefore its reduced cross-linking (O'Neill et al. 2001). In addition, the tobacco *nolac-H18* mutant (nonorganogenic callus with loosely attached cells), artificially generated by T-DNA transformation, has defects in the glucuronic acid of rhamnogalacturonan II of pectin, suggesting that the mutation drastically reduced the formation of borate cross-linking of rhamnogalacturonan II (Iwai et al. 2002).

Experiments with a borate transporter (BOR1) also confirmed the relationship between B and RG-II. In the wild type, about 90% of RG-II was present as the dimeric form (dRG-II-B), both at low and sufficient B supply. In the *bor1-1* mutant, about 60% of RG-II was in its monomeric form (mRG-II) at low B supply, whereas more than 85% of it was present as dRG-II-B at sufficient B supply. However, similar to the wild type, mRG-II derived from the *bor1-1* mutant was able to form dRG-II-B in vitro in the presence of borate and lead. Sugar composition of cell wall fractions was similar in both genotypes. This suggests that the polysaccharide composition in the cell wall was not strongly affected by the *bor1-1* mutation. The observed difference in dimerization of RG-II at low B supply is most likely due to a reduced B concentration in the shoots of the *bor1-1* mutant (Noguchi et al. 2003).

Borate-dependent molecules are not limited to RG-II. There are B-polyhydric alcohol complexes identified from phloem extracts (Hu et al. 1997), a bacterial signaling molecule and its sensor protein (Chen et al. 2002), as well as several antibiotics (Hunt, 2003).

B MOBILITY

B deficiency in crops is more widespread than deficiency of any other micronutrient. Nutritional disorders in vegetables include brown heart in rutabaga, turnip and radish roots, and hollow stem in cauliflower and broccoli (Shelp et al. 1995). The occurrence of these disorders even when B is in ample supply suggests that they are physiological in nature and related to the mobility of B in the plant (Shelp et al. 1995). The relative mobility of an element within a plant has important physiological and agricultural implications. It is because the ability of a plant species to survive or to yield optimally during a period of nutrients stress is a consequence of both its ability to obtain nutrients from the soil under limiting conditions and the extent to which the nutrients can be supplied through redistribution from other tissues within the plant.

B is generally considered to be phloem immobile. It has been observed for years that plants grown with an adequate B supply have B concentrations that decrease from old leaves to young leaves. B deficiency symptoms typically occur in meristematic tissues, while B toxicity symptoms occur first in margins of old leaves. All this indicates that B is an immobile element in some species. For example, there is considerable experimental evidence that in some species B is almost immobile. In squash and tomato, B deficiency

symptoms developed rapidly upon transfer of plants from B-replete to B-deficient growth conditions. The leaf B content established prior to transfer to B-deficient conditions did not decrease, while the growth of apical tissues was completely inhibited (Oertli, 1993; Hu and Brown, 1994). B cannot readily be redistributed in most species, therefore even a brief disruption in soil B supply results in growth depression and yield loss. The extent of the damage depends upon the duration of the deficiency and the stage of plant growth at which it occurs (Dell and Huang, 1997). However, this does not occur in plants which produce sugar alcohols such as sorbitol (Brown and Hu, 1996). Such plants mainly include species within the genera *Pyrus*, *Malus* and *Prunus*.

Although in some plants B has been proved to be immobile, it does not exclude the possibility of B transport in the phloem. In broccoli (*Brassica oleracea* var. *italica*), Shelp (1988) found B concentrations in the phloem sap higher than in the xylem sap. Shelp and coworkers (Shelp et al. 1987; Shelp and Shattuck, 1987; Shelp, 1988) also reported that the ratios of B concentrations in developing sinks to those in old leaves were higher with a continuous supply of growth-limiting B than with adequate B. Campbell et al. (1975) also concluded that transport of B to developing peanut fruit occurs in the phloem. Therefore, immobility of B in some plants may be the result of formation of stable and immobile B complexes within the cell (Loomis and Durst, 1992; Brown and Hu, 1994). However, the extent of B mobility found in plants in a particular experiment is affected by the ability of the leaves to absorb boric acid, the size and photosynthetic activity of their source leaves, and the strength of different sinks. Genetic variability can also be considerable (Stangoulis et al. 2000).

Experiments with broccoli and lupin explored whether B retranslocation depends on plant-B status and external-B supply. B acquired during inflorescence development was an important source of B for reproductive structures, but the relative importance of B acquired before and after inflorescence emergence appeared to be species dependent. The occurrence of B retranslocation was not dependent upon the induction of B deficiency (Marentes et al. 1997).

B and sugar alcohols

B mobility is closely related to the presence of polyols. The pattern of B distribution within shoot organs and the translocation of foliar-applied, isotopically-enriched ^{10}B was studied using six tree species including almond (*Prunus amygdalus B.*), apple (*Malus domestica B.*), nectarine (*Prunus persica L. B*), fig (*Ficus carica L.*), pistachio (*Pistacia vera L.*) and walnut (*Juglans regia L.*). In species in which sorbitol is a major sugar (sorbitol-rich) such as almond, apple and nectarine, B is freely mobile. But, in sorbitol-poor species, those that produce little or no sorbitol such as fig, pistachio and walnut, B is largely immobile. Together with the evidence that B forms a stable complex with sorbitol in sorbitol-rich species, it is suggested that B mobility is mediated by the formation and transport of B-sorbitol complexes (Brown et al. 1996).

Introduction utilizing molecular techniques of the gene for sorbitol synthesis into a species can enhance the within-plant nutrient mobility of B (Brown et al. 1999). Enhancing sorbitol synthesis by transforming plants with sorbitol-6-phosphate dehydrogenase gene, a key enzyme for sorbitol production, can facilitate phloem B

transport in both tobacco and rice (Bellaloui et al., 1999; Bellaloui et al., 2002). An increase in B uptake and mobility may contribute to an overall improvement in tolerating low-B soils and B deficiency (Brown et al.1999). Moreover, in B-immobile plants such as tobacco, the transgenic enhancement of within-plant nutrient mobility could be a viable approach to improve plant tolerance of nutrient stress.

A variety of B-polyol complexes have been isolated and characterized from higher plants. The first successful isolation and characterization of soluble B complexes from higher plants were accomplished in 1997 (Hu et al. 1997), from the phloem sap of celery (*Apium graveolens L.*) and the extra-floral nectar of peach (*Prunus persica L.*). In celery phloem sap, B was present as the mannitol-B-mannitol complex. Molecular modeling further predicted that this complex is present in the 3,4 3',4' bis-mannitol configuration. In the extrafloral nectar of peach, B was present as a mixture of sorbitol-B-sorbitol, fructose-B-fructose, or sorbitol-B-fructose. These findings provided a mechanistic explanation for the observed phloem B mobility in these species (Hu et al, 1997).

However, recent studies are somewhat contradictory. A study of deciduous forest trees proved that B mobility does not require the presence of polyols as expected, and it appeared that to some degree remobilization occurs in many plant species (Lehto et al. 2004). Extensive B mobility was found in *Sorbus aucuparia*, *Prunus padus* and *Ulmus glabra*. The first two species contain high levels of sorbitol, while in *Ulmus glabra*, only trace amounts of B-complexing polyols were detected. A medium level of B mobility was observed in growing leaves in A*lnus incana*, *Fraxinus excelsior*, *Betula pubescens* and

Larix sibirica after ^{10}B isotope labeling of mature leaves of seedlings. Mannitol in *Fraxinus* and pinitol in *Larix* may also complex with B to facilitate remobilization. Another finding suggesting that B mobility is not closely related to polyol presence is in *Alnus glutinosa* which has almost identical concentration of polyols as *A. incana*, a closely related species. Yet *A. glutinosa* did not remobilize B. One explanation is that in plants with limited B mobility, the small amounts of polyols are not necessarily loaded to the phloem in mature leaves and unloaded in new leaves, which would be the prerequisite for B mobility. Sorbitol is closely related to other plant monosaccharides, including other polyols, and the very small amounts detected may in some cases be transitional phases in metabolic reaction chains (Lehto et al. 2004).

A more detailed study was conducted by the same group on Scots pine and Norway spruce (Lehto et al. 2000; Lehto et al. 2004) with no controversial results. ^{10}B -enriched boric acid was applied onto the needles of both species. Small but significant increases in the ^{10}B isotope were found in the new stem and needles of both species, after a dormancy period and 9 weeks of growth. The increases were given credit to the possible presence of B-polyol complexes in these polyol-rich species.

Other mechamisms of B phloem mobility

Other mechanisms for B phloem mobility may be involved in plants which do not produce polyols. Other soluble B complexes may be biologically important (Brown et al., 2002), such as B-fructose (Hu et al., 1997) and B-malic acid (Dembitsky et al., 2002). Complex formation with other compounds and their translocation might explain the B

mobility in species that do not contain polyols. In addition, multiple mechanisms of B efficiency were observed even within one species. A study on three cultivars of Canola (*Brassica napus L.*) showed that applications with ^{10}B labeled boric acid retranslocated from older leaves to younger leaves in one cultivar while the sink remained unknown for the other two cultivars (Stangoulis et al. 2001).

Interestingly, fungi are involved in B mobility in some plants. Experiments using ^{10}B /^{11}B isotope on silver birch showed that B was up by the mycorrhizal mycelia and transported to the host plant in this species combination (Lehto et al. 2004).

B TRANSPORTER

B uptake

The subject of B uptake was controversial long before the discovery of a B transporter. There was evidence supporting both active and passive uptake of B in higher plants. The major form of B exists in living cells as boric acid, a weak acid with pK_a's of 9.14, 12.74 and 13.8. However, at normal cytosol pH, boric acid exists mostly as an uncharged H_3BO_3, which should make it easy to permeate cell membranes and thus making active pumping unnecessary. However, a study on sunflower root B pools suggested a even more complicated mechanism (Dannel et al. 2000). Control plants precultured with high B supply (100 μM) showed a linear response of the ^{10}B concentrations in the root cell sap and in the xylem exudate to the differential short-term ^{10}B supply, and this was not affected by metabolic inhibitor treatments. In the control precultured with low B supply (1 μM), the response of the ^{10}B concentrations in the root cell sap and xylem exudate to

the differential short-term ^{10}B supply appeared to be a combination of a saturable and a linear component. This suggested that B uptake into the root symplasm, as well as xylem loading, are preformed by two transport mechanisms, with the linear components representing B transport by passive diffusion.

