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Date November 30, 1988

THE INFLUENCE OF BORON ON THE MORPHOLOGICAL DEVELOPMENT AND CELL WALL COMPOSITION OF *BRASSICA OLERACEA* VAR. *ITALICA*

A Thesis

Presented for the

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Degree

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ABSTRACT

Broccoli (Brassica oleracea var. italica (L.) cv. 'Premium Crop') was grown in perlite and supplied with nutrient solutions containing 0.08, 0.16, 0.32, 0.81, 1.62, or 8.10 $mg \cdot L^{-1}$ boron. Growth measurements were taken at the 5-, 10-, 15-leaf, and mature (20-leaf) stages of development. These measurements included fresh and dry weights of a mature upper and lower leaf, upper and lower leaf area, percent dry weight of pith and exterior stem samples, and fresh weight of florets and stem. Boron did not have a pronounced effect on growth until the plants had reached the 15-leaf stage. However, at maturity, stem fresh weight increased as boron concentration increased up to 1.62 $mg \cdot L^{-1}$, then declined at 8.10 $mg \cdot L^{-1}$ (boron toxicity). Boron deficient plants (0.08 $mg \cdot L^{-1}$) had higher percent dry leaf weights than plants with sufficient boron (0.81 $mg \cdot L^{-1}$). Floret weight increased as boron concentration increased from 0.08 to 1.62 $mg \cdot L^{-1}$. Maximum floret weight was achieved by plants that had leaf and stem boron concentrations of 20 to 100 $\mu g \cdot g^{-1}$ and 12 to 20 $\mu g \cdot g^{-1}$, respectively, at maturity. Hollow stem, cell wall content, and mineral analysis measurements were taken at maturity. Mature boron deficient plants had a greater incidence of hollow stem and increased amounts of cell wall in the stem interior and exterior. Increased calcium concentration was also found in stem interior tissue and extracted cell walls of boron deficient plants. Boron deficiency decreased the covalently and ionically-associated pectin fractions of the cell wall and slightly increased the

hemicellulosic and cellulosic fractions. This may indicate a relationship between cell wall composition and hollow stem under boron deficient conditions.

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PART I

OVERVIEW

CHAPTER I

INTRODUCTION

Broccoli has become an important vegetable crop in recent years due to increased demand from health conscious individuals seeking high quality fresh produce. Increasing production costs have created an economic motive for increased yields of high quality produce. Broccoli is subject to several physiological disorders such as hollow stem, brown bead formation, and stem scaling which are all associated with boron deficiency. These disorders have an adverse effect on market quality and boron deficiency may also decrease yield. In order to develop methods of improving quality and increasing yield at the lowest cost possible, the metabolic role of boron and the optimal range of boron in the plant must be determined.

Boron deficiency is the most widespread deficiency of the micronutrients (Gupta 1979), perhaps because boron is both easily leached from the soil and is less available under drought conditions. Most plant-available boron comes from soil organic matter and from soil surface particles. Less than 5% of the boron in the soil is available to the crop (Gupta 1979). Many soils, including some in Tennessee, are deficient in boron. In addition, boron uptake is influenced by temperature and light intensity (Gupta et al. 1985). Higher temperatures and greater light intensities increase transpiration and, therefore, boron uptake. The amount of boron in various aged plant

parts differs due to low mobility (Gupta et al. 1985). This low mobility may lead to boron deficiency or toxicity symptoms in one part of the plant without these symptoms being present in other parts of the plant which may be more economically or metabolically important.

Even though boron is one of the seven essential micronutrients for higher plants, its metabolic role remains unclear (Gupta et al. 1985). Although boron has been reported to be involved in cell division, cell wall metabolism, sugar transport, and membrane integrity (Gupta 1979; Gupta et al. 1985), the exact role of boron in these processes is uncertain. The difficulty of determining the exact role of this nutrient may be due to problems in separating primary effects from secondary changes due to the effects of boron deficiency or toxicity on growth (Pollard et al. 1977). Also, many boron experiments subject plants only to extreme solutions (i.e. containing no boron, or sufficient boron) with no intermediate concentrations. Different concentrations of boron may give varying levels of responses and aid in determining the range of boron which is critical for plant growth. In addition, plants require only small amounts of boron which requires accurate and precise analysis.

Vegetables, particularly cole crops, require more boron than most other crops (Gupta 1979). Boron deficiency causes reduced yield and quality of many crops. In cole crops, internal browning of the stem is often observed in connection with boron deficiency (Gupta 1979). In broccoli, this internal browning is also known as "hollow stalk," "hollow heart," or "hollow stem" because in advanced cases of boron

deficiency the pith area develops a visible cavity. Mengel and Kirkby (1979) described boron deficiency symptoms in cauliflower, another cole crop, as cork-like material in the interior and on the exterior of the plant. However, Gupta and Cutcliffe (1975) found no relationship between the occurrence of hollow stem and boron concentrations in soil or plant tissues. Although hollow stem has been described, the exact nature of the disorder has not been ascertained. Since boron has been shown to affect cell walls (Kouchi and Kumazawa 1975), it is possible that boron affects the cell walls of pith cells, thus leading to hollow stem.

Brassica oleracea var. italica (green sprouting broccoli) is a good species for investigations into the physiological role of boron because it has a high requirement for boron (Gupta et al. 1985) and because it is particularly susceptible to hollow stem. Most of the information published concerning boron in plant material has used plant segments or tissue culture techniques which examine one part of the plant without considering the entire plant. Also, various degrees of boron deficiency have not been thoroughly examined.

The objectives of this research were: (1) to determine the effects of boron concentration on developmental changes in the cell wall and its components and on dry matter partitioning during the life cycle of Brassica oleracea var. italica, and (2) to relate these factors to the occurrence of hollow stem in broccoli.

CHAPTER II

LITERATURE REVIEW

BROCCOLI

The origins of Brassica oleracea var. italica (L.) (green sprouting broccoli) (Tiedjens 1943) can be traced to the coasts of the Mediterranean where many wild forms are still found. Observations of what may have been broccoli were recorded by the Romans. Broccoli was found in the United States as early as 1806 although it was not widely grown until extensive advertising broadened its appeal in the 1920's.

The closest relative of broccoli is cauliflower, Brassica oleracea var. botrytis (L.). Taxonomic separation of broccoli and cauliflower was recent (Prakash and Hinata 1980). B. oleracea var. italica was differentiated from B. oleracea var. botrytis by the presence of axillary buds which are small, single stem inflorescences formed in the leaf axes of broccoli. Broccoli was considered to be an evolutionary predecessor of cauliflower. Genetically, they are both diploids with eighteen chromosomes.

Broccoli is an annual with a large, central group of inflorescences in addition to the small axillary inflorescences. The central inflorescences consist of several short stems branching off the main stem. These divide further into multiple closely-spaced branches which support the edible flower buds. If the plant is allowed to bolt, the branches loosen and elongate. The buds open to flowers and

eventually become seed pods. Plants may obtain a height of approximately 90 cm prior to bolting. The leaves are large, dark green, and arise directly from the main stem. Beginning at the bottom of the stem, the leaves progressively increase in size to approximately half-way up the stem, then decrease in size near the inflorescences. Leaves nearest the inflorescences are small and elongated while the primary leaves are large and oval.

Broccoli is a cool season crop with optimum temperatures for production ranging from 16°C to 21°C (60°F to 70°F) (Seelig 1971). Broccoli grows best in well drained, slightly alkaline soil. It can be either directly seeded or transplanted into the field. Broccoli is harvested prior to the loosening of the inflorescences and the opening of the flower buds. The main stem is cut 9 to 25 cm below the inflorescences and all leaves are stripped. Occasionally, a second harvest of the axillary shoots is done. Mechanical harvesting is not common as time-to-maturity varies greatly even within a single variety. After harvest, heads are promptly cooled and shipped.

BORON IN SOIL

Boron deficiency is the most widespread deficiency of the micronutrients (Gupta 1979). Many soils are deficient in boron because it is easily leached from the soil and is less available under drought conditions. Most plant-available boron comes from soil organic matter and from soil surface particles. Less than 5% of boron in the soil is available to the crop (Gupta 1979). Boric acid $B(OH)_3$ is the

predominant form of boron in agricultural soils (Evans and Sparks 1983).

BORON IN PLANTS

Boron is recognized as one of seven essential micronutrients for higher plants, but its role in plant metabolism has not been fully explained (Gupta et al. 1985). RNA synthesis, growth and division of cells, photosynthesis, membrane integrity, calcium transport and metabolism, cell wall synthesis, and IAA metabolism all have been suggested as processes in which boron may be involved. Yet, the role of boron has not been precisely defined in any of these processes. The lack of firm conclusions about these effects may be due to the difficulty of separating primary metabolic effects of boron from secondary changes due to boron's effects on growth (Pollard et al. 1977). Because it is an essential micronutrient, boron deficiency or toxicity in the plant causes abnormal growth and thus induces secondary changes.

One process boron was associated with was meristematic growth. Boron appeared to be involved in meristematic growth by interaction with the hormone IAA and by control of sugar use in the meristem (Adams 1978). A continuous supply of boron to the roots was necessary as few plants can redistribute boron from leaves to the roots even under deficiency conditions. Dicots were also found to require more boron than monocots. An earlier study (Cohen and Lepper 1977) also showed that boron was required for meristematic activity and might act as a

regulator of cell division; but boron was not essential for cell elongation.

Another plant process boron has been associated with was changes in the cell membrane structure and integrity (Pilbeam and Kirkby 1983). Goldbach (1985) used cell suspensions to demonstrate that boron was essential for membrane function and integrity. Gauch (1972) speculated that the negative charge of sugar-borate complexes would enable the complex to pass through cell membranes easier than neutral sugar molecules.

Through its affect on membranes, boron affected photosynthesis. Boron increased both the phospholipid content of the chloroplast membrane and the photosynthetic rate of sunflowers (Hudak and Herich 1976). Boron deficiency also indirectly resulted in chloroplast destruction by inducing the destruction of stroma lamellae and single grana. Boron toxicity decreased chlorophyll content and photosynthetic rate of Cucurbita pepo (Lovatt and Bates 1984). Oertli and Kohl (1961) reported that in various plant species, boron toxicity symptoms are caused by excessive boron accumulation at the site of injury.

Boron has also been shown to affect the metabolism of nucleic acids and carbohydrates (Pilbeam and Kirkby 1983) and be involved in regulating metabolic activity between the glycolytic and pentose-phosphate shunt pathways (Lee and Aronoff 1967). Boron has been shown to be important in RNA synthesis. Therefore, boron deficiency occurs first in tissue with high RNA synthesis levels, such as meristems (Mengel and Kirkby 1982).

Before boron can affect plant process, it must be absorbed and translocated to the site of action. However, boron absorption and translocation in plants is not well understood. It is probably absorbed as undissociated boric acid (Mengel and Kirkby 1982). Uptake of boron by plants was primarily passive, with some active uptake occurring. Absorption was reduced under stress conditions such as drought, high soil pH conditions, and high soil calcium content (Goss 1973). Once in the plant, boron was relatively immobile compared to other micronutrients. Transpiration rate had a direct influence on the transport of boron in the plant, which accounts for the accumulation of boron in leaf margins. This fact may also indicate that boron was translocated in the xylem. Xylem transport of boron is further suggested by the fact that boron was absent from phloem sap. Boron was slowly lost from plant tissue due to leaching. Increased relative humidity resulted in a decreased transpiration rate and increased the amount of boron in roots, with the reverse also holding true (Halbrooks et al. 1986). Relative humidity did not affect the amount of shoot boron. Translocation of boron to shoots was suggested to be a mechanical sink effect.

Due to boron's low mobility in the plant, the amount of boron in plant tissue was closely associated with the relative age of that tissue relative to the age of the plant as a whole (Gupta et al. 1985). Boron moved passively, and there was a lack of transport by the phloem (Kohl and Oertli 1961). Shelp and Shattuck (1987) found that boron had limited phloem mobility but was redistributed from mature to young

leaves and roots when plants are boron starved. Martini and Thellier (1980) found that most of foliar-applied boron on white clover leaves stayed at the application point, but the remaining amount was very mobile, moving quickly from older to younger plant parts.

Cole crops such as broccoli are excellent for investigations into the role of boron in plants as these crops have a high physiological requirement for boron (Gupta 1979, Gupta et al. 1985). In cole crops, boron deficiency often caused internal browning of the stem (Gupta 1979). Mengel and Kirkby (1979) described boron deficiency symptoms in cauliflower as cork-like material in the interior and on the exterior of the plant. This internal browning or cork-like area degraded into hollow spaces within the stem. This was known as hollow heart, hollow stalk, or hollow stem. Hollow stem lowered the quality of broccoli by degrading the appearance of the product. Shattuck et al. (1986) described hollow stem as small elliptical cracks in the inner stem tissue. These cracks may increase and combine into one large hollow space. Not only do these cracks detract from overall quality but they may also facilitate pathogenic activity. The cracks promote the growth of pathogens by exposing inner tissue to the environment at harvest. Hollow stem has been found to be influenced by many factors. Reduced plant spacing, decreased application of nitrogen, and slowed maturation decrease the incidence of hollow stem. Also, cultivars vary in their susceptibility to hollow stem. Gupta and Cutcliffe (1975) found no relationship between the occurrence of hollow stem and boron levels in either soil or plant tissue even though boron deficiency

decreased yields. Boron concentration was eight to nine parts per million in the leaf tissue. Boron and hollow stem have been linked in cauliflower and broccoli with head tissue boron levels of 37 to 47 $\mu g \cdot g^{-1}$ (Shelp and Shattuck 1987, Shelp et al. 1987). Symptoms of hollow stem were found in greenhouse broccoli grown in the absence of boron (Shelp and Shattuck 1987, Shelp 1988). Increased head size was not associated with greater incidence of hollow stem (Shattuck et al. 1986). This indicated decreased yields and increased hollow stem can occur under conditions of boron deficiency. As scientific instrumentation has improved so that minute quantities of boron can be detected, the physical and physiological link of boron and hollow stem should be re-examined.

Environmental conditions have been linked to hollow stem (Gupta and Cutcliffe 1975) and to boron deficiency indicating a possible relationship between the two disorders. Boron absorption was influenced by temperature and light intensity (Gupta et al. 1985). Boron accumulation was enhanced in the presence of higher temperatures, higher root temperatures, higher light intensity, and increasing daylight hours. More boron was accumulated under high temperature and light conditions than could be explained by passive uptake alone (Bowen 1972). Forno et al. (1979) examined the link between temperature and boron deficiency and found that lower temperature decreased the amount of boron in cassava plants. They concluded that this effect was due to both a lower rate of boron uptake due to lower temperatures and a reduction in the size of the root system.

Both deficient and toxic levels of boron slowed maturity of broccoli (Petracek and Sams 1987). Leaf boron concentrations ranged from 5 ppm under deficiency to 210 ppm under toxicity. Deficient levels also resulted in stem scaling, high phenol and fiber levels, and small buds. Slightly toxic levels of boron increased inflorescence size. Boron deficiency also resulted in reduced stomatal conductance, reduced chlorophyll levels, and reduced net photosynthetic rates. Francois (1987) reported reduced yields with boron levels of 134 $mg.kg^{-1}$ and greater in leaf tissue, but found no leaf injury at any rate of toxicity used up to a maximum level of 943 $mg.kg^{-1}$. Increasing boron levels can increase yield and boron deficiency can decrease yield through effecting metabolic processes. The optimum level of boron for maximum yield remains to be established.

BORON AND OTHER NUTRIENTS

Boron also affected long distance transport of many nutrients (nitrogen, phosphorus, calcium, magnesium, zinc, iron). Phosphorus uptake was depressed during early stages of boron deficiency (Goldbach 1985). Alvarez-Tinaut et al. (1979a, 1979b) found that boron stimulated uptake and distribution of nitrogen, phosphorus, potassium, calcium, and magnesium in tomatoes. Also, reduced nitrate uptake was found during boron deficiency (Goss 1973). Boron deficiency or excess also resulted in low zinc uptake, while boron toxicity increased zinc translocation to shoots. Copper uptake was not affected by boron (Alvarez-Tinaut et al. 1979b). Boron toxicity in Spanish groundnuts

had no effect on either phosphorus, magnesium, or copper content, but decreased potassium and increased calcium, manganese, zinc, and iron concentrations (Blamey and Chapman 1979). Boron concentration in leaf tissue was unaffected by phosphorus or potassium but added nitrogen increased boron content in cauliflower leaves (Cutcliffe and Gupta 1980). Because boron affects other minerals, secondary effects caused by changes in these minerals must be taken into account.

CELL WALLS

Cell walls serve several functions; structural support, defines cell environment, and defines relationships between cells (Burgess 1985). Basic structure and function of the plant cell wall has been reviewed in Goodwin and Mercer's Introduction to Plant Biochemistry (1983). The cell wall is formed in layers which are constructed by the plasmalemma (plasma membrane) and associated enzymes. The cell wall is neither chemically nor physically homogeneous. The cell wall is made of three parts: the primary wall, the secondary wall, and the middle lamella. The middle lamella is the first portion formed, with the primary wall being deposited on it and the secondary wall being formed last. This structure results in new layers being closest to the protoplast which results in non-uniform thickening. The thickness of the cell wall portions depends on the type and function of the cell. The chemical components of a plant cell wall include polysaccharides, lignin, proteins, water, and incrusting substances (cuticle) (Burgess 1985). The middle lamella is primarily pectins with no cellulose. The

primary and secondary walls are primarily cellulose with less pectin. Cellulose is by far the most abundant cell wall component formed by the plasmalemma. Pectin and hemicellulose are formed by the Golgi cisternae.

BORON AND CELL WALLS

Kouchi and Kumazawa (1975, 1976) have done extensive work on the effect of boron on cell walls in tomato root tips. They reported a thickening of the cell wall in the apical region with insufficiently developed intercellular spaces. Using an electron microscope, changes in structure due to boron deficiency have been examined. Cortical cells were distorted in shape and arrangement and apparently accumulated an unknown substance and showed irregular thickening and serrated structure. Disorganization of the middle lamella was also seen. The irregular thickening was accompanied by abnormal development of the Golgi secretory vesicles. These results may have been caused by alterations in wall synthesis or breakdown of wall structure. The effect on the cell wall may be due to the effect on the Golgi vesicles. Golgi vesicles incorporate pectic substance and hemicellulose into the cell wall. Therefore, the changes in the Golgi apparatus may result in changes in the carbohydrate composition of the wall. Even though Kouchi and Kumazawa found that there was a decrease in fresh weight under boron deficiency, the amount of cell wall was either not changed or only slightly increased. There was no change in the cell wall composition. Lack of boron accelerated the process of cell aging. The

effects were primarily in the region of elongation and progressed into the meristematic region. This damage was not associated with any degradation of the membrane system. Boron deficient chloroplasts contained more starch, and the cell wall appeared thick with rough edges (Lee and Aronoff 1966). Hirsch and Torrey (1980) also studied boron deficiency in sunflower cells and found deficiency increased cell wall thickness and decreased membrane integrity. Phenolic compounds accumulated in boron deficient tobacco leaves, which may indicate a role for boron in lignin biosynthesis (Watanabe et al. 1961). These phenolic compounds may be lignin precursors.

Boron deficiency increased the hemicellulose content of cell walls in the oil-palm (Elaeis guineensis) (Rajaratnam and Lowry 1974). Boron deficiency increased peroxidase, while hydroxyproline was not effected in Helianthus annuus (Shive and Barnett 1973). Lewis (1980b) and Dutta and McIlrath (1964) found a direct relationship between boron concentration and both lignin synthesis and xylem differentiation. Collenchyma cell walls were thinner and phloem and ground parenchyma wall thickness was increased under boron deficiency (Spurr 1957). In studies of boron deficiency on cell wall polysaccharides in carrots (Goldbach and Amberger 1986), the total cellulose decreased. Boron has been suggested to alter properties of the plasmalemma which affect cell wall deposition. Since boron affects both cell walls and the development of hollow stem, it is possible boron effects hollow stem by affecting the cell walls in the pith area of broccoli.

BORON AND CALCIUM

Yamauchi et al. (1986) have done extensive studies on boron and calcium which are associated with different cell wall components. The amount of calcium and boron associated with the cell wall was greater than half the total amount in the tomato leaf tissue. Boron deficiency resulted in increased calcium uptake and inhibited calcium translocation to the upper leaves of tomato. This might have been due to differing growth rates within the plant. Boron deficiency resulted in increased lignin and cellulose in the cell walls, which indicated more mature plant cells. The amount of calcium associated with pectic constituents was decreased under boron deficiency. Yamauchi et al. (1986) suggested that boron deficiency accelerated senescence of younger tissue by influencing the rate of replacement of pectin with lignin and cellulose. Therefore, boron may affect cell wall structure by affecting the relationship between calcium and pectin. However, Tang and Dela Fuente (1986) found no connection between calcium availability and boron concentration. Konno et al. (1984) found that calcium starved cucumber roots contained less pectic polysaccharide, therefore supporting the theory that calcium is involved in preventing the breakdown of pectic polysaccharides. In any investigation of boron and cell walls, the effect of calcium must be taken into account due to the close associations between calcium and boron and calcium and cell walls.

LIST OF REFERENCES

REFERENCES

Adams, C. A. 1978. Boron as a plant nutrient. Information, Chilean Nitrate Agricultural Service #140. Nitrate Corporation of Chile Ltd., London EC2, UK.

Alvarez-Tinaut, M. C., A. Leal, I. Agui, and L. Recalde. 1979a. Physiological effects of the boron-manganese interaction in tomato plants. II. The uptake and translocation of macroelements. Anales de Edafologia y Agrobiologia 38:991-1012. (Abstract)

Alvarez-Tinaut, M. C., A. Leal, M. Gomez, and L. Recalde. 1979b. Physiological effects of the boron-manganese interaction in tomato plants. III. Uptake and translocation of the microelements Mn, Cu and Zn. Anales de Edafologia y Agrobiologia 38:1013-1029. (Abstract)

Benson, N. R., E. S. Deyman, and I. C. Chmelir. 1961. Translocation and reuse of boron in broccoli. Plant Physiology 36:296-301.

Blamey, F. P. C. and J. Chapman. 1979. Boron toxicity in Spanish groundnuts. Agrochemophysica 11:57-59.

Bowen, J. E. 1972. Effect of environmental factors on water utilization and boron accumulation and translocation in sugarcane. Plant and Cell Physiology 13:703-714.

Burgess, J. 1985. An Introduction to Plant Cell Development. Cambridge University Press, Cambridge, MS.

Cohen, M. S. and R. Lepper, Jr. 1977. Effect of boron on cell elongation and division in squash roots. Plant Physiology 59:884-887.

Cutcliffe, J. A. and U. C. Gupta. 1980. Effects of added nitrogen, phosphorus, and potassium on leaf tissue boron concentration of three vegetable crops. Canadian Journal of Plant Science 60:571-576.

Delmer, D. P. Cellulose biosynthesis. 1987. Annual Review of Plant Physiology 38:59-290.

Dutta, T. R. and W. J. McIlrath. 1964. Effects of boron on growth and lignification in sunflower tissue and organ cultures. Botanical Gazette 125:89-96.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Inc., New York.

Evans, C. M. and D. L. Sparks. 1983. On the chemistry and mineralogy of boron in pure and mixed systems: A review. Communications in Soil Science and Plant Analysis 14:827-846.

Forno, D. A., C. J. Asher, and D. G. Edwards. 1979. Boron nutrition of Cassava and the boron temperature interaction. Field Crops Research 2:265-279.

Francois, L. E. 1986. Effect of excess boron on broccoli, cauliflower, and radish. Journal American Society for Horticultural Science 111:494-498.

Gaines, T. P. and G. A. Mitchell. 1979. Boron determination in plant tissue by azomethine-H method. Communications in Soil Science and Plant Analysis 10:1099-1108.

Gauch, H. G. 1972. Inorganic Plant Nutrition. Dowden, Hutchinson and Ross, Stroudsburg, PA.

Goldbach, H. 1985. Influence of boron nutrition on net uptake and efflux of ^{32}P and ^{14}C glucose in Helianthus annuus roots and cell cultures of Daucus carota. Journal of Plant Physiology 118:431-438.

Goldbach, H. and A. Amberger. 1986a. Influence of boron deficiency on 3H I3ylAA uptake and efflux in cell cultures of Daucus carota L. Plant Growth Regulation 4:81-86.

Goldbach, H. and A. Amberger. 1986b. Influence of boron nutrition on cell wall polysaccharides in cell cultures of Daucus carota L. Journal of Plant Physiology 123:263-269.

Goodwin, T. W. and E. I. Mercer. 1983. Introduction to Plant Biochemistry. Second edition. Pergamon Press, Oxford.

Goss, J. A. 1973. Physiology of Plants and Their Cells. Pergamon Press Inc., New York.

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiology of Plants 62:25-31.

Gupta, U. C. 1968. Relationship of total and hot-water soluble boron, and fixation of added boron to properties of Podzol soils. Soil Science Society of America Proceedings 32:45-48.

Gupta, U. C. 1979. Boron nutrition of crops. Advances in Agronomy 31:273-307.

Gupta, U. C. and J. A. Cutcliffe. 1975. Boron deficiency in cole crops under field and greenhouse conditions. Communications in Soil Science and Plant Analysis 6:181-188.

Gupta, U. C., Y. W. Jame, C. A. Campbell, A. J. Leyshon, and W. Nicholaichuk. 1985. Boron toxicity and deficiency: A review. Canadian Journal of Soil Science 65:381-409.

Halbrooks, M. C., L. A. Peterson, and T. T. Kozlowski. 1986. Effects of transpiration rate on boron uptake by roots and translocation to shoots of table beets. Journal of Plant Nutrition 9:1157-1170.

Hirsch, A. M. and J. G. Torrey. 1980. Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. Canadian Journal of Botany 58:856-866.

Hudak, J. and R. Herich. 1976. Effect of boron on the ultrastructure of sunflower chloroplasts. Photosynthesis 10:463-465.

Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Jarvis, M. C. 1982. The proportion of calcium-bound pectin in plant cell walls. Planta 154:344-346.

Jarvis, M. C., M. A. Hall, D. R. Threlfall, and J. Friend. 1981. The polysaccharide structure of potato cell walls: Chemical fractionation. Planta 152:93-100.

Jones, T. M. and P. Albersheim. 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. Plant Physiology 49:926-936.

Kohl, H. C. and J. J. Oertli. 1961. Distribution of boron in leaves. Plant Physiology 36:420-424.

Konno, H., T. Yamaya, Y. Yamasaki, and H. Matsumoto. 1984. Pectic polysaccharide breakdown of cell walls in cucumber roots grown with calcium starvation. Plant Physiology 76:633-637.

Kouchi, H. and K. Kumazawa. 1975. Anatomical responses of root tips to boron deficiency: II. Effect of boron deficiency on the cellular growth and development in root tips. Soil Science and Plant Nutrition 21:137-150.

Kouchi, H. and K. Kumazawa. 1976. Anatomical responses of root tips to boron deficiency: III. Effect of boron deficiency on subcellular structure of root tips, particularly on morphology of cell wall and its related organelles. Soil Science and Plant Nutrition 22:53-71.

Lee, S. G. and S. Aronoff. 1966. Investigations on the role of boron in plants. III. Anatomical observations. Plant Physiology 41:1570-1577.

Lee, S. G. and S. Aronoff. 1967. Boron in plants: A biochemical role. Science 158:798-799.

Lewis, D. H. 1980. Boron, lignification and the origin of vascular plants: A unified hypothesis. New Phytologist 84:209-229.

Lovatt, C. J. 1985. Evolution of xylem resulted in a requirement for boron in the apical meristems of vascular plants. New Phytologist 99:509-522.

Lovatt, C. J. and L. M. Bates. 1984. Early effects of excess boron on photosynthesis and growth of Cucurbita pepo. Journal of Experimental Botany 35:297-305.

Martini, F. and M. Thellier. 1980. Use of an (n,α) nuclear reaction to study the long-distance transport of boron in Trifolium repens after foliar application. Planta 150:197-205.

McNeil, M. 1984. Structure and function of the primary cell walls of plants. Annual Review of Biochemistry 53:625-663.

Mengel, K. and E. A. Kirkby. 1979. Principles of Plant Nutrition. 2nd edition. <<Der Bunc>> AG, Bern, Switzerland.

Mengel, K. and E. A. Kirkby. 1982. Principles of Plant Nutrition. 3rd edition. International Potash Institute. Worblaufen-Bern/Switzerland.

Moore, P. J. and L. A. Staehelin. 1988. Immunogold localization of the cell-wall-matrix polysaccharides rhamnogalacturonan I and xyloglucan during cell expansion and cytokinesis in Trifolium pratense L.; implication for secretory pathways. Planta 174:433-445.

Oertli, J. J. and H. C. Kohl. 1961. Some consideration about the tolerance of various plant species to excessive supplies of boron. Soil Science 92: 243-247.