Hu and Brown (1997) proposed that B uptake, under conditions of adequate or excessive B supply, is the result of passive assimilation of undissociated boric acid ($B[OH]_3$). This conclusion was based largely on the theoretical predictions of membrane permeability proposed by Raven (1980); however, accumulation against concentration gradient exists (Brown et al. 2002).

bor1

A B transporter was first found as a result of study of the *Arabidopsis thaliana* mutant *bor1-1* (Noguchi et al. 1997). The mutant was discovered by a defect in root-shoot translocation of B. Compartmental analysis of B in wild type and *bor1-1* mutant plants of *Arabidopsis thaliana* proved that the reduced B content in shoots of the mutant plants at low B supply only were mainly the B contents in the water soluble fractions (cell sap), but not the B in the water insoluble residue (WIR). The results suggested that the *bor1-1* mutation has little or no effect on the binding of B in the cell wall, since B in WIRs mainly represents cell wall bound B (Noguchi et al. 2000). Uptake experiments with ^{10}B-enriched tracer B demonstrated that B taken up through roots was preferentially transported to young leaves compared to old leaves in the wild-type plants under a low B supply. Such a preferential transport to young leaves was not evident in the mutant plants,

suggesting that in *Arabidopsis thaliana* plants B is preferentially transported to young organs under a low B supply and that this transport process is controlled at least in part by the *BOR1* gene (Takano et al. 2001). Further analysis using ^{10}B showed that roots of the mutants contain adequate levels of B, while the plants still suffer from reduced B delivery to shoots due to the impaired xylem loading. The patterns of B increases in root cell saps in both wild type and the mutant plants are the same, suggesting that uptake into roots occurs mainly by passive transport. The concentration of tracer B in xylem exudates of the *bor1-1* plants also followed a linear concentration dependence, whereas in the wild-type plants a combination of saturable and linear concentration dependence was observed, suggesting a B transporter in the wild type (Takano et al. 2002).

It was a mutation in the *BOR1* gene that led to symptoms of B deficiency (Takano. 2001). The *BOR1* locus is located on the lower arm of chromosome 2 (Noguchi et al., 2000). It is delimited in a 15.1-kilobase (kb) region between newly generated molecular markers at positions 19,383 kb and 19,399 kb. Nucleotide sequences of this region in the genome of *bor1-1* and *bor1-2* mutants (ethylmethane sulphonate mutants) revealed that each mutant contains a different single base substitution in the hypothetical open reading frame (ORF) *At2g47160*, each causing a different amino-acid substitution in the predicted protein. A genomic DNA fragment containing wild-type *At2g47160* was then introduced into the bor1-1 mutant and demonstrated to complement the mutation, establishing that *At2g47160* corresponds to the *BOR1* gene. Comparison between the cDNA and genomic sequences revealed that *BOR1* has 12 exons. On the basis of the nucleotide sequence, *BOR1* was predicted to encode a polypeptide of 704 amino acids containing 10 putative

transmembrane domains. The mutations found in the *bor1-1* and *bor1-2* alleles were located within the second transmembrane domain (Takano et al. 2002).

Subcellular localization of the *BOR1* gene product was determined using a construct containing green fluorescent protein (GFP) under the control of the *BOR1* promoter region. This suggested that BOR1 is a plasma-membrane-localized protein, which was consistent with its putative transporter function. Cell-type specificity expression showed that *BOR1* localized in the pericycle, located at the outmost layer of the stele and inside the endodermis (Takano et al. 2002).

Phylogenetic analyses showed that BOR1 is a membrane protein related to the family of mammalian anion exchangers known as *SLC4*. Also, this B transporter fell into a clade with the yeast protein *YNL275w*, human BTR1 and six other *Arabidopsis* proteins: *At1g15460, At1g74810, At3g06450, At3g62270, At4g32510* and *At5g25430*. Phylogenetically, the yeast transporter seems to be an intermediate between the anion exchangers and BOR1, and so could potentially transport both bicarbonate and borate. Most importantly, the human BTR1 protein, named as being a possible bicarbonate-like transporter, also falls into the same clade as BOR1, suggesting its possible role as a B transporter (Frommer and Von Wiren, 2002). One other interesting feature of BOR1 and its yeast homolog YNL275w shown by the yeast study is that they both have B-efflux transport activity (Takano et al. 2002).

The phylogenetic analysis gave no firm indication of the function of the six other *Arabidopsis* proteins. However, some might serve as B transporters that use other coupling mechanisms and that provide a route, for example, for importing B into cells. Many years ago, biophysical studies (Lucas, 1975) had indicated that bicarbonate and borate may use the same transporter. Thus, some of the anion-exchange transporters similar to YNL275w may transport bicarbonate as well as borate, for example to facilitate the supply of CO_2 for photosynthesis. Study of this transporter family may therefore shed light not only on the functions of B in metabolism but perhaps also on CO_2 movement in plants. Given the close relationship of bicarbonate and B transporters among anion exchangers, it could be that relatives of the active bicarbonate transporter from cyanobacteria may transport borate. Finally, some aquaporins may be permeable to borate and serve in B transport (Dordas et al. 2001; Ruiz, 2001).

There is another interesting feature of BOR1. The surprising similarity of the transport systems for B and bicarbonate points to a similarity in the binding forms of these two substrates. In plant cells a high cytoplasmic pH allows the formation of the borate anion, whereas in kidney cells bicarbonate formation from CO_2 is also enzymatically facilitated. Given the phylogenetic relation between the proteins, the simplest hypothesis is that BOR1 also transports anions, and that perhaps borate transport is coupled to the antiport of a counterion in the same way as bicarbonate. BOR1 could then function as a borate/chloride anion exchanger using the chemical gradient established by certain chloride channels (X-QUAC channels). Alternatively, it could use proton coupling, instead of chloride coupling, to export borate by a secondary active route. Another

possibility is that the negative membrane potential in the pericycle would allow borate anions to be exported by *BOR1*-mediated uniport (diffusion through a transporter without coupling to a second ion). Electrophysiological analyses of BOR1 in various settings should help to decide this matter (Frommer et al. 2002).

The mammalian homolog of AtBor1 was also studied and proved to be a B transporter. BTR1 (Bicarbonate Transporter Related Protein-1) was cloned as a putative bicarbonate transporter-related protein. BTR1 mRNA was reported to be widely expressed in various tissues, but most strongly in kidney, salivary glands, testis, thyroid and trachea. Moreover, it may also be responsible for anion transport mechanisms hitherto unaccounted for in these tissues (Parker et al. 2001).

The mammalian BTR1 has unique transport features. In the absence of borate, it conducts Na^+ and OH^- (H^+). In the presence of borate, BTR1 functions as an electrogenic Na^+-coupled borate *c*otransporter. This is a voltage-regulated, electrogenic transporter with shallow inward rectification when mediating Na^+-$B(OH)_4^-$ influx and with steep outward rectification when mediating Na^+-$B(OH)_4^-$ efflux. Based on its transport features, Parker and his colleagues (2001) renamed the transporter as NaBC1. NaBC1 (BTR1) plays a central role in mediating the stimulating and toxic effects of borate on cell growth and proliferation.

Recently, a novel mutant line 8-21 that requires a high concentration of B for normal growth has been found in *Arabidopsis thaliana* (Aoki et al. 2004). Experiments showed

that the concentrations of B in the shoot and the root were the same in both wild-type and the mutant plants, suggesting that the mutant could not utilize B efficiently. Moreover, Line 8-21 was not allelic to *bor1-1* (Noguchi et al. 1997). A significant portion of F2 plants from the crosses between the wild type and the mutant grew poorly on a low B media, suggesting segregation of the mutation.

MATERIALS AND METHODS

Plant Material for B analysis

Sour cherries (*Prunus Cerasus* L.cv.Montmorency) trees at the MSU's Horticulture Teaching & Research Center (HTRC), Holt, MI were used. Trees were spaced at 16 feet (4.88 m) between rows and 12 feet (3.66 m) between trees (227 trees per acre, 561 per hectare). Trees were planted in May 1988 and grown in Marlette fine sandy loam soil type. Trees at the HTRC were treated with increasing levels of B from 2002 till 2004 and sampled following treatments.

B applications

Commercial Solubor DF (greater than 80% sodium pentaborate decahydrate, and less than 20% boric acid, equivalent to 17.5% elemental B) was used for foliar applications from 2002 till 2004. Trees were sprayed to the extent that the Solubor DF solution started to drip. Three levels of B (including the –B control) were applied each year. In 2002, 18 trees on the south end of the cherry tree plot at the HTRC were assigned randomly into three treatments: 0 ppm (control), 25 ppm, or 50 ppm of B. In 2003, 36 trees on the north end of the cherry tree plot at the HTRC were assigned randomly into three treatments: 0 ppm (control), 100 ppm, or 200 ppm of B. In 2004, at the HTRC, the 36 trees tested in 2003 were treated with even higher concentrations of B: 0 ppm (control), 400 ppm and 800 ppm. Also, only in 2004, 18 trees at the Clarksville Horticulture Experiment Station (CHES) were treated with 0 ppm (control), 400 ppm or 800 ppm of B. In 2002, foliar B sprays were started 29 days after full bloom (AFB) and were continued about every 5 days thereafter unless there was rain predicted on the day of spray. In 2003 and 2004, the

first sprays were 26 days and 17 days after the full bloom. The spray intervals of the two years were 5 days and 7 days, respectively. The sprays began mid May and ended late June.