Petracek, P. D. and C. E. Sams. 1987. The influence of boron on the development of broccoli plants. Journal of Plant Nutrition 10:2095-2107.

Pilbeam, D. J. and E. A. Kirkby. 1983. The physiological role of boron in plants. Journal of Plant Nutrition 6:563-582.

Pollard, A. S., A. J. Parr, and B. C. Loughman. 1977. Boron in relation to membrane function in higher plants. Journal of Experimental Botany 28:831-841.

Prakash, S. and K. Hinata. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Botanica 55. Borgstroms, Motala.

Rajaratnam, J. A. and J. B. Lowry. 1974. The role of boron in the oil-palm (Elaeis guineensis). Annals of Botany 38:193-200.

SAS Institute Inc. 1985. SAS User's Guide: Basics, Version 5 Edition. Cary, North Carolina.

Seelig, R. A. 1971. Fruit and vegetable facts and pointers. Fresh Fruit and Vegetable Association. Washington, D. C.

Shattuck, V. I. and B. J. Shelp. 1987. Effect of boron nutrition on hollow stem in broccoli (Brassica oleracea var. italica). Canadian Journal of Plant Science 67:1221-1225.

Shattuck, V. I., B. J. Shelp, A. Loughton, and R. Baker. 1986. Environmental stability of yield and hollow stem in broccoli (Brassica oleracea var. italica). Canadian Journal of Plant Science 66:683-688.

Shelp, B. J. 1988. Boron mobility and nutrition in broccoli (Brassica oleracea var. italica. Annals of Botany 61:83-91.

Shelp, B. J. and V. I. Shattuck. 1987. Boron nutrition and mobility, and its relation to hollow stem and the elemental composition of greenhouse grown cauliflower. Journal of Plant Nutrition 10:143-162.

Shelp, B. J., V. I. Shattuck, and J. T. A. Proctor. 1987a. Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. I. Rutabaga. Communications in Soil Science and Plant Analysis 18:187-219.

Shelp, B. J., V. I. Shattuck, and J. T. A. Proctor. 1987b. Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. II. Radish. Communications in Soil Science and Plant Analysis 18:187-219.

Shive, J. B. Jr. and N. M. Barnett. 1973. Boron deficiency effects on peroxidase, hydroxyproline, and boron in cell walls and cytoplasm of Helianthus annuus L. hypocotyls. Plant Cell Physiology 14:573-83.

Spurr, A. R. 1957a. Boron in morphogenesis of plant cell walls. Science 126:78-80.

Spurr, A. R. 1957b. The effect of boron on cell wall structure in celery. American Journal of Botany 44:637-650.

Tang, P. M. and R. K. DelaFuente. 1986a. Boron and calcium sites involved in indole-3-acetic acid transport in sunflower hypocotyl segments. Plant Physiology 81:651-655.

Tang, P. M. and R. K. DelaFuente. 1986b. The transport of indole-3-acetic acid in boron and calcium deficient sunflower hypocotyl segments. Plant Physiology 81:646-650.

Tiedjens, V. A. 1943. The Vegetable Encyclopedia and Gardener's Guide. The New Home Library. New York.

Thomas, R. L., R. W. Sheard, and J. R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agronomy Journal 59:240-243.

Veierskov, B. and L. Meravy. 1985. Changes in carbohydrate composition in wheat and pea seedling induced by calcium deficiency. Plant Physiology 79:315-317.

Watanabe, R., W. J. McIlrath, J. Skok, W. Chorney, and S. H. Wender. 1961. Accumulation of scopoletin glucoside in boron-deficient tobacco leaves. Archives of Biochemistry and Biophysics 94:241-243.

Yamauchi, T., T. Hara, and Y. Sonoda. 1986a. Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant and Cell Physiology 27:729-732.

Yamauchi, T., T. Hara, and Y. Sonoda. 1986b. Effects of boron deficiency and calcium supply of the calcium metabolism in tomato plant. Plant and Soil 93:223-230.

PART II

EFFECTS OF BORON ON THE MORPHOLOGICAL DEVELOPMENT OF *BRASSICA OLERACEA* VAR. *ITALICA*

CHAPTER I

INTRODUCTION

Boron is an essential micronutrient but has an undefined role in plant metabolism (Gupta et al. 1985) perhaps due to the difficulty of separating primary and secondary effects (Pollard et al. 1977). Boron has limited mobility within plants (Gupta et al. 1985, Mengel and Kirkby 1982). This limited mobility often results in localized boron deficiency within some tissues, even when other tissues have sufficient boron for normal growth. Cole crops have a high boron requirement (Gupta 1979) and localized boron deficiency within the stem can result in hollow stem, and in inflorescence brown bead formation (Petracek and Sams 1987). Hollow stem is also influenced by environmental conditions (Gupta and Cutcliffe 1975), perhaps through higher temperatures and higher light intensity increasing the amount of boron accumulated (Gupta et al. 1985). Slowed maturation has been associated with decreased incidence of hollow stem (Shattuck et al. 1986) and with boron deficiency (Petracek and Sams 1987). In addition, boron deficiency may limit photosynthesis and stomatal conductance (Petracek and Sams 1987, Hudak and Herich 1976, Lovatt and Bates 1984). It has also been suggested that boron deficiency may affect carbohydrate movement and metabolism (Gupta et al. 1985). Boron deficiency decreased yields and both boron deficiency and toxicity slowed maturity of broccoli (Petracek and Sams 1987). Boron stimulated phosphorus,

nitrogen, potassium, calcium, and magnesium absorption (Alvarez-Tinaut et al. 1979, Goss 1973). Because boron affects so many plant processes, boron may affect the partitioning of plant growth.

The objective of this investigation was to determine the effects of boron availability within the plant on partitioning of growth among various plant organs at various stages of development.

CHAPTER II

MATERIALS AND METHODS

Three experiments were planted for this study over a two year period. Experiment one had a limited range of treatments. Experiment two had a wider range of treatments and the results presented here are from experiment two unless otherwise indicated. Experiment three had the same range of treatments as experiment two, but no deficiency symptoms were observed. Slower growth due to cooler temperatures may have lowered the amount of boron needed to provide the plant with sufficient levels of tissue boron in experiment three.

The broccoli cultivar Premium Crop was greenhouse grown. Temperatures were maintained between 16°C and 32°C (60 and 90°F). In the summer this was accomplished by placing black fabric screening over the greenhouse to shade the plants. An evaporative cooling system with fans circulating cool air across the plants was also used. In the winter, steam heating with a blower system was used to heat the air inside the greenhouse.

Plants were seeded directly into perlite in boron-free 27 liter plastic pots. The plants were watered daily with distilled, deionized water. A complete nutrient solution (Epstein 1972; Appendix A) which varied only in boron content was applied to the plants daily. In experiment one three concentrations of boron were used: 8.10 $mg \cdot L^{-1}$, 0.81 $mg \cdot L^{-1}$, 0.08 $mg \cdot L^{-1}$. Solutions used in experiment two and three

contained one of the following molarities of boron: 7.493×10^{-5}M (0.81 mg•L^{-1}), 7.493×10^{-4}M (8.10 mg•L^{-1}), 7.439×10^{-6}M (0.08 mg•L^{-1}), 1.4986×10^{-4}M (1.62 mg•L^{-1}), 1.4986×10^{-5}M (0.16 mg•L^{-1}), and 2.9972×10^{-5}M (0.32 mg•L^{-1}). In experiment one the experimental design consisted of three plants per replication, one of each of the three treatments, all of the mature stage. The experimental design in experiment two and three was a randomized complete block with 24 plants in a replication, one of each of the six treatments at each of four harvest stages. Treatment means were compared utilizing linear contrasts within the SAS system (SAS Institute Inc., 1985). The normal treatments (0.16 mg•L^{-1}, 0.32 mg•L^{-1}, 0.81 mg•L^{-1}, 1.62 mg•L^{-1}) which all exhibited normal growth were contrasted with each other and few differences were found in any of the tests, therefore the normal treatments are generally considered as one unit. The toxic treatment (8.10 mg•L^{-1}) was compared to the deficient treatment (0.08 mg•L^{-1}) and separately to the normal treatments (0.16, 0.32, 0.81, 1.62 mg•L^{-1}). The deficient treatment was contrasted with the normal treatments. Contrasts which tested significant at the 0.01 level were considered "highly significant," and those significant at the 0.05 level were considered "significant." The 0.10 level was also tested to determine if some factors were significant at lower probability levels.

The four growth stages in experiment two and three were: (1) 5-leaf stage, (2) 10-leaf stage, (3) 15-leaf stage, and (4) head maturity (approximately the 20-leaf stage). For the 5-leaf stage, approximately 40 plants were used as a treatment unit in order to

obtain the amount of material needed for the laboratory procedures. Experiment one used only the mature stage.

Several types of plant material were used: whole stems, interior and exterior stem, florets, young and old leaves. The type of material used depended on the age of the plant at harvest. Plants harvested at the 5-leaf stage were combined within a single replication and treatment due to the extremely small size. Stems from the 10-leaf stage were used intact but stems from plants harvested at the 15-leaf and mature stage in experiments two and three were divided into exterior and interior portions. The stem was separated into these portions by peeling the exterior epidermis and vascular tissue from the interior pith. Separation allowed comparison of the effects of boron on the two portions. At the mature stage, florets were shaved off the stem for analysis.

Various leaves and stem portions were used for mineral analysis. At the 5-leaf stage, all leaves were combined for testing. At the 10-leaf stage, the leaves were divided into two sets, one to five and six to ten. At the 15-leaf stage, leaves 7 and 12 were used; and at the mature stage, leaves 12 and 16 were used. These leaf divisions were chosen to represent mature young and mature old leaves on the plant. In addition, at maturity, a subsample of the stem exterior and interior was also dried and analyzed for mineral content. Fresh and dry weights were also recorded for all leaf and stem portions, with averages calculated for the 5- and 10-leaf stages.

Samples were analyzed for nitrogen, calcium, magnesium, potassium, and boron. Nitrogen analysis was done on experiment one samples only. When there was insufficient plant material for complete testing, boron was analyzed first, then nitrogen. If enough material remained, the other minerals were tested. The plant material was dried in a forced air oven at 70°C in paper bags. It was then ground using a Wiley™ mill with a 30 mesh screen and stored in sterile plastic bags. The material was weighed using an analytical balance. Nitrogen analysis was performed using an automated colorimetric procedure utilizing the Technicon AutoAnalyzer (Thomas et al. 1967; Appendix A). The procedure for calcium, magnesium, and potassium analysis utilized atomic absorption (Appendix A). An analysis for boron content utilized Jackson's (1958) curcumin procedure (Appendix A).

CHAPTER III

RESULTS AND DISCUSSION

The effects of boron concentration on the morphology of broccoli were investigated using fresh and dry weights and mineral analysis. Dry matter production and partitioning data were recorded and analyzed. From this study, boron seems to have the greatest physical effect on the plant at the visible bud stage and during head formation. At the visible bud stage (approximately the 20-leaf stage) the boron deficient plants (0.08 $mg \cdot L^{-1}$) developed callus tissue on the stem epidermis (scaling). Other deficiency symptoms observed were degradation in the pith (hollow stem) and brown bead formation. Plants receiving toxic levels of boron (8.10 $mg \cdot L^{-1}$) developed necrosis of the leaf margins early in the life cycle. This necrosis (yellowing, burning) progressed as the plant aged.

Except where noted, only results from plants in experiment two are used. Plants from experiment three exhibited no hollow stem and boron concentration was higher in all treatments than in the lowest normal treatment (0.16 $mg \cdot L^{-1}$) of experiment two. Experiment one had fewer treatments and fewer types of data taken than in experiment two, and results were similar.

BORON CONTENT

Boron was analyzed to determine the relationship between the amount present in the plant and the amount provided to the plant. Increasing treatment levels of boron increased the boron content of plant tissues (Tables B-1, B-2, B-3). There was no significant difference among the normal treatments. This would indicate that the plant somehow compensates for the amounts of boron supplied, either by concentrating or excluding the boron. For this reason, the normal treatments (0.16, 0.32, 0.81, 1.62 $mg \cdot L^{-1}$) were generally considered together as treatments resulting in normal growth. Treatment levels below (0.08 $mg \cdot L^{-1}$) or above (8.10 $mg \cdot L^{-1}$) the middle four normal treatments resulted in deficiency or toxicity symptoms, respectively. Treatment contrasts of boron content for toxic vs. deficient, toxic vs. normal, and normal vs. deficient treatments were all highly significant (0.01 level) for all four ages in both young and old leaves (Tables B-1, B-2). For stem exterior toxic vs. deficient and toxic vs. normal contrasts were highly significant, while normal vs. deficient were not (Table B-3). Contrasts of toxic vs. either deficient or normal treatments in the stem interior were highly significant and normal vs. deficient were significantly different (Table B-3). Florets showed highly significant differences for all three contrasts (Table B-3). Sufficient boron content in both the young and older leaves of the mature stage ranged from 15 to 100 $\mu g \cdot g^{-1}$. Sufficient boron in younger and older leaves in the 5-leaf stage ranged from 24 to 84 $\mu g \cdot g^{-1}$, 10-leaf ranged from 20 to 100 $\mu g \cdot g^{-1}$, and 15-leaf

ranged from 15 to 80 $\mu g \cdot g^{-1}$. Sufficient boron in stem interior and exterior ranged from 10 to 17 $\mu g \cdot g^{-1}$. Normal boron content of florets ranged from 6 to 52 $\mu g \cdot g^{-1}$. These ranges are the tissue boron concentrations over all leaf stages which allowed normal development of the plant as a whole. The greatest head weight occurred near the upper end of each of the ranges. These tests confirm a direct relationship between treatment level and tissue boron level. Under deficiency conditions, boron is apparently transported into the stem portions, raising the boron content to levels comparable to the sufficient treatments. These ranges can be used to detect boron deficiency at early stages. They may also allow the broccoli producer the option of adding boron to fields with low but sufficient levels of boron in the soil in order to produce maximum yields without going into boron toxicity.

CALCIUM CONTENT

Calcium, which is closely linked to cell walls, was analyzed to determine if boron deficiency was associated with calcium concentration. There were no significant boron treatment effects on calcium content in either young or older leaves at any plant age (Tables B-4, B-5). Toxic vs. normal treatments were significantly different for calcium in the stem exterior and normal vs. deficient treatments were different for calcium in stem interior (Table B-6). There was insufficient data to analyze contrasts of calcium content in florets (Table B-6). Younger leaves on the plant have less calcium than older

leaves. The same results were found in young tomato leaves (Yamauchi et al. 1986). Florets contained much less calcium than did stem, and stem tissue contained less than leaves. Because the deficient level stem interior contains less calcium, hollow stem could be connected to deficient levels of calcium in the pith area. Calcium content is not associated to boron level in the leaves. Therefore, boron deficiency is associated with the transport of calcium to leaves rather than to the pith area. Calcium deficiency is not directly associated with cell wall calcium content (Tables B-47, B-48). Calcium deficiency must be within the cell itself as calcium content of the cell wall is increased under boron deficiency (Table B-47). Yamauchi et al. (1986a) found most of the calcium in the tissue to be associated with the cell wall. Therefore, the calcium that is present in the pith area may be actively partitioned into the cell wall, changing the wall components, and thereby causing a breakdown of the wall to wall cohesion or a breakdown of cell wall component adhesion.