Table 1. B treatments from 2002 to 2004

	B concentration (μg/L)	Spray time	Total B a year (g/tree)
2002	0	From May 22th till July 9th	0
South end	25	7 applications	0.25
	50		0.50
2003	0	From May 19th till June 27th	0
North end	100	12 applications	1.69
	200		3.37
2004	0	From May 24th till June 29th	0
North end	400	6 applications	4.73
	800		9.45

Two g/ tree a year is the normal amount growers use (Hanson et al. 1987).

Fruit quality assessments

Fruit, 10 per tree, were selected at random on well exposed limbs for determinations of fresh weight, pulling force, total soluble solids, and drain weight (percentage of pulp weight over total flesh weight after freezing and thawing; pulp weight = total flesh weight - juice weight). These parameters were measured at 5-day intervals starting approximately one month AFB.

Fruit pulling force is the force it takes to remove a fruit from its pedicel. It was measured with a mechanical force gage (Hunter Spring, Hartfield, Pennsylvania). Total soluble

solids were read with a pocket refractometer (Pocket PAL-1, ATAGO). For drain weights, fruits were pitted prior to freezing at -18°C for 24 h. The frozen fruits were then thawed in 50 mL Corning tubes containing 4 grams of 3 mm diameter glass beads. The addition of the beads helped to separate the juice and pulp. Immediately after the fruits thawed, the tubes were centrifuged at 1,000 g for 5 min. The juice and pulp were separated and weighed. The drain weight (pulp) was calculated as the percentage of the pulp weight over total pitted fruit weight.

Colorimetric Analysis of B

Colorimetric analyses of B were performed as described by Lopez (1993) using a BioSpec-1601 spectrophotometer (Shimadzu) and UVProbe 2.00 software (Shimadzu).

Preparation before conducting colorimetric analysis

Cherry bud, flower, leaf, and fruit were sampled randomly on well exposed limbs, 10 per tree. Tissues were freeze-dried till constant weight. Samples were ground and an aliquot of around 0.5 g per sample was weighed, ashed at 550 °C for 6 h and then dissolved in 1 ml of 3 N HNO_3.

Splat Test

The splat test is designed to mimic mechanical harvesting. Fruits were dropped two meters, bouncing twice on 45° inclined wooden boards before they hit the floor. Fruits were then collected and classified into three categories: good, soft but not cracked, and cracked.

Field plot design and Statistical Analysis

A complete random design (CRD) with subsamples (2 subsamples per plot, 6 plots per treatment) and repeated measures (sampling days) was used as the field plot design.

Statistical analysis was performed on treatment means using either PROC GLM or PROC MIXED procedures in SAS version 8.0 (SAS Institute, Cary, N.C.). Error bars represent 95% confidence intervals.

RESULTS AND DISCUSSION

Foliar application of B influences fruit B concentration

B treatments did not influence fresh weight, pulling force, total soluble solids, and drain weight three years in a row, from 2002 to 2004 (drain weight data not shown), with concentrations of B in the sprays ranging from 0 to 800 ppm (Figures 1, 2 and 3). Fresh weight, pulling force, and total soluble solids data were otherwise typical of normal development.

Since pulling force is related to abscission, the lack of B treatment effects on pulling force indicates that B does not influence abscission. Since total soluble solids (TSS) level represents TSS accumulation or transport of photosynthetic products into the fruit, the lack of B treatment effects on TSS indicates that B did not influence TSS dynamics in the fruit.

Fruit B concentrations (Figures 4 to 11) were measured in order to know whether foliar B applications resulted in transport into the fruit. Mesocarp (flesh) and pit B were measured separately. Data are presented in two ways: μg of B per fruit and μg of B per gram of dry weight (Figures 4, 5, 8 and 9). B effects on fruit B concentration in 2002 are not shown: the highest concentration of B in the 2002 sprays was 50 ppm. The first B effects were observed in 2003 on 57 days after full bloom (AFB), in fruit mesocarp from trees treated with 200 ppm B. However, there were no significant differences in mesocarp

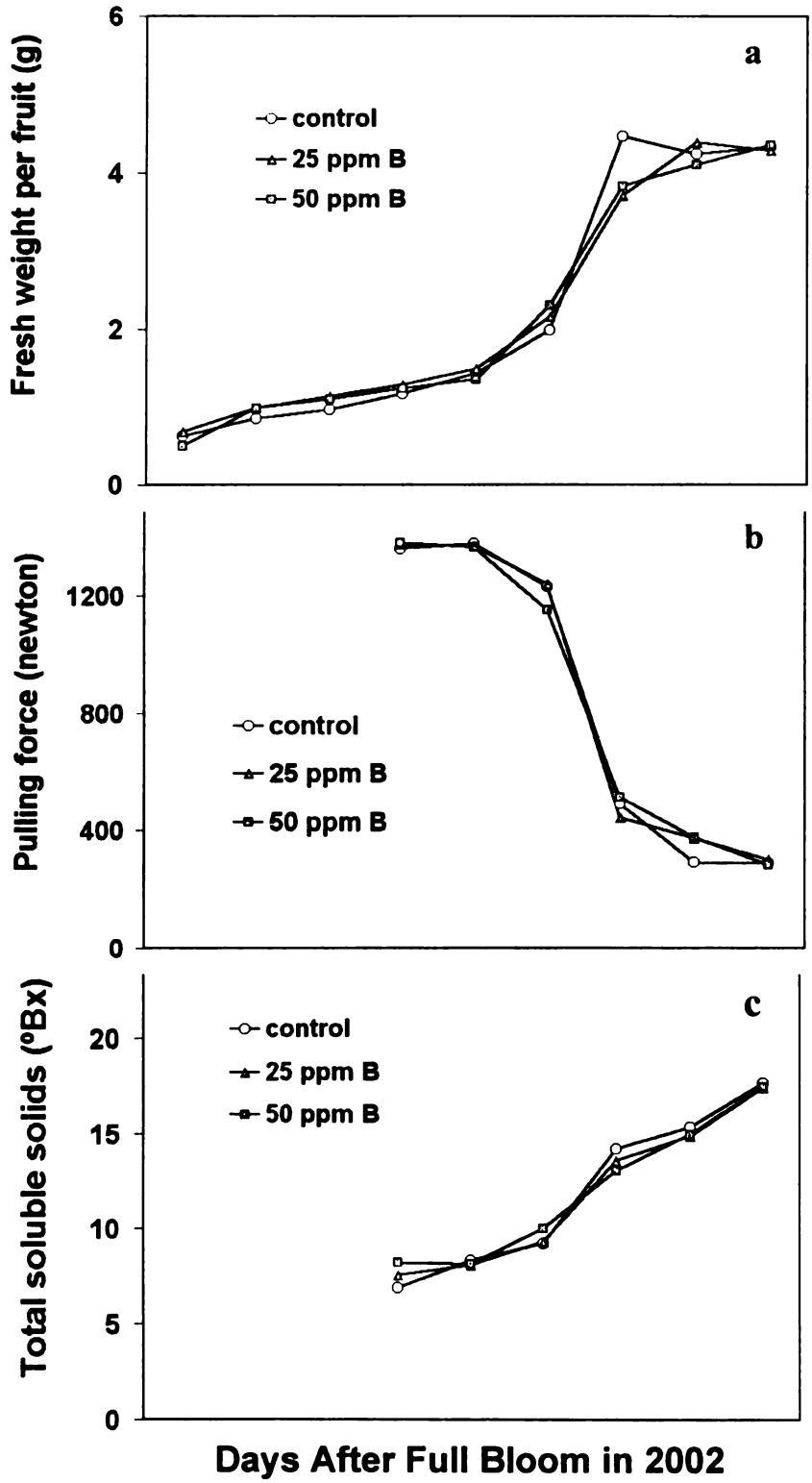

Figure 1. 2002 fruit quality parameters.
a. Fresh weight per fruit. b. Pulling force per fruit. c. Degree of total soluble solids (°Brix) per fruit. Data shown are the means of 12 replicates.

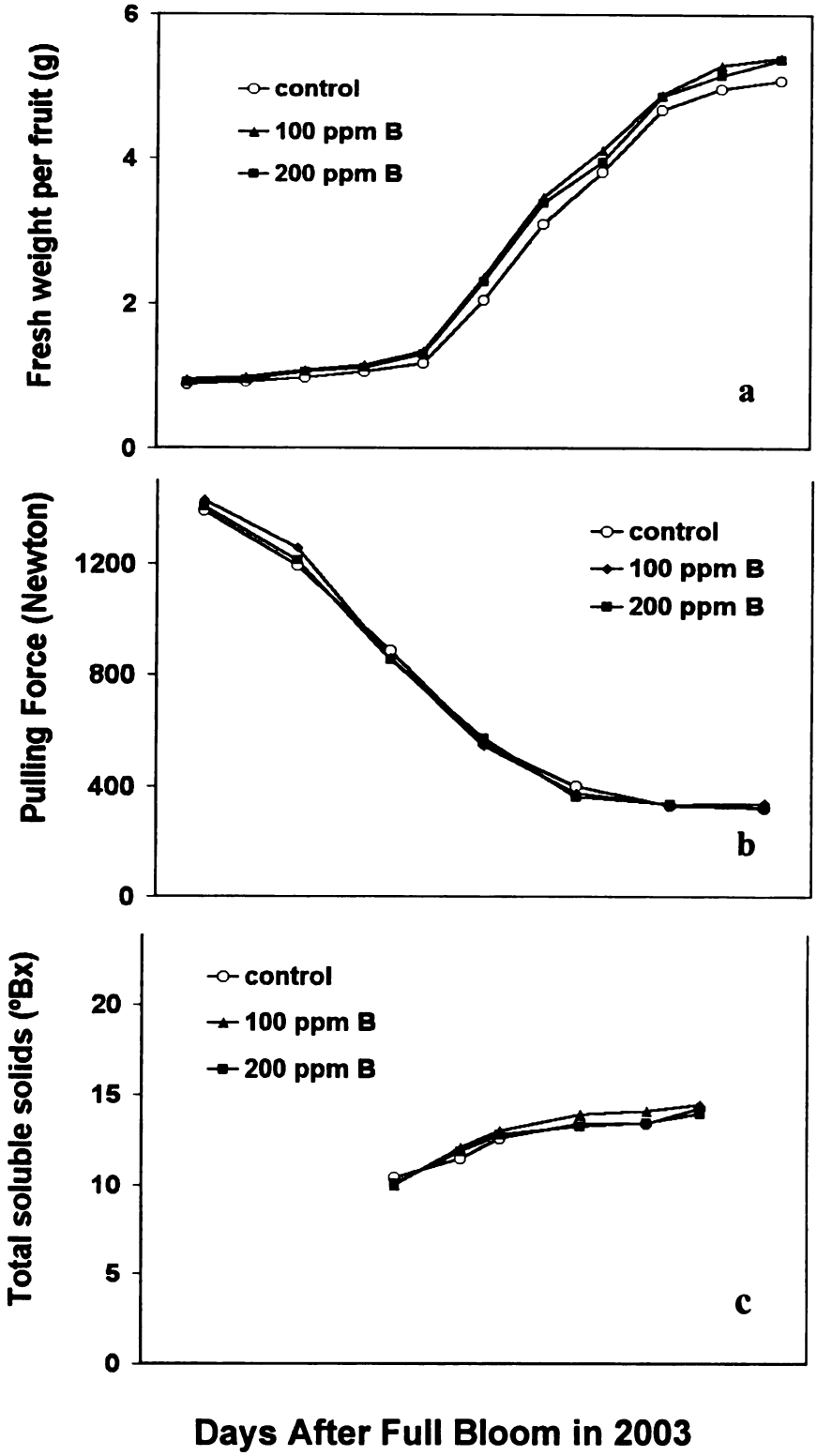

Figure 2. 2003 fruit quality parameters.
a. Fresh weight per fruit. b. Pulling force per fruit. c. Degree of total soluble solids (°Brix) per fruit. Data shown are the means of 12 replicates.

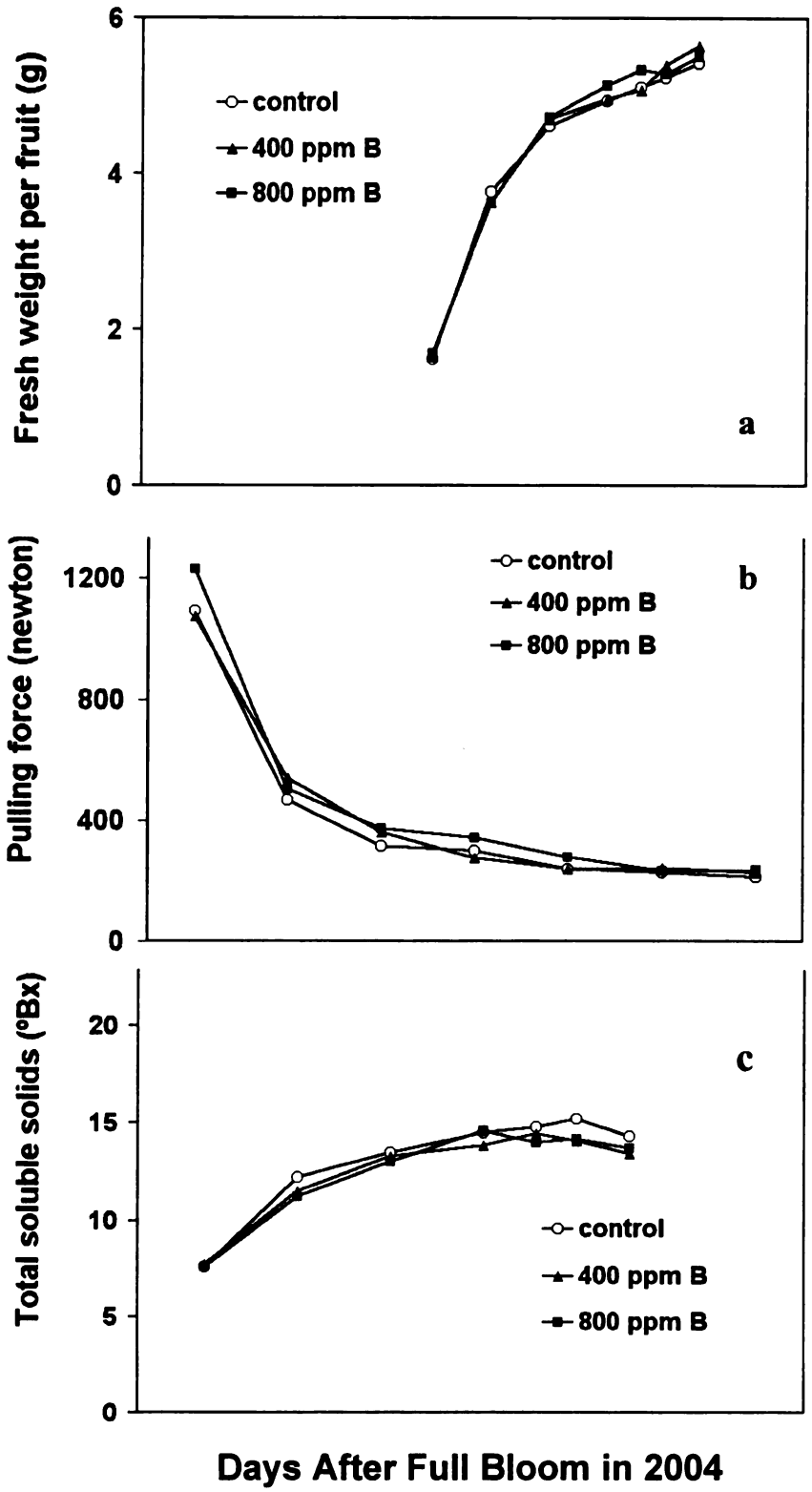

Figure 3. 2004 fruit quality parameters.
a. Fresh weight per fruit. b. Pulling force per fruit. c. Degree of total soluble solids (°Brix) per fruit. Data shown are the means of 12 replicates.

B concentrations between the control and 100 ppm treatments, nor between 100 ppm and 200 ppm treatments. On 63 and 68 days AFB 2003, there were significant differences in mesocarp B among all the treatments (0, 100 and 200 ppm); while on 72 days AFB 2003, B treatment effects on mesocarp were only observed between the control and 200 ppm B.

Interestingly, in 2004, with higher B applications (0, 400 and 800 ppm), significant treatment differences in mesocarp B levels were observed starting from 21 days AFB and continuing until the last day of sampling, 67 days AFB. However, differences between 400 ppm and 800 ppm were only significant on some sampling dates. One possible reason is that 400 ppm was high enough so that fruit B accumulation (perhaps as cell wall binding capacity) may have been saturated (Figures 6 and 7). Note that in 2003, foliar spray of B at 100 ppm was applied 12 times in total, making the total amount of applied B (1.7 g of B per tree) close to the annual amount growers use (growers apply once a year 1 lb of B over 227 trees per acre, or 2 g of B per year).

Our observations, especially the increase in B fruit levels after sprays stopped, indicated three things: 1. B is mobile in sour cherry, which is consistent with previous studies (Hanson, 1991); 2. B from foliar B application is translocated into the fruit; 3. B accumulation in the fruit is dose-dependent, e.g. with higher B applications, the treatment effects were evident much earlier in 2004 than in 2003.

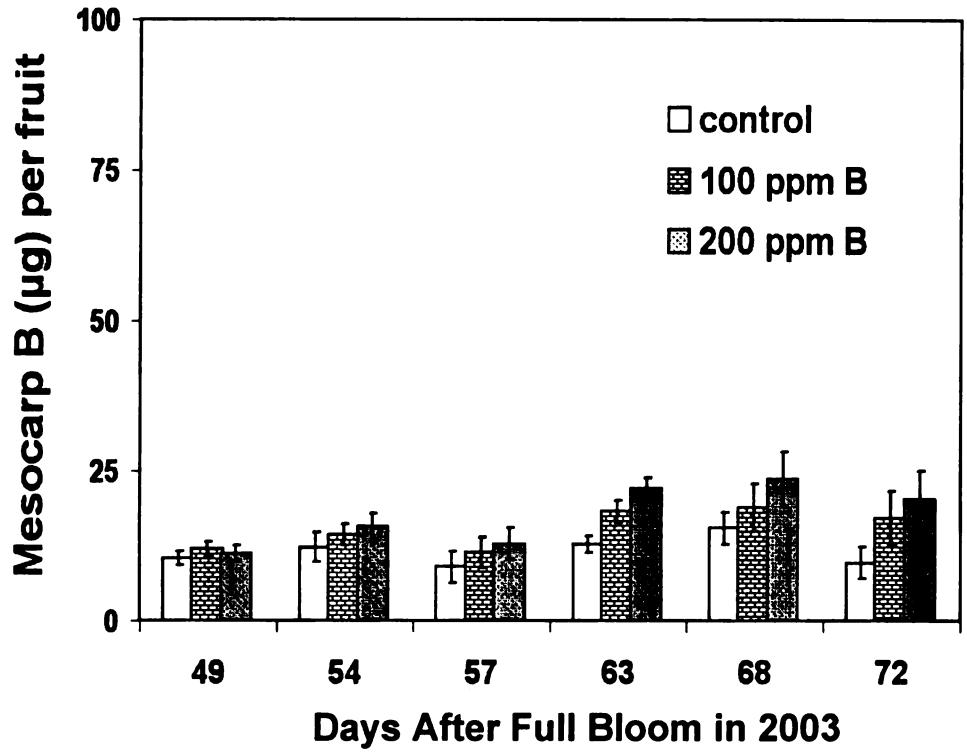

Figure 4. Amount of mesocarp B (µg) on a per fruit basis, 2003. Data shows mean ± SE of 10 replicates