MAGNESIUM CONTENT

Magnesium uptake and distribution has been found to be increased by increasing boron (Alvarez-Tinaut et al. 1979). Therefore, magnesium content was examined to determine if the effects of boron were due to boron's effects on magnesium. Treatment contrasts of magnesium concentration were not significant for younger or older leaves except for the total (young) leaves in the 5-leaf stage (Tables B-7, B-8). Toxic vs. deficient treatment contrasts tested different (0.10

probability level) and toxic vs. normal treatment contrasts tested highly significantly different (0.01 level). No differences were seen in florets (Table B-9). However, toxic vs. normal treatment contrasts showed a low level of difference for stem exterior and interior (Table 9). Toxic levels of boron resulted in high levels of magnesium. Also, normal vs. deficient treatments were different for stem interior with deficiency of boron also resulting in high magnesium levels. Younger and older leaves and other plant parts differed little in magnesium content and content varied little as the plant matured. Boron toxicity generally increased magnesium content, however the differences among treatments were so slight that this probably did not greatly affect plant growth.

POTASSIUM CONTENT

Potassium uptake and distribution was found to increase with increasing boron uptake (Alvarez-Tinaut et al. 1979). Therefore, potassium content was examined to determine if boron effects on the plant were caused by boron's effects on potassium content. In examining the potassium content of leaves, no significant differences were found in the older or younger leaves, except for older leaves at the 10-leaf stage (Table B-10, B-11). Toxic vs. normal and normal vs. deficient contrasts were different at that stage. Florets also had no significant differences in potassium content (Table B-12). Stem exterior tested different for toxic vs. normal treatments (Table B-12). Stem interior potassium content was highly significantly different for

the normal vs. deficient contrast (Table B-12). Potassium content did not vary widely among plant portions except in florets and stem interior. Florets have slightly less potassium than do leaves or stems. Stem interior had much more potassium than other plant parts. Toxicity of boron decreased the amount of potassium in the plant. This agrees with previous findings (Blamey and Chapman 1979). As with magnesium, the slight differences in potassium content probably do not constitute the primary effects seen from boron deficiency but rather slight secondary effects which are not considered symptoms of boron deficiency.

YIELD PARAMETERS

Boron's affect on yield through its effects on florets and stems was also examined. Increasing the amount of boron increased the fresh weight of the florets, up to the point of toxicity where it decreased (Table B-13). This indicates high levels of boron may be beneficial to the production of broccoli for commercial purposes, even though the higher levels do cause slight toxicity symptoms in leaves. Experiment three plants do not seem to follow this pattern, however the boron concentrations in the tissue of plants from even the deficient treatment fell within the normal concentrations in the plants of experiment two. Therefore, the optimum level of boron concentration occurred at much lower treatment levels in plants in experiment three. The critical levels of boron for floret tissue are 6 to 52 $\mu g \cdot g^{-1}$, with the upper end of the range resulting in the greatest floret weight.

The wide critical range indicates the plant can develop normally with low levels but higher levels increase yield. Contrasts between toxic vs. deficient and normal vs. deficient treatments were highly significant. Decreased photosynthetic rates (Petracek and Sams 1987) are probably responsible for decreased carbohydrate levels (Pilbeam and Kirkby 1983) and therefore decreased floret yield. Head diameter showed no significant differences among treatments (Table B-14). Broccoli grown in the greenhouse did not produce heads as large or as domed as those normally produced in the field, therefore head diameter was not a good indicator of possible commercial yields.

The effects of boron on days to harvest was examined to determine if the growing season could be effected by boron deficiency or toxicity. The amount of time required for plants in each treatment to reach a certain leaf stage was not effected by boron treatment at any growth stage except at maturity (Table B-15). At the mature stage, toxic vs. normal contrasts had a low level of significance. At maturity, the toxic treatment had the lowest amount of time. Therefore, boron probably does not affect the length of the growing season.

Another yield factor, stem weight, was also examined. Boron content did not significantly affect fresh stem weight in the 10- or 15-leaf stages (Table B-16). However, toxic vs. normal contrasts showed highly significant differences at the 5-leaf stage. The toxic level treatment decreased stem weight. However, the samples comprising the 5-leaf stage is a composite of many stems, therefore, this effect

may be due to competition within the pot rather than depressed weight due to boron toxicity. Normal vs. deficient treatment contrasts also show significant differences at maturity, with the deficient treatment having the least stem weight. Like florets, the 1.62 $mg \cdot L^{-1}$ level of the middle treatments had the highest fresh weight. As in florets, more photosynthesis under sufficient boron may have resulted in more carbohydrate being transported into the tissue and therefore higher fresh weights.

Epidermal scale formation in the boron deficient plants at maturity resulted in excess callus formation, which may have affected stem weight. Therefore, at the mature stage, the stem was divided into external and internal tissue and the percent dry weight was determined for each tissue type. External stem tissue in the boron deficient plants had more dry weight than that of the other treatments. However, the overall growth of these plants is sufficiently stunted to prevent this scaling from increasing the fresh weight of the mature stem. Boron deficient plants exhibited greater pith degradation (hollow stem) at maturity. Plants in the deficiency treatment were therefore expected to have less internal dry weight. However, the toxic boron treatment (8.10 $mg \cdot L^{-1}$) had less internal dry weight, while the boron deficient plants had greater dry weight than other treatments (Table B-17). This indicates that the boron deficient plants had more dry matter but less fresh stem weight, therefore less water content than the other treatments. The interior and exterior showed highly significant differences in the toxic vs. deficient and toxic vs. normal

contrasts. Normal vs. deficient treatment contrasts were significant at the 0.10 level.

Stem diameter was also examined for a possible connection to yield and boron content. Boron treatments had some effect on stem diameters (Table B-18). Contrasts of toxic vs. deficient and normal vs. deficient showed slight differences (0.10 probability level). The deficient treatment level resulted in the greatest stem diameter. It is unlikely that larger stem diameter could be associated with lower stem fresh weight. Therefore, the larger diameter found in boron deficiency must be due to other factors. However, as the differences were minor, no real importance was connected with this finding.

HOLLOW STEM

Hollow stem damage in the pith area of the stem lowers the quality of broccoli. Therefore, the connection of hollow stem and boron was examined. Hollow stem was rated from zero (no symptoms) to 5 (severe damage). The early stages of 5 and 10 leaves had no symptoms. At the 15-leaf stage symptoms were beginning to appear in the deficient treatment. The mature stage, after the visible bud stage and growth, showed extreme hollow stem in the deficient treatment (Table B-19). Hollow stem symptoms lessened with increasing boron levels (Table B-19). The highest normal treatment (1.62 $mg \cdot L^{-1}$) showed no hollow stem. However, the toxic level (8.10 $mg \cdot L^{-1}$) showed slight symptoms, possibly due to damage by the extreme amount of boron present. Toxic vs. deficient and normal vs. deficient treatment

contrasts tested highly significant. Hollow stem volume was also calculated and it followed the same trends (Table B-20). There is a direct association between boron treatments and hollow stem. Hollow stem and callusing symptoms appear to worsen under high temperature and light conditions. Temperature data indicates that hollow stem was more severe under temperatures of 21°C to 32°C (69 to 90°F) than under temperatures of 13°C to 29°C (56 to 85°F). It appears that hollow stem is caused by a combination of high temperatures and low boron levels. However, the question of what hollow stem is physiologically has not been answered. Since the pith area had more dry matter under boron deficiency (Table B-17), it is not a lack of matter by degradation or malformation. As boron effects cell walls (Kouchi and Kumazawa 1975, 1976; Rajaratnam and Lowry 1974; Goldbach and Amberger 1986), hollow stem may actually be caused by a breakdown in the cell wall structure, causing weakness and therefore cracking. Accelerated aging (Yamauchi et al. 1986) in the pith area may also affect the strength of the bonds.

Another physical symptom of boron imbalance is leaf burn caused by boron toxicity. Toxicity symptoms were observed with the 1.62 $mg \cdot L^{-1}$ and 8.10 $mg \cdot L^{-1}$ treatment plants throughout the life cycle. These included yellowing and necrosis of leaf edges with cupping of the leaves. Toxicity symptoms at the 1.62 $mg \cdot L^{-1}$ boron were slight. This supports the findings of Oertli and Kohl (1961) that the injury occurs at the position of probable highest boron content in the tissue. Investigation into the boron content of leaf margins should show high

boron levels at injury sites. Leaf margins are the most likely areas of high boron content due to the transport of boron to the leaf edge by xylem through transpiration during the process of guttation.

LEAF DEVELOPMENT

Partitioning of growth is best examined by comparing the growth of leaves as compared to the rest of the plant. However, leaves do not show the same clear trends in overall results as stem or floret tissue. The upper, younger leaves showed few differences among treatments. Deficient plants tend to have a higher percent dry weight than normal plants and toxic plants tend to have the lowest ratio. Leaves from younger plants tend to have more water and less dry matter (Table B-21). Contrasting toxic vs. deficient or normal treatments show significant differences. The ratio of dry weight to leaf area (specific leaf weight) showed the same highest ratio in deficient plants and lowest in toxic plants (Table B-22). Toxic vs. deficient treatment contrasts in the mature stage are highly significant. Toxic vs. normal and normal vs. deficient also show some significant differences. Analysis of dry weight showed significant differences of toxic vs. deficient and high significance at normal vs. deficient contrast (Table B-23). A significant difference was shown for the normal vs. deficient treatment of leaf fresh weight (Table B-24). The toxic treatment, therefore, has more water than other treatments. Deficient and toxic treatments have more leaf area than normal treatments. Boron deficiency increased leaf dry matter. Critical

boron levels for young leaf tissue were 15 to 89 $\mu g \cdot g^{-1}$ in the mature stage.

Older, lower leaves did not show any clear trends in percent dry weight, even when comparing younger and older plants. However, younger plants did show a lower specific leaf weight than did older plants. Critical boron levels were 17 to 97 $\mu g \cdot g^{-1}$ in the mature stage. Treatment contrasts of percent dry weight show significant differences in the toxic vs. deficient and toxic vs. normal tests in the 10-leaf stage (Table B-25). No contrast differences were seen in the 15-leaf stage but the mature stage were different at 0.10 probability level in the toxic vs. normal treatment contrasts. In dry weight to leaf area, the 10-leaf stage showed significant differences in the toxic vs. normal contrast (Table B-26). No significance was seen in the 15-leaf stage, but the mature stage plants showed highly significant differences in the toxic vs. deficient treatments and significant differences in the toxic vs. normal treatments. Mature stage dry weight (Table B-27) showed highly significant differences in the toxic vs. deficient contrasts, significant differences in the toxic vs. normal treatments, and some differences in the normal vs. deficient treatments. The same general trends in fresh weight of leaves were evident at the mature stage (Table B-28). The deficient treatment level resulted in greater dry matter than other treatments. The differences in treatment effects in the 10-leaf stage may be due to differing maturation rates during this stage.

In comparing young and old leaves at the same stage of development, differences in boron content and dry matter were expected. However, there were little differences in the range of boron contained in leaves of different ages. This does not agree with previous studies which found translocation of boron between different ages of plant parts (Shelp and Shattuck 1987). Younger leaves were higher in percent dry weight, indicating less water in younger than older leaves. This finding does not support a direct relationship between boron and dry weight of leaves. Higher fresh weights in the deficient treatment of both young and old leaves indicate that matter is not being transported from leaves to stem and floret tissue under boron deficiency. This is probably due to restriction of carbohydrate flow from the primary site of synthesis in the leaves to the stem and floret tissue. As boron deficiency degrades membrane structure (Pilbeam and Kirkby 1983), carbohydrate flow may be restricted by degraded membranes.

CHAPTER IV

CONCLUSIONS

Dry matter partitioning is not significantly affected by boron concentration in young plants. When the plant reaches maturity, however, boron concentration does affect partitioning and thus morphology. Deficiency results in lower fresh weights in florets and stem, and an increase in percent dry weight in stem exterior and younger leaves. The increase in stem exterior weight may be due to scaling. Deficiency also increased hollow stem, perhaps through degradation of the cell wall or through affecting cell wall composition or cohesion. The specific leaf weight (ratio of dry weight to leaf area) of younger leaves was increased under boron deficiency due to deposition of cell wall material or to carbohydrate accumulation. No effects were seen in older leaves. Toxicity decreased stem fresh weight, the percent dry weight in stem exterior and younger leaves as well as the specific leaf weight in younger leaves. Dry matter accumulation occurs in young leaves at the expense of stems and florets under boron deficiency. Optimum boron concentration for florets may be near toxic for leaves since the 1.62 $mg \cdot L^{-1}$ treatment exhibited some leaf toxicity symptoms. The same trend is seen for internal and external tissue. Therefore, optimum leaf boron levels for maximum floret production range from 15 $\mu g \cdot g^{-1}$ to 97 $\mu g \cdot g^{-1}$ which is close to the previously reported range of 21 to 100 $\mu g \cdot g^{-1}$ (Petracek and Sams 1987).

There were a few deficiency symptoms at the lower end of this range and slight toxicity symptoms at the upper end. Younger leaves had higher dry matter accumulation than older leaves but no extreme differences were found in boron content between young and older leaves. Boron toxicity increased calcium content but did not significantly effect magnesium or potassium content.

These results indicate broccoli production can be increased on soils with low and normal amounts of boron as even levels of boron toxic to leaves increased both the fresh and dry weights of the florets.

LITERATURE CITED

LITERATURE CITED

Alvarez-Tinaut, M. C., A. Leal, I. Agui, and L. Recalde. 1979a. Physiological effects of the boron-manganese interaction in tomato plants. II. The uptake and translocation of macroelements. Anales de Edafologia y Agrobiologia 38:991-1012. (Abstract)

Alvarez-Tinaut, M. C., A. Leal, M. Gomez, and L. Recalde. 1979b. Physiological effects of the boron-manganese interaction in tomato plants. III. Uptake and translocation of the microelements Mn, Cu and Zn. Anales de Edafologia y Agrobiologia 38:1013-1029. (Abstract)

Blamey, F. P. C. and J. Chapman. 1979. Boron toxicity in Spanish groundnuts. Agrochemophysica 11:57-59.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives John Wiley and Sons, Inc., New York.