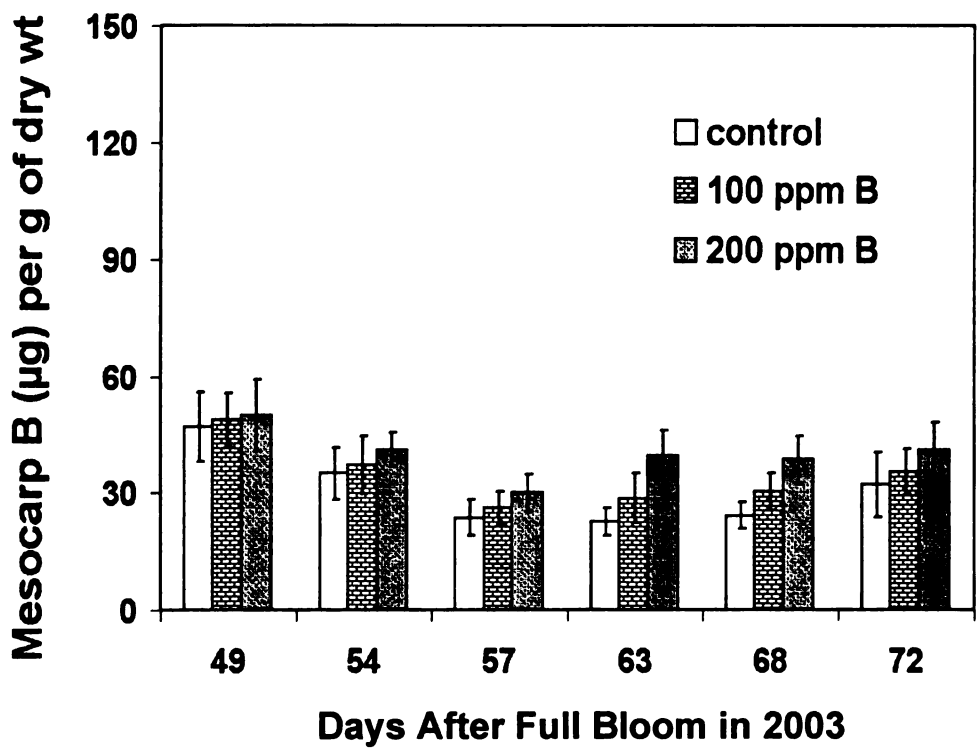

Figure 5. Amount of mesocarp B (µg) on a per dry weight (g) basis, 2003. Data shows mean ± SE of 10 replicates.

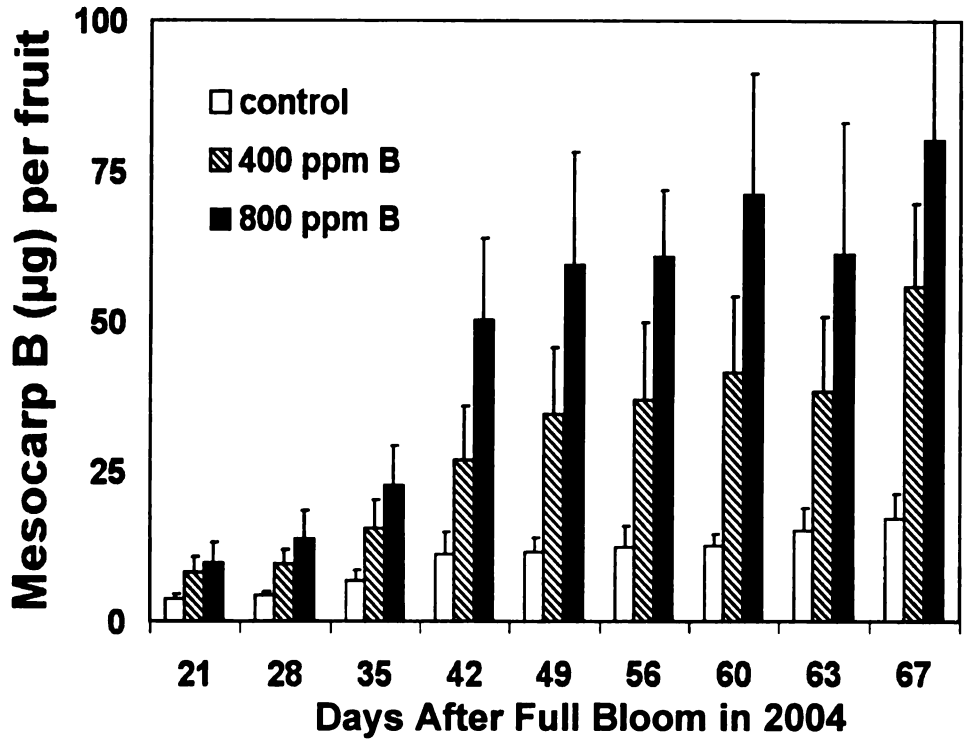

Figure 6. Amount of mesocarp B (µg) on a per fruit basis, 2004. Data shown are the means ± SE of 10 replicates.

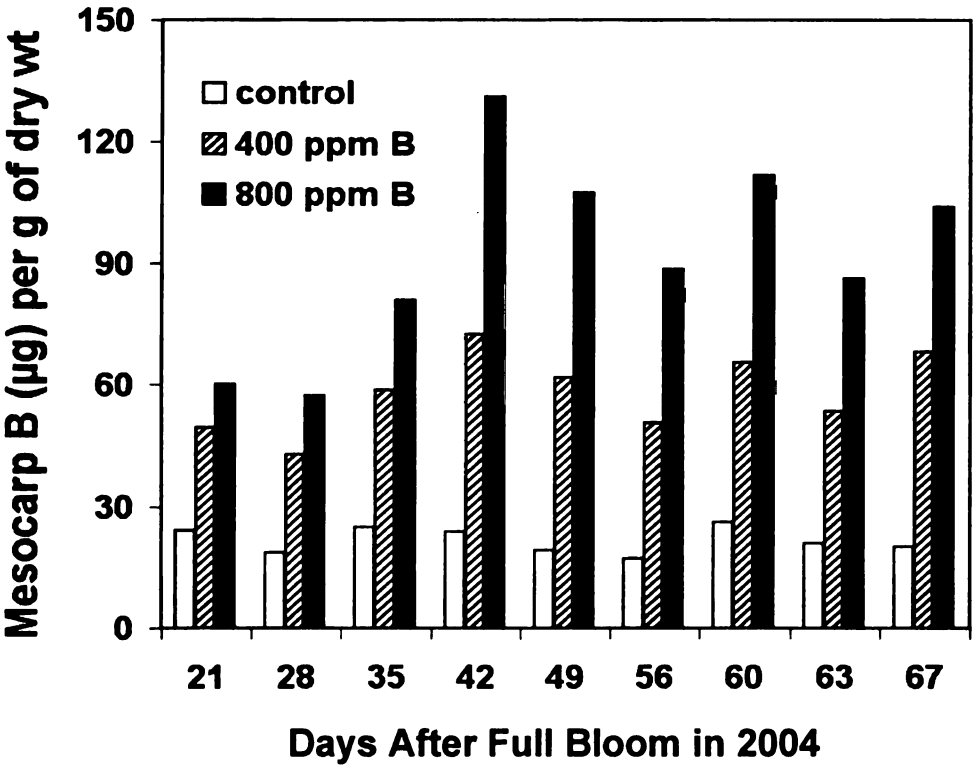

Figure 7. Amount of mesocarp B (µg) on a per gram of dry weight basis, 2004. Data shown are the means ± SE of 10 replicates.

Pit B levels

In contrast with mesocarp (around 20 μg of B per fruit in the control and up to 80 μg of B per fruit with 800 ppm B treatment), there were only trace amounts of B in pits (less than 3 μg of B per fruit for all the treatments; Figures 8 and 9). Considering that the pit could not be separated from mesocarp until 42 days AFB (2004), at which time the pit already completed the hardening process, one explanation is that B applications may not get into pit after pit hardening. However, although the amount measured was small, there was still a significant treatment effect.

In 2004, from 42 to 67 days AFB, there were always significant treatment effects on μg of B per pit and μg of B per g of dry weight, but the differences between 400 ppm B and 800 ppm B were insignificant on some sampling dates (Figures 10 and 11). The differences were, however, always consistent, whether expressed as μg of B per fruit or as μg of B per g of dry weight.

On some sampling dates, all three B treatments differed on both amount of B per pit and amount per dry weight basis. While on other dates, there were no significant differences between 400 ppm and 800 ppm (Tables 1 and 2). One explanation is that pit hardening started early so that pit development was complete before B sprays had an effect.

Table 2. Amount of mesocarp B (μg) per fruit in 2004.
Treatments as described in 'Materials and methods'. Data shows means ±SE of 10 replicates. DAFB= Days After Full Bloom. Means with the same letter are not significantly different.

DAFB / Treatment	42	49	56	60	63	67
800 ppm B	50.3 (±13.4) [a]	59.5 (±18.6) [a]	60.8 (±11.0) [a]	71.2 (±20.1) [a]	61.2 (±21.9) [a]	80.4 (±28.3) [a]
400 ppm B	26.8 (±9.1) [b]	34.4 (±11.2) [b]	38.4 (±13.8) [b]	41.4 (±12.7) [b]	38.1 (±12.5) [b]	55.4 (±14.3) [b]
control	11.1 (±3.7) [c]	11.6 (±2.3) [c]	11.8 (±3.7) [c]	12.5 (±2.1) [b]	15.1 (±3.8) [c]	17.1 (±4.1) [c]

Table 3. Amount of pit B (μg) per fruit in 2004

Treatments as described in 'Materials and methods'. Data shows means ±SE of 10 replicates. DAFB= Days After Full Bloom. Means with the same letter are not significantly different.