Goldbach, H. and A. Amberger. 1986. Influence of boron deficiency on 3H I3yIAA uptake and efflux in cell cultures of Daucus carota L. Plant Growth Regulation 4:81-86.

Goss, J. A. 1973. Physiology of Plants and Their Cells. Pergamon Press Inc., New York.

Gupta, U. C. 1979. Boron nutrition of crops. Advances in Agronomy 31:273-307.

Gupta, U. C. and J. A. Cutcliffe. 1975. Boron deficiency in cole crops under field and greenhouse conditions. Communications in Soil Science and Plant Analysis 6:181-188.

Gupta, U. C., Y. W. Jame, C. A. Campbell, A. J. Leyshon, and W. Nicholaichuk. 1985. Boron toxicity and deficiency: A review. Canadian Journal of Soil Science 65:381-409.

Hudak, J. and R. Herich. 1976. Effect of boron on the ultrastructure of sunflower chloroplasts. Photosynthesis 10:463-465.

Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Kouchi, H. and K. Kumazawa. 1975. Anatomical responses of root tips to boron deficiency: II. Effect of boron deficiency on the cellular growth and development in root tips. Soil Science and Plant Nutrition 21:137-150.

Kouchi, H. and K. Kumazawa. 1976. Anatomical responses of root tips to boron deficiency: III. Effect of boron deficiency on

subcellular structure of root tips, particularly on morphology of cell wall and its related organelles. Soil Science and Plant Nutrition 22:53-71.

Lovatt, C. J. and L. M. Bates. 1984. Early effects of excess boron on photosynthesis and growth of Cucurbita pepo. Journal of Experimental Botany 35:297-305.

Mengel, K. and E. A. Kirkby. 1982. Principles of Plant Nutrition. Third edition. International Potash Institute. Worblaufen-Bern/Switzerland.

Oertli, J. J. and H. C. Kohl. 1961. Some consideration about the tolerance of various plant species to excessive supplies of boron. Soil Science 92: 243-247.

Petracek, P. D. and C. E. Sams. 1987. The influence of boron on the development of broccoli plants. Journal of Plant Nutrition 10:2095-2107.

Pilbeam, D. J. and E. A. Kirkby. 1983. The physiological role of boron in plants. Journal of Plant Nutrition 6:563-582.

Pollard, A. S., A. J. Parr, and B. C. Loughman. 1977. Boron in relation to membrane function in higher plants. Journal of Experimental Botany 28:831-841.

Rajaratnam, J. A. and J. B. Lowry. 1974. The role of boron in the oil-palm (Elaeis guineensis). Annals of Botany 38:193-200.

SAS Institute Inc. 1985. SAS User's Guide: Basics, Version 5 Edition. Cary, North Carolina.

Shattuck, V. I., B. J. Shelp, A. Loughton, and R. Baker. 1986. Environmental stability of yield and hollow stem in broccoli (Brassica oleracea var. italica). Canadian Journal of Plant Science 66:683-688.

Shelp, B. J. and V. I. Shattuck. Boron nutrition and mobility, and its relation to hollow stem and the elemental composition of greenhouse grown cauliflower. Journal of Plant Nutrition 10:143-162. 1987.

Thomas, R. L., R. W. Sheard, and J. R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agronomy Journal 59:240-243.

Yamauchi, T., T. Hara, and Y. Sonoda. 1986. Effects of boron deficiency and calcium supply of the calcium metabolism in tomato plant. Plant and Soil 93:223-230.

PART III

INFLUENCE OF BORON CONCENTRATION ON CELL WALL DEVELOPMENT OF *BRASSICA OLERACEA* VAR. *ITALICA*

CHAPTER I

INTRODUCTION

Boron has been shown to effect cell walls and their composition (Kouchi and Kumazawa 1976). Boron deficiency increased hemicellulose in oil-palm (Rajaratnam and Lowry 1974) and decreased cellulose in carrots (Goldbach and Amberger 1986). Boron deficiency caused irregular thickening of cell walls in tomato (Kouchi and Kumazawa 1976) and in sunflower (Hirsch and Torrey 1980) and influenced hollow stem formation in broccoli (Petrachek and Sams 1987). Boron has also been associated with the uptake and deposition of calcium, essential to cell wall development (Yamauchi et al. 1986). Boron has also been associated with cell wall calcium (Yamauchi et al. 1986). Hollow stem may be induced by a change in the content or composition of the cell wall.

The objective of this investigation was to determine what effects boron availability has on the development of cell walls in broccoli and to associate cell wall changes with the development of hollow stem.

CHAPTER II

MATERIALS AND METHODS

Three experiments were planted over a two year period. Experiment one had a limited range of treatments. Experiment two had a wider range of treatments and the results are presented here unless otherwise noted. Experiment three had the same range of treatments, but the tissue boron was sufficient to eliminate any deficiency symptoms, probably due to slowed growth.

The broccoli cultivar Premium Crop was greenhouse grown. Temperatures were maintained between 16°C and 32°C (60 and 90° F).

Plants were seeded directly into perlite, in boron-free plastic pots. The plants were watered daily with distilled, deionized water. A complete nutrient solution (Epstein 1972; Appendix A) which varies only in boron content was applied to the plants daily.

Experiment one used mature stage plants for analysis. The four growth stages in experiment two and three were: (1) 5-leaf stage, (2) 10-leaf stage, (3) 15-leaf stage, and (4) head maturity. For the 5-leaf stage approximately 40 plants were used as a plant unit in order to obtain the amount of material needed for the laboratory procedures.

Cell wall extraction and fractionation was done using the methods of Jarvis et al. (1981) and Jarvis (1982) as modified by Gross (1984) (Appendix A). Several types of plant material were used: whole stems, interior and exterior stem, and florets. The type of material used

depended on the age of the plant at harvest. Plants harvested at the 5-leaf stage had very small stems which could not be divided into interior and exterior stem tissue. Also, individual stems did not weigh enough to be used for extraction. Therefore, stems were combined within a single replication of a treatment. Plants harvested at the 10-leaf stage were large enough so that a single stem could be used per replication for each treatment for extraction. However, this single stem was not large enough to be separated into interior and exterior tissue. Plants harvested at the 15-leaf and mature stages were divided into the exterior and interior portions, which were extracted separately. The stem was separated into these portions by peeling the exterior epidermis and vascular tissue away from the interior pith.

A test for starch contamination in the extracted cell wall was done using the iodine procedure of Jones and Albersheim (1972) (Appendix A).

The calcium, magnesium, potassium, and boron content of cell walls was also determined. When there was insufficient plant material for complete testing, boron was analyzed first, then the other minerals were tested. These tests were done using dried cell wall material. The material was weighed using an analytical balance. The procedure for calcium, magnesium, and potassium utilized atomic absorption analysis (Appendix A). An analysis for boron content was done using Jackson's (1958) curcumin procedure (Appendix A).

CHAPTER III

RESULTS AND DISCUSSION

One purpose of this study was to determine if the hollow stem was caused by degradation of the cell walls located in the center of the stem. The effects of boron toxicity on cell wall material were also tested. Fractionation was used to determine what portion of the cell wall was most affected by boron deficiency and toxicity.

It was found that boron effected cell walls, especially in association with hollow stem. Increases in cell wall weight were found in plants which had a high degree of hollow stem. In contrasts of the normal treatments (0.16, 0.32, 0.81, 1.62 $mg \cdot L^{-1}$) with each other, little or no significant differences were found in any stage or tissue type. Also, plants in these treatments exhibited normal growth. For these reasons, in most tests the normal treatments were considered together. Results are from experiment two, except where noted. Experiment three plants exhibited no hollow stem and were therefore not used. Experiment one did not have the number of treatments nor the range of data taken and was therefore considered too incomplete for extensive use.

CELL WALL CONTENT

Cell walls were extracted from fresh tissue to determine if boron has an effect on the amount of total cell wall present in the tissue. The ratio of dry cell wall weight to fresh tissue weight did not differ among treatments in the early stages (Table B-29). Treatment contrasts were not significant in the 5-, 10-, or 15-leaf stages with the exception of toxic vs. normal in the 5-leaf stage which was significant at the 0.10 probability level. Mature stage treatment contrasts showed varying degrees of significance (Table B-30). For the stem interior, the toxic vs. deficient treatments tested highly significant, as did the normal vs. deficient. The toxic vs. normal treatment contrasts were also significant. For the stem exterior, toxic vs. deficient contrasts were significant and toxic vs. normal treatments were significant at 0.10 level of probability. Normal vs. deficient treatment contrast was not significant. For florets, toxic vs. deficient was highly significant, toxic vs. normal had low significance, and normal vs. deficient was significant (Table B-30). The boron deficient treatment resulted in greater amounts of dry extracted cell wall to fresh tissue (3.5 g•100 g^{-1} FW for interior and 14 g•100 g^{-1} FW for exterior) than the normal treatments (2.6 g•100 g^{-1} FW interior, 12 g•100 g^{-1} FW exterior). Toxic treatments resulted in lower wall amounts of 2 g•100 g^{-1} FW and 9 g•100 g^{-1} FW for interior and exterior cell wall respectively. Boron deficiency resulted in greater cell wall formation, particularly in the interior which is the area of hollow stem development. These data suggest hollow stem is not due to cell

wall degradation or lack of formation but may be due to malformation or degradation of wall to wall cohesion. The degradation of wall cohesion may be due to disorganization of the middle lamella (Kouchi and Kumazawa 1975, 1976). Florets also showed higher amounts of cell wall in the deficient and lower amounts in the toxic treatments. Florets in the normal treatments were approximately 7 g•100 g^{-1} FW cell wall weight. Florets at the upper level of the normal treatments had the highest cell wall weight, second only to the deficient treatment. These data suggest that increases in floret fresh weight reported earlier (Table B-13) were due to actual increases in cell wall rather than increased water content. Increases in the amount of cell wall may be due to increases in parenchyma cell wall thickness in the pith area and increases in collenchyma cell walls in the epidermis (Spurr 1957).

Changes in the amount of cell wall over the life of the plant were also examined. The ratio of dry cell wall to fresh weight increased as the plant matured. The 5-leaf stage was approximately 5 g•100 g^{-1} FW, the 10-leaf stage was a little over 6 g•100 g^{-1} FW, the 15-leaf stage was approximately 12 g•100 g^{-1} FW for the exterior and 2 g•100 g^{-1} FW for the interior. In the mature stage the normal treatments resulted in 2.6 g•100 g^{-1} FW for the interior and 12 g•100 g^{-1} FW for the exterior.

In order to eliminate the effect of cell water, the amount of cell wall was compared to the amount of dry tissue. The ratio of cell wall to dried stem tissue in the mature stage stem interior showed highly significant differences in the toxic vs. deficient and normal vs. deficient contrasts while stem exterior showed no significant contrasts

(Table B-31). Deficient treatments of stem interior had significantly greater cell wall weights compared to dried weight than other treatments. From Part I of this experiment (Table B-17), it can be seen that the stem interior and exterior had highly significant differences between toxic and other treatments for the ratio of dry tissue to fresh weight and in stem exterior the deficient treatment had a significantly higher ratio than other treatments. Therefore, the differences among treatments in the stem interior may be due to actual changes in wall weight rather than changes in water content. The increased dry to fresh weight of the exterior may be due to lignin deposit in the exterior tissue. Lignin is dissolved by strong acids (Goodwin and Mercer 1983) and acetic acid was used in the extraction process. If the lignin is the only factor which differs and it is eliminated through cell wall extraction, the differences in weight would disappear.

CELL WALL BORON CONTENT

Cell wall boron content was determined to examine whether the cell wall changes were associated with differing amounts of boron or if the changes were occurring as a secondary effect. Testing for boron content confirmed that hollow stem, increased cell wall content, and low boron levels occur in the same tissue. Treatment contrasts of toxic vs. deficient and normal vs. deficient were significant in the 5-leaf stage (Table B-32). No significant differences were seen in the 10-leaf stage, and the 15-leaf stage stem interior was significant for

the same contrasts as the 5-leaf stage. The 15-leaf stem exterior contrasts of normal vs. deficient tested highly significant and the toxic vs. deficient were significant at the 0.10 probability level. Contrasts for the mature stage stem interior and exterior toxic vs. deficient treatment was highly significant, and the normal vs. deficient treatment was significant (Table B-33). In florets, toxic vs. deficient treatments and normal vs. deficient treatments were both highly significant contrasts. This confirms that the tissue most effected by boron deficient treatments had the least amount of boron present in the tissue. This response supports a direct relationship between boron and hollow stem rather than an indirect, secondary effect. Hollow stem was found in the 15-leaf stage only in boron deficient tissue (Table B-19). In the mature stage, hollow stem was the most severe in the most deficient tissue. The 10-leaf stage had no clear differences in any treatments which may be due to an over supply of boron or a lag in the growth curve, decreasing the amount of boron needed. Critical boron levels for the mature stage ranged from 57 to 74 $\mu g \cdot g^{-1}$ for stem interior, 28 to 38 $\mu g \cdot g^{-1}$ for stem exterior, and 24 to 32 $\mu g \cdot g^{-1}$ for florets.

CELL WALL MINERAL CONTENT

To determine if hollow stem was an effect of the disorganization of the cell wall, the calcium content was examined. Calcium is associated with the pectin fraction of the middle lamella. The calcium content of the cell wall showed no significant treatment contrasts in

the 5- or 10-leaf stages (Table B-34). The 15-leaf stage had significant effects in the normal vs. deficient contrast. The mature stage stem interior showed significantly more calcium in the boron deficient wall with toxic vs. deficient and normal vs. deficient contrasts testing highly significant (Table B-35). This is opposite results found in dried whole tissue where increased calcium was associated with increased boron concentration (Table B-6). Therefore, the increased calcium may be associated with increased cell wall production in the interior. Stem exterior showed highly significant contrast between toxic vs. normal and a 0.10 probability level in toxic vs. deficient contrasts. High levels of calcium in the toxic treatment of the exterior agrees with the trend found in dry tissue (Table B-6). Florets showed no significant differences in any contrasts.

Magnesium and potassium content was examined to determine if changes in the cell wall was associated with changes in the content of these elements. Magnesium and potassium showed few differences in any tissue (Tables B-36, B-37, B-38, B-39). This indicates boron deficiency increases calcium deposition in the wall, but does not effect potassium or magnesium content of the wall, and therefore changes in the wall are probably not secondary effects from these minerals.

CELL WALL FRACTIONS

The fact that boron content changes the total cell wall does not indicate exactly what is changed. Therefore, selected samples were

fractionated to pinpoint the location in the wall where changes were taking place. The cell walls of the plants in experiment one were fractionated into four parts (Table B-40). There were no extreme differences among treatments, however the deficient treatment was lower in ionically-associated and covalently-associated pectin fractions and higher in hemicellulosic and cellulosic fractions than in normal or toxic levels. Ionically-associated pectin, hemicellulosic and cellulosic fractions had no significant treatment contrasts. Covalently-associated pectin showed significant contrast effect in normal vs. deficient treatments. These results suggest that boron deficiency is associated with increases in total wall content due to the additive result of minor increases in hemicellulosic and cellulosic fractions. Boron deficiency has been reported to increase cellulose, decrease pectin (Yamauchi et al. 1986), and decrease hemicellulose (Kouchi and Kumazawa 1976). Decreases in pectin support the possibility that hollow stem occurs due to disorganization of the middle lamella. It is possible that the rapid growth during floret development causes physical stresses which pull the cells apart at the middle lamella.

CHAPTER IV

CONCLUSIONS

The amount of boron present in cell wall tissue is associated with the physiological disorder hollow stem. Tissues deficient in boron have a higher degree of hollow stem and a greater amount of cell wall. Minor increases in the cell wall fractions of hemicellulosic and cellulosic fractions may be responsible for the overall cell wall increase in boron deficient plants. Boron deficiency also increased calcium content in extracted cell walls, as opposed to the decreased calcium concentration in whole tissue. This increase in calcium may be due to increased cell wall formation. Boron deficiency does not effect the wall through decreasing the amount of wall present, but it may effect the wall to wall cohesion, or cell wall component adhesion. Separation may be occurring at the middle lamella due to lack of pectin or altered pectin structure due to an excess of calcium in the wall. Further study of the wall components and their chemical makeup will be needed to study this effect.

LITERATURE CITED

LITERATURE CITED

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives John Wiley and Sons, Inc., New York.

Goldbach, H. and A. Amberger. 1986. Influence of boron nutrition on cell wall polysaccharides in cell cultures of Daucus carota L. Journal of Plant Physiology 123:263-269.

Goodwin, T. W. and E. I. Mercer. 1983. Introduction to Plant Biochemistry. 2nd edition. Pergamon Press, Oxford.

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiology Plant 62:25-31.

Gupta, U. C., Y. W. Jame, C. A. Campbell, A. J. Leyshon, and W. Nicholaichuk. 1985. Boron toxicity and deficiency: A review. Canadian Journal of Soil Science 65:381-409.

Hirsch, A. M. and J. G. Torrey. 1980. Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. Canadian Journal of Botany 58:856-866.

Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Jarvis, M. C. 1982. The proportion of calcium-bound pectin in plant cell walls. Planta 154:344-346.

Jarvis, M. C., M. A. Hall, D. R. Threlfall, and J. Friend. 1981. The polysaccharide structure of potato cell walls: Chemical fractionation. Planta 152:93-100.

Jones, T. M. and P. Albersheim. 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. Plant Physiology 49:926-936.

Kouchi, H. and K. Kumazawa. 1975. Anatomical responses of root tips to boron deficiency: II. Effect of boron deficiency on the cellular growth and development in root tips. Soil Science and Plant Nutrition 21:137-150.

Kouchi, H. and K. Kumazawa. 1976. Anatomical responses of root tips to boron deficiency: III. Effect of boron deficiency on subcellular structure of root tips, particularly on morphology of cell wall and its related organelles. Soil Science and Plant Nutrition 22:53-71.

Petracek, P. D. and C. E. Sams. 1987. The influence of boron on the development of broccoli plants. Journal of Plant Nutrition 10:2095-2107.

Rajaratnam, J. A. and J. B. Lowry. 1974. The role of boron in the oil-palm (Elaeis guineensis). Annals of Botany 38:193-200.

SAS Institute Inc. 1985. SAS User's Guide: Basics, Version 5 Edition. Cary, North Carolina.

Spurr, A. R. 1957. The effect of boron on cell wall structure in celery. American Journal of Botany 44:637-650.

Yamauchi, T., T. Hara, and Y. Sonoda. 1986. Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant Cell Physiology 27:729-732.

APPENDIXES

APPENDIX A

PROCEDURES

NUTRIENT SOLUTION

Chemicals

KNO_3	MW 101.11 use 101.11 grams
$Ca(NO_3)_2.4H_2O$	MW 236.16 use 236.16 grams
$NH_4H_2PO_4$	MW 115.08 use 115.08 grams
$MgSO_4 \cdot 7H_2O$	MW 246.49 use 246.49 grams
KCl	MW 74.55 use 3.728 grams
H_3BO_3	MW 61.84 use 4.638 grams
$MnSO_4 \cdot H_2O$	MW 169.01 use 0.338 grams
$ZnSO_4 \cdot 7H_2O$	MW 287.55 use 0.575 grams
$CuSO_4 \cdot 5H_2O$	MW 249.71 use 0.125 grams
Na_2MoO_4	MW 241.95 use 0.121 grams
$FeSO_4$	MW 278.02 use 5.560 grams
NaEDTA	MW 372.24 use 7.414 grams

Procedure

1. Dissolve NaEDTA in 400 ml of heated distilled, deionized water. Dissolve $FeSO_4$ in another 400 ml water. Pour NaEDTA solution into $FeSO_4$ solution. Bring to 1000 ml volume.
2. Dissolve each of the other chemicals in separate 1000 ml solutions.
3. Add 1 ml of each solution per liter of final nutrient mixture into container. (Example: 20 ml of KNO_3 solution into a 20 liter jug)
4. Adjust final nutrient mixture with NaOH to pH 6.0.

Reference

Epstein, Emanuel. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Inc. New York.

NITROGEN ANALYSIS

Procedure

1. Place 0.20 gms of dry, milled tissue into 125 ml Erlenmeyer flask. Add 10 ml H_2SO_4,, leave overnight to digest.

2. Heat at 200°C or more until approximately 5 ml is left in flask, approximately 2.5 hours. Remove from hot plate, let cool.

3. Add 20 ml 35% H_2O_2. Heat until clear. Heat for 45 more minutes. [If samples remain clouded, let cool and add more peroxide. Reheat to clear plus 45 minutes.]

4. Add distilled water to cool, approximately 100 ml. Let completely cool.

5. Bring to 250 ml volume in volumetric with distilled water. Shake and allow to stabilize.

6. Run sample on auto analyzer.

Reference

Thomas, R. L., R. W. Sheard, and J. R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. Agronomy Journal 59:240-243.

ANALYSIS PROCEDURE FOR CALCIUM, MAGNESIUM, AND POTASSIUM

Procedure

1. Weigh 0.5 grams dried, milled sample into 18 mm test tube. Mark sample number by arrangement of test tubes in rack.
2. Ash sample for 4 hours to overnight at 500 to 525°C. Let cool.
3. Add 4 ml 4N HCl. Vortex on low.
4. Add 11 ml distilled, deionized water. Vortex on low.
5. Let silica settle 1-2 minutes.

For Calcium and Magnesium:

A. Pick up 1 ml of sample, dispense 10 ml, using auto-dilutor, into 16 mm tubes.

B. Pick up 1 ml of diluted sample, dispense 10 ml into clean 16 mm tube.

C. Add 1 ml 5% lanthium chloride solution (for a 0.45% lanthium solution).

For Potassium:

A. Do #1 and 2 from above dilution, then pick up 1 ml from second dilution, dispense 10 ml into another clean 16 mm tube.

B. Add 1 ml 5% lanthium chloride solution (optional).

6. Read on atomic absorption instrument, according to specifications posted.

Modification for Cell Walls

Use 0.10 grams dried sample and do one dilution.

ANALYSIS PROCEDURE FOR BORON

Reagents

Curcumin-Oxalic Acid: Dissolve 0.1 grams curcumin and 12.5 grams oxalic acid in 80 ml ethanol. Bring to 100 ml volume.

95% ethanol

0.1 N HCl

Procedure

1. Weigh 0.5 grams dried, milled tissue into crucibles. Ash at 500°C for 4 hours to overnight. Let cool.
2. Pipette 10 ml of 0.1N HCl into crucible. Stir. Filter through #2 filters into 30 ml cups.
3. Pipette 1 ml of sample into 50 ml cup. Add 4 ml curcumin-oxalic acid reagent. Swirl to mix.
4. Place cups into 55°C drying oven. Dry completely--approximately 3 hours. Let cool.
5. Pipette 25 ml ethanol into cups. Stir.
6. Place sample into colorimeter tubes and take reading.

Calculation

ppm boron in tissue = μg boron in 1 ml solution x 20

Modification for Cell Walls

Use only 0.1 grams tissue.

Reference

Jackson, M. L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

CELL WALL EXTRACTION PROCEDURE FOR BROCCOLI

Chemicals

HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, MW=238.3, powder)

Chloroform (high purity, analytical reagent grade)

Methanol (99.9% assay, analytical reagent grade)

Acetone (high grade, analytical reagent grade)

Phenol (loose crystals, FW 94.11, analytical reagent grade)

Acetic Acid (glacial, assay 99.7%, reagent grade)

Reagents

1. 20mM HEPES-NaOH pH 6.9 To approximately 750ml of distilled deionized water add 0.4766 grams of HEPES. Add NaOH to correct pH to 6.9 and bring to 1 L volume.

2. Phenol:acetic acid:H_2O, 2:1:1 w/v/v Phenol burns--wear lab coat, apron, mask and gloves when mixing. To a 500g bottle of phenol crystals add 250 ml glacial acetic acid and 250 ml of DD H_2O. (mix in bottle to avoid contact with phenol, if possible)

3. Chloroform:methanol, 1:1 v/v Dangerous vapors, wear mask. Add equal parts chloroform and methanol.

4. Acetone

5. P_2O_5, phosphorus pentaoxide used as a drying agent.

Mixed reagents can be stored under refrigeration for no more than two weeks.

Procedure

1. To 25 g of fresh tissue, add 50 ml HEPES-NaOH and homogenize with a Polytron for 1.5 min. Move to hood for next step.

2. Filter homogenate through Miracloth. Wash residue with 30ml HEPES-NaOH. Wash residue again with 30ml phenol:acetic acid:water (inactivates enzymes).

Discard liquid in waste container properly marked for hazardous waste disposal.

3. Remove from filter and suspend in 30ml chloroform:methanol for 10 minutes or more. During that time, gently homogenize suspension with Polytron for 30 seconds.

4. Filter suspension through a course grade sintered-glass filter. Wash residue with 30ml chloroform:methanol.

5. Remove from filter and suspend residue in 30ml of acetone for 10 min. Filter through miracloth and wash with 75ml acetone.

Discard liquid in second, separate, waste container also properly marked.

6. [Optional] Shift residue to a vacuum filtration system and vacuum filter for app. 10 min. to remove acetone.

7. Place material in non-plastic container with open top. Place roughly four tablespoons P_2O_5 in glass or ceramic dish and place dish in vacuum desiccator. Place material in the desiccator and pull vacuum for one hour. If possible, dry at 25 to 37 degrees C. Leave in desiccator (under heat) for 3 days and record weight. Dry to constant weight under vacuum.

8. Record final weights.

9. Perform iodine test for starch (see attached).

Modification for Florets: Florets have a high amount of protein and must be handled somewhat differently to extract all the cell wall content. Use 40 or 50 ml of the reagents to wash and suspend material and wash with approximately 100 ml acetone. Also vacuum filter the sintered glass filters in step 4. These filters must be cleaned with NoChromix each time they are used as the material lodges in the filters under the vacuum filtering.

References

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiology of Plants 62:25-32.

Jarvis, M. C. 1982. The proportion of calcium-bound pectin in plant cell walls. Planta 154:344-346.

Jarvis, M. C., M. A. Hall, D. R. Threlfall, and J. Friend. 1981. The polysaccharide structure of potato cell walls: Chemical fractionation. Planta 152:93-100.

CELL WALL FRACTIONATION PROCEDURE FOR BROCCOLI

Chemicals

Na^+-acetate (trihydrate, 99+%)

CDTA (trans-1,2-diaminocyclohexane-N,N,N′N′-tetraacetic acid monohydrate, 98%, $C_6H_{10}[N(CH_2CO_2H)_2]_2.H_2O$

Sodium carbonate (Na_2CO_3 monohydrate, analytical reagent grade)

Potassium hydroxide (KOH, analytical reagent grade)

Sodium borohydrate ($NaBH_4$, 98%)

Phosphorus pentaoxide (P_2O_5)

Acetic acid (glacial, assay 99.7%, reagent grade)

Reagents

1. 50mM sodium acetate with 50 mM CDTA pH 6.5 Dissolve 6.87g Na^+-acetate in approximately 750 ml distilled deionized water. Add 18.218g CDTA and stir. Use NaOH to correct pH to 6.5. Bring to 1 L volume with water.

2. 50 mM Na_2CO_3 with 2 mM CDTA Dissolve 6.2g Na_2CO_3 in approximately 750 ml distilled deionized water. Add 0.7287g CDTA and stir. Bring to 1 L volume with water.

3. 4 M KOH with 100mM $NaBH_4$ Dissolve 257.98g KOH in 500 ml distilled deionized water. Add 3.86g $NaBH_4$. Bring to 1 L volume with water.

Procedure

1. Weigh 0.5g dried cell wall (see previous procedure) into a 125 ml Erlenmeyer flask. Add 50 ml Na^+-acetate-CDTA reagent. Stopper. Place into a 25°C shaking waterbath for 6 hours. Pour into a fritted glass filter (course grade). Wash with 15 ml additional reagent. Pour

resulting filtrate into a 12" length of dialysis tubing (Spectra/Por 1: 6000 to 8000 MWCO). Suspend tube in distilled deionized water for 72 hours. Remove and replace water 2-3 times daily. After the 72 hours, either freeze for later tests by placing tube in secure sterile plastic bag or freeze-dry by placing in suitable container such as a strong plastic beaker, float on liquid nitrogen, and place in freeze-dryer. Continue freeze drying until a constant weight is obtained, then weigh on an analytical balance. Record weights. Store in closed container over P_2O_5. The resulting material is the ionically-associated pectin fraction.

2. Return residue from previous step to Erlenmeyer flask. Add 50 ml Na_2CO_3-CDTA reagent. Stopper. Stir constantly for 20 hours at 4°C then 1 hour at 25°C. Filter through fritted glass filter (course grade) and wash with 15 ml of same reagent. Place beaker with filtrate in an ice bath and add acetic acid to neutralize filtrate to pH 6.5. Repeat dialysis and drying/storing as above. This is the covalently bound pectin fraction.