DAFB / Treatment	42	49	56	60	63	67
800 ppm B	2.2 (±0.6) [a]	2.0 (±0.7) [a]	1.9 (±0.7) [a]	2.3 (±0.5) [a]	2.1 (±0.5) [a]	1.8 (±0.4) [a]
400 ppm B	1.4 (±0.3) [a]	1.5 (±0.3) [a]	1.2 (±0.3) [b]	1.8 (±0.4) [b]	1.8 (±0.5) [a]	1.8 (±0.3) [a]
control	1.0 (±0.3) [b]	0.8 (±0.1) [b]	0.7 (±0.1) [c]	1.3 (±0.2) [c]	1.1 (±0.2) [b]	1.2 (±0.2) [b]

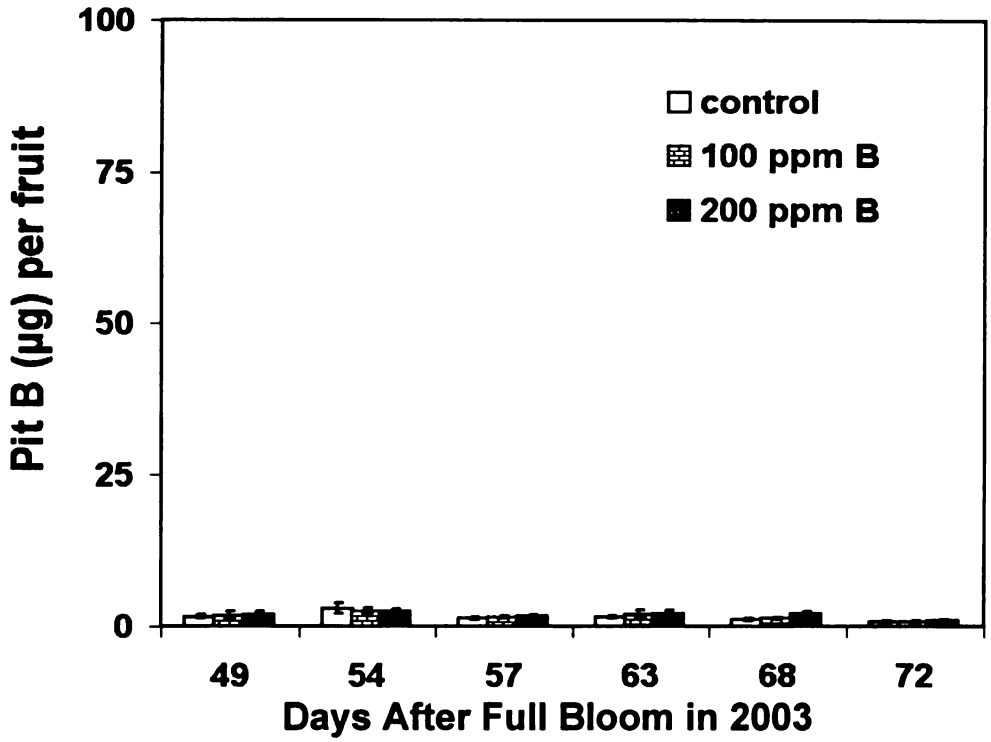

Figure 8. Amount of pit B (µg) on a per fruit basis, 2003.
Data shown are the means ± SE of 10 replicates.

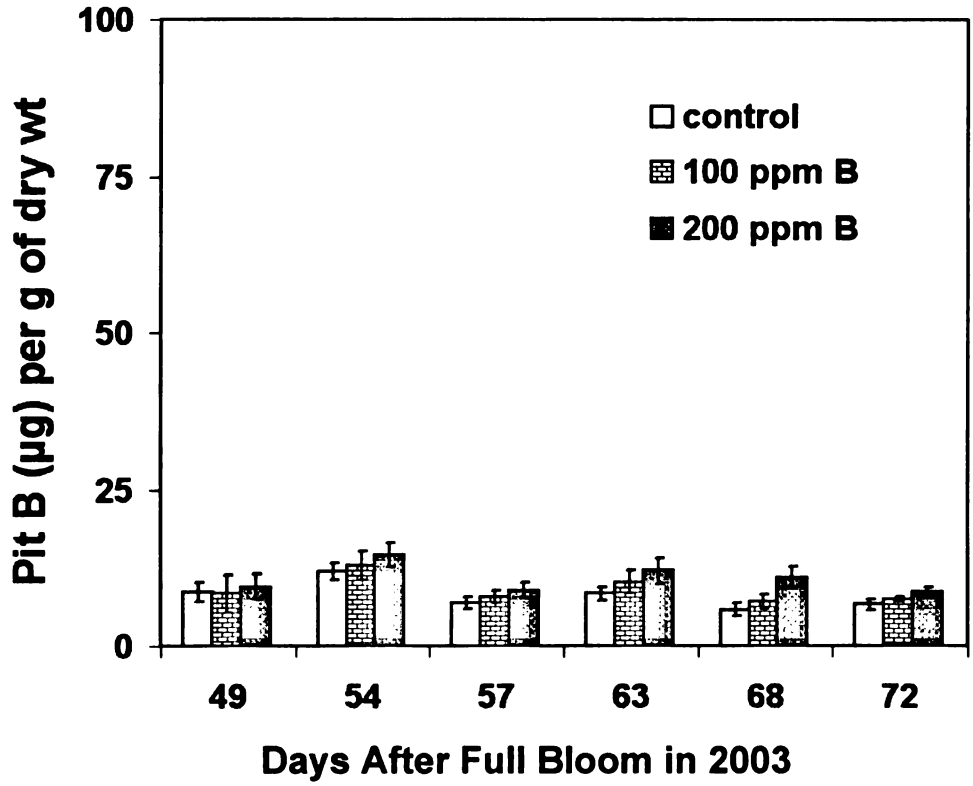

Figure 9. Amount of pit B (µg) on a per dry weight (g) basis, 2003.
Data shown are the means ± SE of 10 replicates.

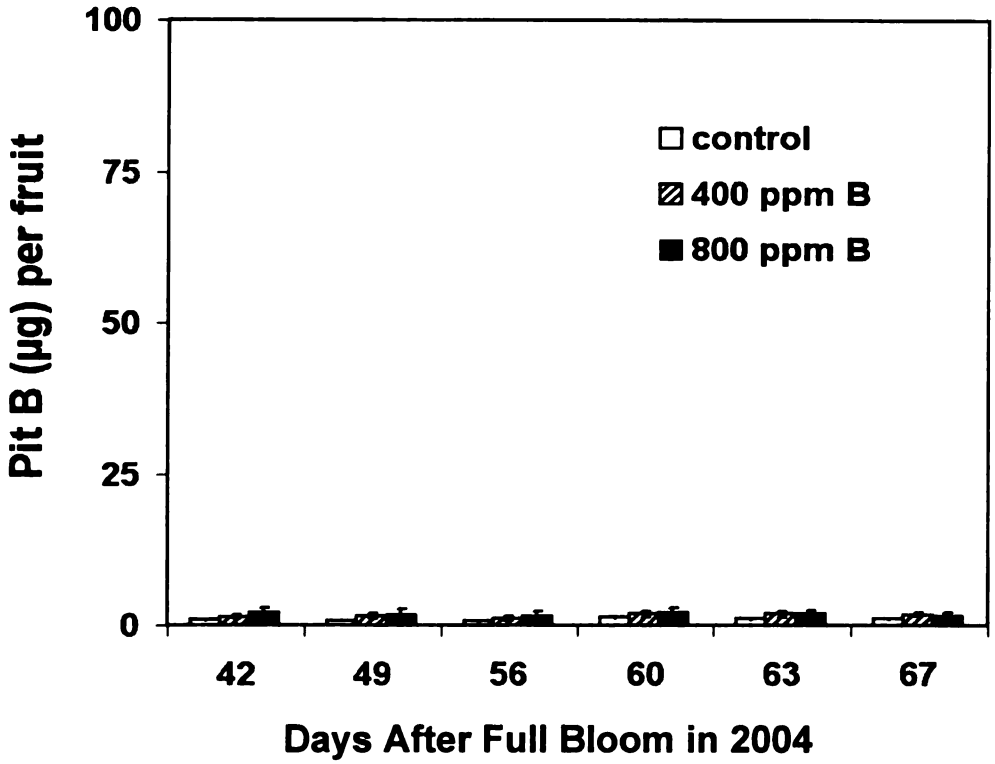

Figure 10. Amount of pit B (µg) on a per fruit basis, 2004.
Data shown are the means ± SE of 10 replicates.

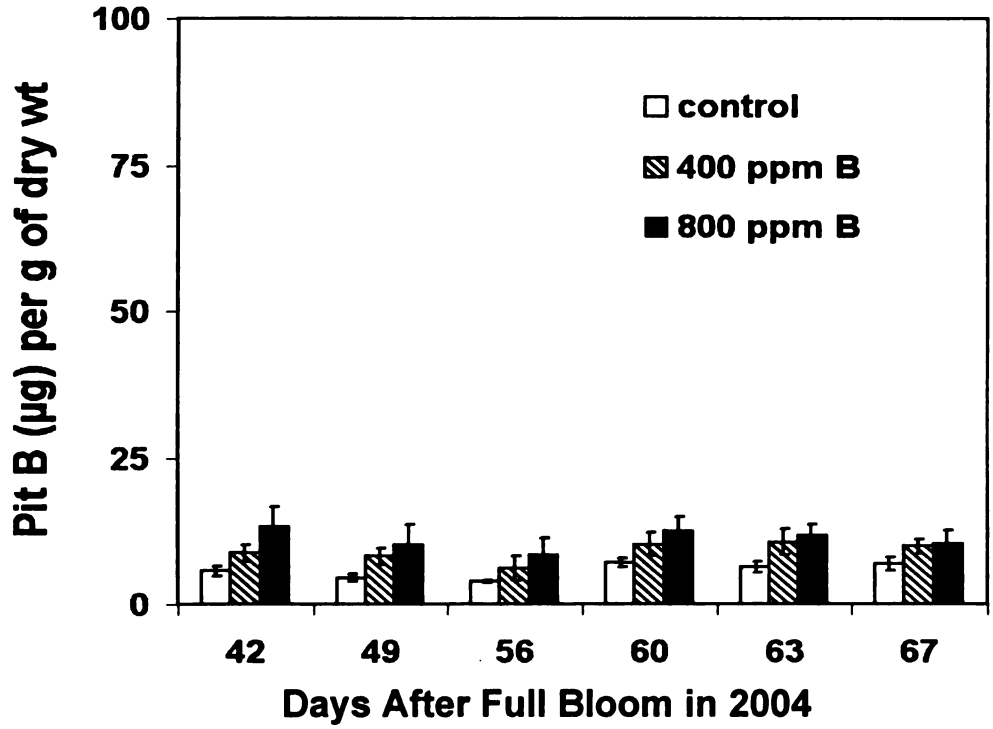

Figure 11. Amount of pit B (µg) on a per gram of dry weight basis, 2004.
Data shown are the means ± SE of 10 replicates.