3. Return residue from previous step to Erlenmeyer flask. Add 50 ml of KOH-$NaBH_4$ reagent. Replace air in flask with N_2 gas and stopper. Place in shaking waterbath at 25°C for 1 hour. Filter through fritted glass filter (course grade) and wash with 15 ml of same reagent. Place beaker with filtrate in ice bath and add 14 ml acetic acid while in hood. With beaker still in ice, neutralize to pH 6.5 with additional acetic acid (1-2 ml). Repeat dialysis and drying/storing as above. This is the hemicellulosic fraction.

4. Wash residue remaining in the filter with 100 ml distilled deionized water. Freeze as above for later tests or freeze dry as described in section one. (no dialysis) This is the cellulosic fraction.

NOTE: Fritted glass filters must be cleaned with NoChromix after procedure or next set of samples will not filter properly and vacuum filtering must be used.

References

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiology of Plants 62:25-32.

Jarvis, M. C. 1982. The proportion of calcium-bound pectin in plant cell walls. Planta 154:344-346.

Jarvis, M. C., M. A. Hall, D. R. Threlfall, and J. Friend. 1981. The polysaccharide structure of potato cell walls: Chemical fractionation. Planta 152:93-100.

IODINE TEST FOR STARCH

Reagent

Dissolve 10g KI (potassium iodide) and 2.5g I (iodine) in 30ml of H_2O.

Procedure

Place 0.01g dried cell wall in small Pyrex or Kimex test tube. Add 1 ml distilled deionized water. Heat at 60°C for 10 minutes in block heater. Pour liquid onto spot plate. Add 1-2 drops iodine reagent. Blue color reaction means starch is present.

References

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiology of Plants 62:25-32.

Jarvis, M. C. 1982. The proportion of calcium-bound pectin in plant cell walls. Planta 154:344-346.

Jarvis, M. C., M. A. Hall, D. R. Threlfall, and J. Friend. 1981. The polysaccharide structure of potato cell walls: Chemical fractionation. Planta 152:93-100.

APPENDIX B

DATA AND STATISTICS

Table B-1. Effect of boron treatment concentration on the boron content of mature upper (younger) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Boron Content ($\mu g \cdot g^{-1}$)			
	5L	10L	15L	Mature
0.08	13.8	10.3	8.4	5.8
0.16	24.4	20.4	24.4	14.9
0.32	33.9	33.1	26.5	19.1
0.81	57.3	48.4	43.5	51.7
1.62	83.9	72.4	61.6	88.9
8.10	201.6	189.9	192.9	196.2
Contrasts[ab]				
Toxic vs Deficient	***	***	***	***
Toxic vs Normal	***	***	***	***
Normal vs Deficient	***	***	***	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-2. Effect of boron treatment concentration on the boron content of mature lower (older) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Boron Content ($\mu g \cdot g^{-1}$)		
	10L	15L	Mature
0.08	8.2	8.0	7.7
0.16	21.2	15.8	16.9
0.32	32.9	31.1	18.7
0.81	67.9	55.8	55.7
1.62	100.9	80.5	97.1
8.10	193.2	216.9	199.2
Contrasts[ab]			
Toxic vs Deficient	***	***	***
Toxic vs Normal	***	***	***
Normal vs Deficient	***	***	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-3. Effect of boron treatment concentration on the boron content of mature stage florets and stalk portions of broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Boron Content ($\mu g \cdot g^{-1}$)		
	Florets	Stalk Exterior	Stalk Interior
0.08	0.9	8.5	7.3
0.16	5.7	10.8	7.7
0.32	26.8	10.4	8.8
0.81	43.7	17.2	14.9
1.62	52.3	16.8	16.9
8.10	65.4	32.9	33.0
Contrasts[ab]			
Toxic vs Deficient	***	***	***
Toxic vs Normal	***	***	***
Normal vs Deficient	***	NS	*

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-4. Effect of boron treatment concentration on the calcium content of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$)			
	5L	10L	15L	Mature
0.08	28.6	21.9	22.9	15.8
0.16	28.8	22.2	23.1	13.2
0.32	29.1	21.5	22.2	19.9
0.81	29.5	22.5	20.6	14.5
1.62	28.3	21.1	18.0	15.1
8.10	28.3	21.7	21.6	22.4
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-5. Effect of boron treatment concentration on the calcium content of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$)		
	10L	15L	Mature
0.08	--	44.9	25.8
0.16	42.8	38.9	24.6
0.32	37.8	42.3	26.4
0.81	41.2	36.9	26.4
1.62	--	23.7	20.5
8.10	45.4	38.3	32.2
Contrasts[ab]			
Toxic vs Deficient	NE	NS	NS
Toxic vs Normal	NE	NS	NS
Normal vs Deficient	NE	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), nonsignificant (NS), or non-estimatable (NE) due to missing data.

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-6. Effect of boron treatment concentration on the calcium content of stalk portions and florets of broccoli at the mature stage.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$)		
	Stalk Exterior	Stalk Interior	Florets
0.08	5.4	4.0	--
0.16	4.2	3.3	1.1
0.32	3.7	4.0	0.6
0.81	3.6	4.3	1.6
1.62	4.7	4.7	3.1
8.10	5.8	5.5	1.1
Contrasts[ab]			
Toxic vs Deficient	NS	NS	*
Toxic vs Normal	**	NS	NE
Normal vs Deficient	NS	*	NE

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), nonsignificant (NS), or non-estimatable (NE) due to missing data.

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-7. Effect of boron treatment concentration on the magnesium content of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$)			
	5L	10L	15L	Mature
0.08	3.5	4.6	3.5	3.2
0.16	3.8	4.6	2.6	3.9
0.32	3.4	4.5	3.4	3.9
0.81	3.9	4.9	3.3	2.6
1.62	3.1	4.7	3.1	3.2
8.10	4.1	4.8	3.4	4.3
Contrasts[ab]				
Toxic vs Deficient	*	NS	NS	NS
Toxic vs Normal	***	NS	NS	NS
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-8. Effect of boron treatment concentration on the magnesium content of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$)		
	10L	15L	Mature
0.08	--	5.4	3.7
0.16	5.6	5.4	4.0
0.32	5.3	5.7	3.7
0.81	6.3	4.8	3.0
1.62	--	4.7	3.5
8.10	6.6	4.4	4.3
Contrasts[ab]			
Toxic vs Deficient	NE	NS	NS
Toxic vs Normal	NE	NS	NS
Normal vs Deficient	NE	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), nonsignificant (NS), or non-estimatable (NE) due to missing data.

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-9. Effect of boron treatment concentration on the magnesium content of stalk portions and florets of broccoli at the mature stage.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$)		
	Stalk Exterior	Stalk Interior	Florets
0.08	2.9	3.9	3.0
0.16	2.6	2.9	3.9
0.32	2.9	3.1	2.9
0.81	2.9	3.2	3.1
1.62	3.0	3.3	3.0
8.10	3.5	4.1	3.0
Contrasts[ab]			
Toxic vs Deficient	NS	NS	NS
Toxic vs Normal	*	*	NS
Normal vs Deficient	NS	*	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-10. Effect of boron treatment concentration on the potassium content of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$)		
	10L	15L	Mature
0.08	--	39.6	17.8
0.16	23.4	30.1	24.3
0.32	50.2	27.1	19.4
0.81	36.6	26.7	15.4
1.62	--	20.5	19.6
8.10	48.7	36.9	22.3
Contrasts[ab]			
Toxic vs Deficient	NE	NS	NS
Toxic vs Normal	NE	NS	NS
Normal vs Deficient	NE	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), nonsignificant (NS), or non-estimatable (NE) due to missing data.

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-11. Effect of boron treatment concentration on the potassium content of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$)			
	5L	10L	15L	Mature
0.08	34.3	42.4	30.4	16.6
0.16	38.7	34.9	28.7	21.5
0.32	33.3	34.6	31.2	22.0
0.81	31.6	38.2	26.9	13.9
1.62	34.1	37.3	21.9	14.8
8.10	36.5	42.1	28.3	21.0
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	NS	*	NS	NS
Normal vs Deficient	NS	*	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-12. Effect of boron treatment concentration on the potassium content of stalk portions and florets of broccoli at the mature stage.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$)		
	Stalk Exterior	Stalk Interior	Florets
0.08	21.7	84.3	15.5
0.16	23.1	57.9	15.5
0.32	18.5	57.9	8.6
0.81	16.3	57.4	10.9
1.62	20.4	54.5	10.4
8.10	23.6	73.7	8.9
Contrasts[ab]			
Toxic vs Deficient	NS	NS	NS
Toxic vs Normal	*	NS	NS
Normal vs Deficient	NS	***	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-13. Effect of boron treatment concentration on the fresh weight of broccoli florets at the mature stage of three experiments.

Boron Treatment ($mg \cdot L^{-1}$)	Floret Weight (g)		
	Exp 1	Exp 2	Exp 3
0.08	25.67	14.17	40.69
0.16	--	27.49	40.52
0.32	--	30.87	43.11
0.81	29.08	26.54	39.41
1.62	--	35.21	38.54
8.10	39.26	32.59	30.97
Contrasts[ab]			
Toxic vs Deficient	NS	***	**
Toxic vs Normal	NS	NS	***
Normal vs Deficient	NS	***	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-14. Effect of boron treatment concentration on the head diameter of broccoli of two experiments.

Boron Treatment ($mg \cdot L^{-1}$)	Head Diameter (cm)	
	Exp 3	Exp 1
0.08	11	9
0.16	11	--
0.32	11	--
0.81	11	10
1.62	11	--
8.10	10	12
Contrasts[ab]		
Toxic vs Deficient	NS	***
Toxic vs Normal	NS	*
Normal vs Deficient	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-15. Effect of boron treatment concentration on the time required for broccoli to reach various stages of maturity.

Boron Treatment ($mg \cdot L^{-1}$)	Number of Days		
	10L	15L	Mature
0.08	55	68	102
0.16	54	66	97
0.32	55	65	100
0.81	54	68	102
1.62	56	71	103
8.10	56	67	92
Contrasts[ab]			
Toxic vs Deficient	NS	NS	NS
Toxic vs Normal	NS	NS	*
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-16. Effect of boron treatment concentration on the fresh weight of the total stalk at various growth stages of broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Stalk Weight (g)			
	5L	10L	15L	Mature
0.08	0.55	9.77	40.67	126.92
0.16	0.52	9.02	41.23	175.27
0.32	0.59	10.08	38.98	180.27
0.81	0.58	12.24	38.33	179.49
1.62	0.59	8.71	26.23	200.71
8.10	0.46	10.58	31.23	150.90
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	***	NS	NS	NS
Normal vs Deficient	NS	NS	NS	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-17. Effect of boron treatment concentration on the percent dry weight of broccoli stalks at the mature stage.

Boron Treatment ($mg \cdot L^{-1}$)	Percent Dry Wt ($g \cdot 100\ g^{-1}$ FW)	
	Stalk Exterior	Stalk Interior
0.08	19.63	8.37
0.16	17.29	8.08
0.32	17.15	8.05
0.81	17.72	7.80
1.62	17.76	8.36
8.10	13.48	6.77
Contrasts[ab]		
Toxic vs Deficient	***	***
Toxic vs Normal	***	***
Normal vs Deficient	*	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-18. Effect of boron treatment concentration on the stalk diameter of broccoli at the mature stage.

Boron Treatment ($mg \cdot L^{-1}$)	Stalk Diameter (cm) Mature
0.08	2.8
0.16	2.3
0.32	2.4
0.81	2.5
1.62	2.3
8.10	2.3
Contrasts[ab]	
Toxic vs Deficient	*
Toxic vs Normal	NS
Normal vs Deficient	*

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-19. Effect of boron treatment concentration on the degree[a] of hollow stem in the mature stage broccoli stalk.

Boron Treatment ($mg \cdot L^{-1}$)	Hollow Stem Degree
0.08	4.6
0.16	2.0
0.32	0.2
0.81	0.1
1.62	0.0
8.10	0.7
Contrasts[bc]	
Toxic vs Deficient	***
Toxic vs Normal	NS
Normal vs Deficient	***

[a]0=none, 5=severe.

[b]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[c]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-20. Effect of boron treatment concentration on the volume of hollow stem in the mature stage stalk of broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Hollow Stem Volume[c] (cm^3)
0.08	15.6
0.16	2.2
0.32	0.0
0.81	0.0
1.62	0.0
8.10	0.0
Contrasts[ab]	
Toxic vs Deficient	***
Toxic vs Normal	NS
Normal vs Deficient	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

[c]Volume=(3.142)·[(0.5)·(hollow stem diameter)]^2·(hollow stem length).