Leaf B levels

Interestingly, the treatment effect on 2004 leaf B levels (Figures 2.3 a and 2.3 b) was not as significant as the treatment effect on fruit flesh (mesocarp). On 57 days AFB, there was no difference in leaf B level between the three treatments. But on 63 and 67 days AFB, although 400 ppm B and 800 ppm B did not differ significantly, the control was different from the other two treatments (Table 3; Figures 12 and 13). On 67 days AFB, on a per leaf basis, there was no difference between the control and the 400 ppm treatment. It is likely that B is so mobile in sour cherry that it almost immediately relocates to wherever there is an active sink, namely, fruit from 57 days AFB till 63 days AFB. Consequently, leaf B may not be a good standard for growers to judge cherry B deficiency/toxicity levels.

Table 4. Amount of leaf B (μg) per leaf in 2004
Treatments as described in 'Materials and Methods'. Data shows means ± SE of 10 replicates. DAFB= Days After Full Bloom. Means with the same letter are not significantly different.

DAFB / Treatment	53	63	67
800 ppm B	**14.8 (+3.5) [a]**	**11.7 (+ 0.8) [a]**	**9.7 (+ 1.7) [a]**
400 ppm B	**13.7 (+3.3) [a]**	**11.2 (+ 2.2) [a]**	**10.0 (+ 1.3) [b]**
control	**9.7 (+1.3) [a]**	**8.1 (+ 1.0) [b]**	**13.8 (+ 2.3) [b]**

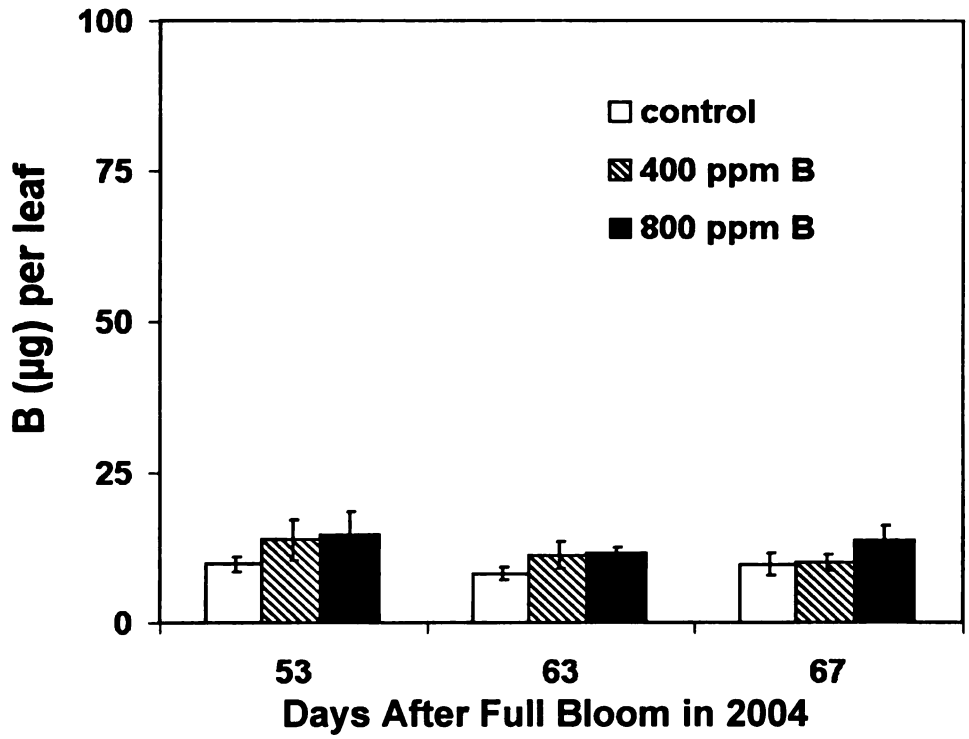

Figure 12. Amount of B (µg) on a per leaf basis, 2004, after 2004 B spray. Data shown are the means ± SE of 10 replicates.

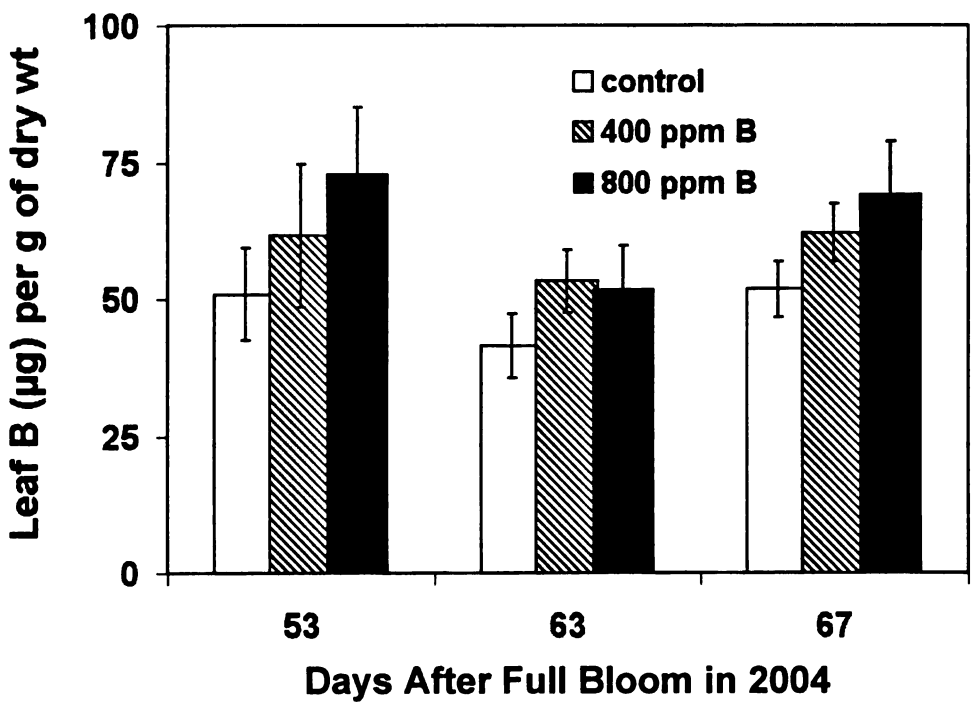

Figure 13. Amount of B (µg) on a per dry weight (g) basis, 2004, after 2004 B spray. Data shown are the means ± SE of 10 replicates.

Effect of the previous year's treatment

There was no significant treatment effect (carry-over effect) from 2003 on 2004 fruits (Figures 14 and 15). The total amount of B per fruit increased over time while the concentration (marked as B per unit dry weight) slightly decreased, probably because fruits grow fast at this early development stage. This increase in B per fruit also indicated a B stock or reserve in the plant. Fruit B levels increased as with time even without B spray (Figure 14), indicating a pool of B in sour cherry was available to new growth. Similar results (no significant treatment effects) were also observed in bud, flower and leaf tissues in 2004 (Figures 16 and 17).

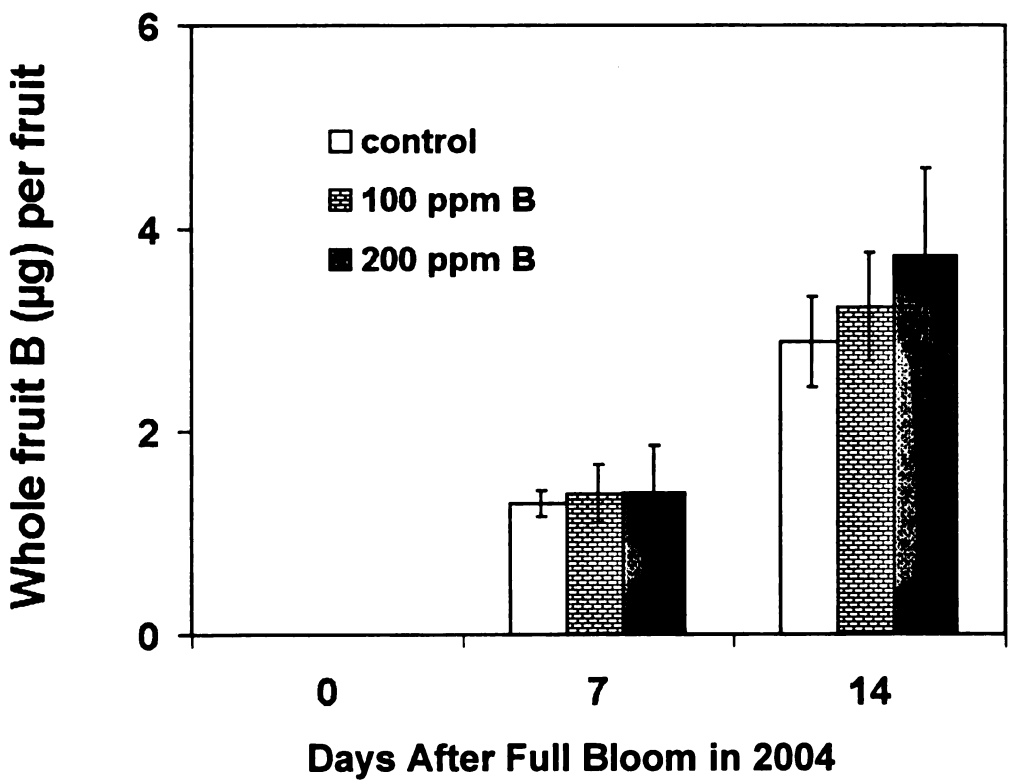

Figure 14. Amount of mesocarp B (µg) on a per fruit basis, 2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

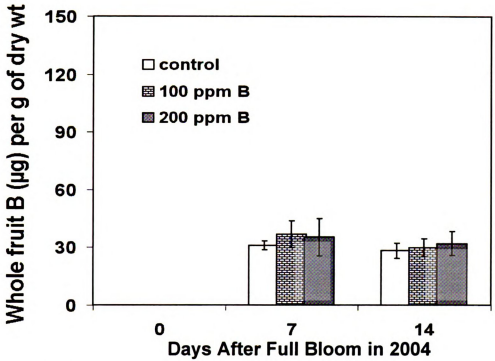

Figure 15. Amount of mesocarp B (µg) on a per dry weight basis, 2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

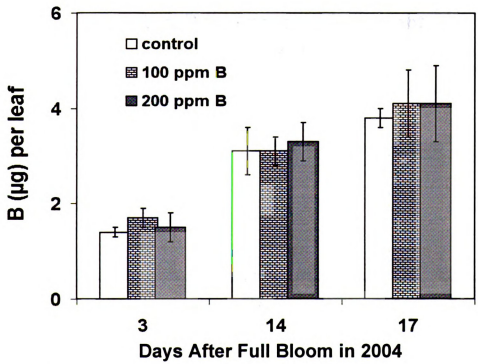

Figure 16. Amount of B (µg) per leaf, 2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

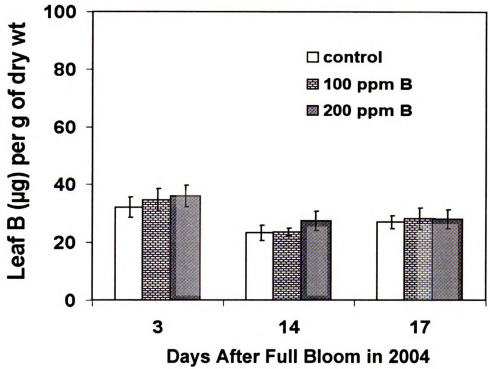

Figure17. Amount of leaf B (µg) per gram of dry weight (g), 2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

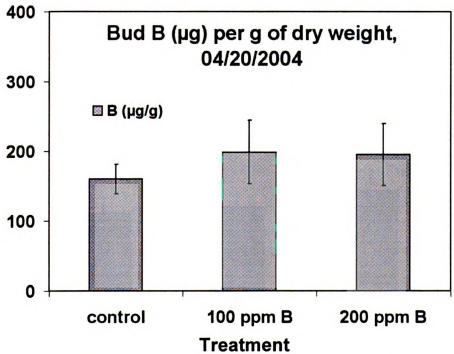

Figure 18. Amount of bud B (µg) on a per gram of dry weight (g) basis, 04/20/2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

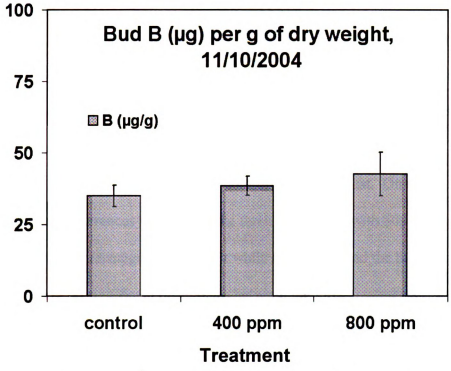

Figure 19. Amount of bud B (µg) on a per gram of dry weight basis, 11/10/2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

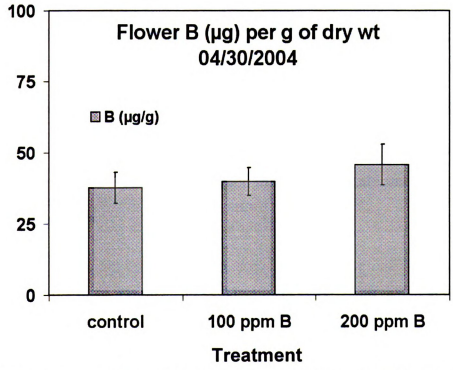

Figure 20. Amount of flower B (µg) on a per gram of dry weight (g) basis, 04/30/2004, before 2004 B spray started. Data shown are the means ± SE of 10 replicates.

Splat test

In 2003, splat tests were carried out on day 68 and 77 AFB. There was no significant difference between 100 ppm, 200 ppm, and control treated fruit (Table 4, Figure 21). In 2004, splat tests were carried out on day 63, 67 and 75 AFB. On day 63 AFB, there was a difference between control and 800 ppm treatments. The 800 ppm treatment had the lowest percentage of good fruit while the control had the highest. On 67 days AFB, there were significant differences between all the three treatments, with 800 ppm treatments having the lowest percentage of good fruit while the control had the highest. On 75 days AFB, there were no significant differences between the three treatments (Figure 22; Table 5); however, all fruits were about to abscise.

Fruits appeared to mature faster in 2004 than in 2003. As shown in Table 1, on 77 days AFB in 2003, the good fruit percentage ranged from 77% to 83%; while in 2004, on 75 days AFB, the good fruit percentage ranged from 8% to 15%. A week after, on 82 days AFB, all the fruits were gone (abscised). Therefore a time adjustment may be needed before we can compare 2004 splat test results with 2003 splat test results. Since 75 days AFB is very late in the harvest season and all fruits become soft eventually, it is within expectations to see low percentages of good fruits and no significant difference between the three treatments.

One reason why there was no significant treatment effect on percent of good fruit observed in 2003 may be due to the low concentration of the B applications. Growers now use in a single application the equivalent of one lb (454 g) of elemental B per acre a

Table 5. Splat test results in 2003
Splat tests were performed within 12 h after fruits were sampled from HTRC. Good fruit were defined as fruit which was not cracked and still firm after the splat test. $\alpha = 0.05$ was used as significance level. Means with the same letter are not significantly different.

Treatment	Percentage of good fruit on Day 68 AFB ($\pm$ SE)	Percentage of good fruit on Day 77 AFB ($\pm$ SE)
100 ppm B	94 %($\pm$ 3%) [a]	77 %($\pm$ 6%) [a]
200 ppm B	95 %($\pm$ 3%) [a]	78 %($\pm$ 10%) [a]
control	95 %($\pm$ 2%) [a]	83 %($\pm$ 4%) [a]

Table 6. Splat test results in 2004
Experimental details are described in Table 2. $\alpha = 0.05$ is used as significance level. Means with the same letter are not significantly different.

Treatment	Percentage of good fruit on Day 63 AFB ($\pm$ SE)	Percentage of good fruit on Day 67 AFB ($\pm$ SE)	Percentage of good fruit on Day 75 AFB ($\pm$ SE)
400 ppm B	61 %($\pm$ 20%) [ab]	78 %($\pm$ 10%) [a]	8 %($\pm$ 13%) [a]
800 ppm B	52 %($\pm$ 16%) [a]	65 %($\pm$ 8%) [b]	15 %($\pm$ 11%) [a]
control	72 %($\pm$ 16%) [b]	92 %($\pm$ 7%) [c]	8 %($\pm$ 10%) [a]

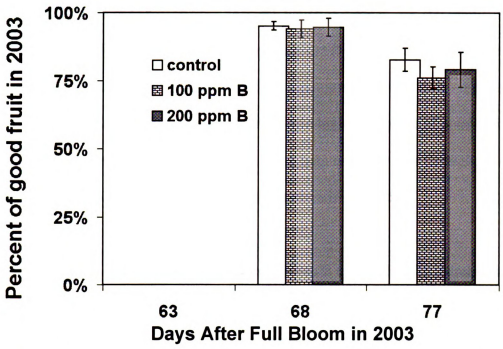

Figure 21. Percentage of good fruit of sour cherry after splat test in 2003. Cherries were treated with three levels of B foliar spray (control, 100 ppm B and 200 ppm B). Data shown are the means ± SE of 10 replicates.

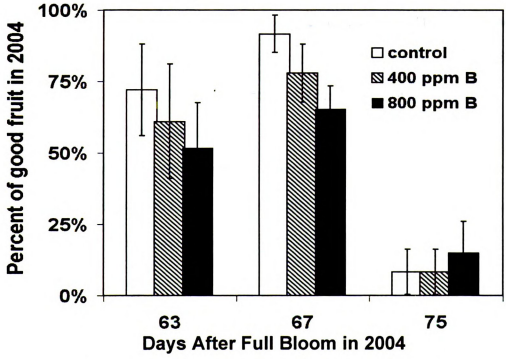

Figure 22. Percentage of good fruit of sour cherry after splat test in 2004. Cherries were treated with three levels of B foliar spray (control, 400 ppm B and 800 ppm B). Data shown are the means ± SE of 10 replicates.

year (227 trees per acre, 561 per hectare), which is close to our 100 ppm B application. As shown in Table 2, early harvest could prevent B treatment effects to some extent (63 days AFB), but a later harvest showed a B treatment effect (67 days AFB). To further understand whether early harvest (marked by a high percentage of good fruit) would necessarily avoid a B treatment effect, more frequent splat tests throughout fruit development with 400/800 ppm B applications are required.

In conclusion, although the correlation between B applications to increase fruit set and percentage of good fruit is not yet clear, evidence here indicates that early or timely harvests can avoid the soft fruit problem. Another option is that a 400 ppm treatment, the equivalent of what growers routinely apply, may also be the threshold of B effects on soft fruit. Since B foliar applications may increase fruit set and a significant B effect will not be observed if B application does not exceed 2 g per tree a year, it would be beneficial for sour cherry industry to use B foliar applications only if fruit B levels are carefully monitored, i.e. less than 50 μg mesocarp B on a per gram of dry weight basis. If a significant B treatment effect on fruit is observed, then B applications or concentrations should be adjusted or simply stopped in order to avoid the possibility of consequent soft fruit problems. However, based on these results, leaf B level is not recommended as a way to monitor fruit B dynamics.

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