Table B-21. Effect of boron treatment concentration on the percent dry weight of upper (younger) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Percent Dry Wt ($g \cdot 100\ g^{-1}$ FW)			
	5L	10L	15L	Mature
0.08	9.34	10.21	13.16	13.59
0.16	9.45	10.12	11.07	11.97
0.32	9.83	9.99	11.27	11.76
0.81	9.74	10.17	11.94	13.14
1.62	8.79	10.02	11.09	11.66
8.10	9.49	9.71	12.61	9.99
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	**
Toxic vs Normal	NS	NS	NS	**
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-22. Effect of boron treatment concentration on the specific leaf weight of upper (younger) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Specific Leaf Weight ($g \cdot cm^{-2}$)			
	5L	10L	15L	Mature
0.08	0.47	0.49	0.83	1.00
0.16	0.47	0.52	0.71	0.81
0.32	0.41	0.51	0.73	0.84
0.81	0.40	0.52	0.75	0.91
1.62	0.38	0.51	0.62	0.84
8.10	0.45	0.54	0.78	0.67
Contrasts[ab]				
Toxic vs Deficient	NS	*	NS	***
Toxic vs Normal	NS	NS	NS	*
Normal vs Deficient	NS	NS	NS	*

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-23. Effect of boron treatment concentration on the dry weight of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Dry Weight (g)			
	5L	10L	15L	Mature
0.08	0.04	0.57	1.87	2.71
0.16	0.05	0.53	1.35	1.58
0.32	0.05	0.54	1.62	1.69
0.81	0.05	0.67	1.58	1.98
1.62	0.05	0.52	0.95	1.79
8.10	0.05	0.68	1.34	1.79
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	**
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	*	NS	*	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-24. Effect of boron treatment concentration on the total fresh weight of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Fresh Weight (g)			
	5L	10L	15L	Mature
0.08	0.48	5.67	14.09	20.14
0.16	0.49	5.21	12.37	13.41
0.32	0.50	5.49	14.29	14.44
0.81	0.48	6.64	13.63	15.43
1.62	0.52	5.16	8.67	14.90
8.10	0.48	6.99	10.72	18.01
Contrasts[ab]				
Toxic vs Deficient	NS	NS	*	NS
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	NS	NS	NS	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-25. Effect of boron treatment concentration on the percent dry weight of lower (older) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Percent Dry Wt ($g \cdot 100\ g^{-1}$ FW)		
	10L	15L	Mature
0.08	10.21	10.79	11.71
0.16	11.63	10.79	11.01
0.32	11.42	11.50	11.66
0.81	11.60	11.48	12.68
1.62	11.39	9.86	11.48
8.10	10.79	11.29	10.14
Contrasts[ab]			
Toxic vs Deficient	**	NS	NS
Toxic vs Normal	**	NS	*
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-26. Effect of boron treatment concentration on the specific leaf weight of lower (older) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Specific Leaf Weight ($g \cdot cm^{-2}$)		
	10L	15L	Mature
0.08	0.45	0.55	0.80
0.16	0.46	0.56	0.71
0.32	0.42	0.56	0.73
0.81	0.43	0.55	0.67
1.62	0.44	0.51	0.84
8.10	0.48	0.54	0.58
Contrasts[ab]			
Toxic vs Deficient	NS	NS	***
Toxic vs Normal	**	NS	**
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-27. Effect of boron treatment concentration on the dry weight of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Dry Weight (g)		
	10L	15L	Mature
0.08	0.15	0.94	2.08
0.16	0.19	1.02	1.53
0.32	0.21	0.92	1.74
0.81	0.20	0.89	1.74
1.62	0.18	0.67	1.96
8.10	0.24	0.92	1.26
Contrasts[ab]			
Toxic vs Deficient	NS	NS	***
Toxic vs Normal	NS	NS	**
Normal vs Deficient	NS	NS	*

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-28. Effect of boron treatment concentration on the fresh weight of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Fresh Weight (g)		
	10L	15L	Mature
0.08	1.26	8.75	17.54
0.16	1.69	9.79	13.93
0.32	1.89	8.01	14.77
0.81	1.76	8.07	13.85
1.62	1.60	6.83	17.29
8.10	2.21	8.22	12.50
Contrasts[ab]			
Toxic vs Deficient	*	NS	***
Toxic vs Normal	NS	NS	**
Normal vs Deficient	NS	NS	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-29. Effect of boron treatment concentration on the ratio of dry extracted cell wall weight to fresh weight of stalk and stalk portions of broccoli at various stages of development.

Boron Treatment ($mg \cdot L^{-1}$)	Dry/Fresh Wt ($g \cdot 100\ g^{-1}$ FW)			
	5L	10L	15L Exterior	15L Interior
0.08	5.18	6.07	11.91	2.03
0.16	5.32	5.99	12.64	2.22
0.32	5.39	6.43	13.27	2.33
0.81	5.52	6.28	13.05	2.09
1.62	5.32	6.17	12.02	2.00
8.10	5.15	6.29	11.83	1.97
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	*	NS	NS	NS
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-30. Effect of boron treatment concentration on the ratio of dry extracted cell wall to fresh weight of broccoli plant portions at the mature stage only.

Boron Treatment ($mg \cdot L^{-1}$)	Dry/Fresh Wt ($g \cdot 100\ g^{-1}$ FW)		
	Stalk Interior	Stalk Exterior	Florets
0.08	3.54	14.18	8.52
0.16	2.80	12.52	6.33
0.32	2.62	11.81	7.19
0.81	2.65	11.84	7.03
1.62	2.66	12.02	7.54
8.10	2.14	9.25	6.07
Contrasts[ab]			
Toxic vs Deficient	***	**	***
Toxic vs Normal	**	*	*
Normal vs Deficient	***	NS	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-31. Effect of boron treatment concentration on the ratio of extracted cell wall to oven dried weight of stalks at the mature stage of broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Stalk Exterior ($g \cdot g^{-1}$)	Stalk Interior ($g \cdot g^{-1}$)
0.08	0.72	0.42
0.16	0.72	0.35
0.32	0.69	0.33
0.81	0.67	0.34
1.62	0.68	0.32
8.10	0.67	0.32
Contrasts[ab]		
Toxic vs Deficient	NS	***
Toxic vs Normal	NS	NS
Normal vs Deficient	NS	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-32. Effect of boron treatment concentration on the boron content of extracted cell walls of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Boron Content ($\mu g \cdot g^{-1}$)			
	5L	10L	15L Stalk Interior	15L Stalk Exterior
0.08	17.2	29.6	22.9	14.8
0.16	28.6	33.6	40.9	28.1
0.32	31.2	27.7	33.9	33.6
0.81	33.6	34.7	44.7	25.1
1.62	23.5	35.3	34.6	37.2
8.10	32.0	35.2	44.0	27.4
Contrasts[ab]				
Toxic vs Deficient	**	NS	**	*
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	**	NS	**	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-33. Effect of boron treatment concentration on the boron content of extracted cell walls of various portions of mature stage broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Boron Content ($\mu g \cdot g^{-1}$)		
	Stalk Interior	Stalk Exterior	Florets
0.08	39.3	15.2	4.9
0.16	50.9	38.2	24.5
0.32	60.5	28.8	29.2
0.81	57.5	36.3	32.7
1.62	74.4	37.2	29.1
8.10	68.6	47.9	33.1
Contrasts[ab]			
Toxic vs Deficient	***	***	***
Toxic vs Normal	NS	NS	NS
Normal vs Deficient	**	**	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-34. Effect of boron treatment concentration on the calcium content of extracted cell walls of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content ($\mu g \cdot g^{-1}$)			
	5L	10L	15L Stalk Interior	15L Stalk Exterior
0.08	415	291	295	342
0.16	431	254	255	310
0.32	445	266	244	292
0.81	433	289	224	297
1.62	451	295	300	273
8.10	390	306	265	306
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	NS	NS	**	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-35. Effect of boron treatment concentration on the calcium content of extracted cell walls of mature stage plant portions of broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content ($\mu g \cdot g^{-1}$)		
	Stalk Interior	Stalk Exterior	Florets
0.08	533	421	231
0.16	285	374	201
0.32	301	400	280
0.81	336	407	243
1.62	286	412	250
8.10	310	485	297
Contrasts[ab]			
Toxic vs Deficient	***	*	NS
Toxic vs Normal	NS	***	NS
Normal vs Deficient	***	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-36. Effect of boron treatment concentration on the potassium content of extracted cell walls of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content ($\mu g \cdot g^{-1}$)			
	5L	10L	15L Stalk Interior	15L Stalk Exterior
0.08	178	92	196	65
0.16	174	67	261	84
0.32	187	76	213	81
0.81	207	99	194	88
1.62	240	101	214	49
8.10	131	134	197	55
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	**	*	NS	NS
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-37. Effect of boron treatment concentration on the potassium content of extracted cell walls of mature stage broccoli, various plant parts.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content ($\mu g \cdot g^{-1}$)		
	Stalk Interior	Stalk Exterior	Florets
0.08	655	183	126
0.16	660	273	89
0.32	681	205	140
0.81	783	218	112
1.62	588	235	138
8.10	618	161	134
Contrasts[ab]			
Toxic vs Deficient	NS	NS	NS
Toxic vs Normal	NS	**	NS
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-38. Effect of boron treatment concentration on the magnesium content of extracted cell walls of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content ($\mu g \cdot g^{-1}$)			
	5L	10L	15L Stalk Interior	15L Stalk Exterior
0.08	61	54	72	50
0.16	63	44	81	53
0.32	64	45	71	49
0.81	67	56	69	51
1.62	70	56	82	42
8.10	54	62	78	48
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	*	NS	NS	NS
Normal vs Deficient	NS	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-39. Effect of boron treatment concentration on the magnesium content of extracted cell walls of mature stage broccoli plant portions.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content ($\mu g \cdot g^{-1}$)		
	Stalk Interior	Stalk Exterior	Florets
0.08	129	76	75
0.16	98	84	66
0.32	118	78	70
0.81	131	87	66
1.62	108	86	75
8.10	113	90	71
Contrasts[ab]			
Toxic vs Deficient	NS	**	NS
Toxic vs Normal	NS	NS	NS
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-40. Effect of boron treatment concentration on the ratio of cell wall fraction to total extracted cell wall of broccoli stalks in experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Fraction/Total Wall ($g \cdot 100\ g^{-1}$)			
	Ionically-Associated Pectin	Covalently-Associated Pectin	Hemicellulose	Cellulose
0.08	12.65	11.19	12.25	41.70
0.81	13.10	15.11	11.49	39.81
8.10	14.73	12.74	11.66	37.31
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	NS	NS	NS	NS
Normal vs Deficient	NS	**	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-41. Effect of boron treatment concentration on the calcium content of younger (upper) leaves of broccoli at mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	10.6
0.81	12.3
8.10	13.3
Contrasts[ab]	
Toxic vs Deficient	**
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-42. Effect of boron treatment concentration on the calcium content of older (lower) leaves of broccoli at the mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	16.1
0.81	16.3
8.10	18.4
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-43. Effect of boron treatment concentration on the calcium content of the stalk of broccoli at the mature stage of experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium Content (10^3 $\mu g \cdot g^{-1}$) Stalk
0.08	5.0
0.81	4.4
8.10	4.0
Contrasts[ab]	
Toxic vs Deficient	*
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81, $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-44. Effect of boron treatment concentration on the magnesium content of younger (upper) leaves of broccoli at the mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	1.1
0.81	1.1
8.10	1.6
Contrasts[ab]	
Toxic vs Deficient	***
Toxic vs Normal	***
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-45. Effect of boron treatment concentration on the magnesium content of older (lower) leaves of broccoli at mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	1.4
0.81	1.3
8.10	1.7
Contrasts[ab]	
Toxic vs Deficient	*
Toxic vs Normal	**
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81, $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-46. Effect of boron treatment concentration on the magnesium content of the stalk of broccoli at the mature stage of experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium Content (10^3 $\mu g \cdot g^{-1}$) Stalk
0.08	1.3
0.81	1.2
8.10	1.2
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81, $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-47. Effect of boron treatment concentration on the potassium content of older (lower) leaves of broccoli at the mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	11.3
0.81	12.1
8.10	13.8
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81, $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-48. Effect of boron treatment concentration on the potassium content of younger (upper) leaves of broccoli at the mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$) Mature
0.08	9.3
0.81	10.2
8.10	12.1
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-49. Effect of boron treatment concentration on the potassium content of the stalk at the mature stage of broccoli, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium Content (10^3 $\mu g \cdot g^{-1}$) Stalk
0.08	23.8
0.81	22.7
8.10	22.1
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81, $mg \cdot L^{-1}$ treatment; deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-50. Effect of boron treatment concentration on the percent dry weight of broccoli florets, experiment three only.

Boron Treatment ($mg \cdot L^{-1}$)	Floret Weight ($g \cdot 100\ g^{-1}$ FW)
0.08	15.01
0.16	15.14
0.32	14.42
0.81	14.78
1.62	16.06
8.10	15.64
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-51. Effect of boron treatment concentration on the time required for broccoli to reach maturity.

Boron Treatment ($mg \cdot L^{-1}$)	Days to Harvest Mature
0.08	118
0.81	116
8.10	121
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	*
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-52. Effect of boron treatment concentration on the fresh weight of total stalk of broccoli at the mature stage of experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Stalk Weight (g) Mature
0.08	70.67
0.81	72.97
8.10	93.00
Contrasts[ab]	
Toxic vs Deficient	***
Toxic vs Normal	**
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-53. Effect of boron treatment concentration on the stalk diameter of broccoli, mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Stalk Diameter (cm) Mature
0.08	2.6
0.81	2.6
8.10	2.7
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	*
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-54. Effect of boron treatment concentration on the hollow stem length in broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Hollow Stem Length (cm)		
	Exp 1 Mature	Exp 2 Mature	Exp 2 15L
0.08	0.4	19.5	2.2
0.16	--	6.7	0.0
0.32	--	0.4	0.0
0.81	0.0	1.5	0.0
1.62	--	0.0	0.0
8.10	0.0	1.2	0.0
Contrasts[ab]			
Toxic vs Deficient	NS	***	***
Toxic vs Normal	NS	NS	NS
Normal vs Deficient	NS	***	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-55. Effect of boron treatment concentration on the hollow stem width in broccoli.

Boron Treatment ($mg \cdot L^{-1}$)	Hollow Stem Width (cm)		
	Exp 1 Mature	Exp 2 Mature	Exp 2 15L
0.08	0.1	0.9	0.2
0.16	--	0.4	0.0
0.32	--	0.1	0.0
0.81	0.0	0.1	0.0
1.62	--	0.0	0.0
8.10	0.0	0.1	0.0
Contrasts[ab]			
Toxic vs Deficient	NS	***	***
Toxic vs Normal	NS	NS	NS
Normal vs Deficient	NS	***	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-56. Effect of boron treatment concentration on the percent dry weight of total leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Percent Dry Wt ($g \cdot 100\ g^{-1}$ FW) 5L	10L
0.08	9.34	10.51
0.16	9.45	10.49
0.32	9.83	10.30
0.81	9.74	10.47
1.62	8.79	10.34
8.10	9.49	9.95
Contrasts[ab]		
Toxic vs Deficient	NS	*
Toxic vs Normal	NS	*
Normal vs Deficient	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-57. Effect of boron treatment concentration on the percent dry weight of total leaves of broccoli at various growth stages, experiment three.

Boron Treatment ($mg \cdot L^{-1}$)	Percent Dry Wt ($g \cdot 100\ g^{-1}$ FW)			
	5L	10L	15L	Mature
0.08	12.40	9.32	11.25	10.27
0.16	12.14	11.32	10.77	11.52
0.32	12.43	10.92	10.58	10.81
0.81	12.57	9.89	10.37	10.69
1.62	12.20	8.92	10.49	11.29
8.10	10.97	11.49	8.56	10.21
Contrasts[ab]				
Toxic vs Deficient	***	NS	***	NS
Toxic vs Normal	***	NS	***	*
Normal vs Deficient	NS	NS	NS	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-58. Effect of boron treatment concentration on the specific leaf weight of total leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Specific Leaf Weight ($g \cdot cm^{-2}$) 5L	10L
0.08	0.47	0.48
0.16	0.47	0.49
0.32	0.41	0.48
0.81	0.40	0.49
1.62	0.38	0.49
8.10	0.45	0.53
Contrasts[ab]		
Toxic vs Deficient	NS	*
Toxic vs Normal	NS	*
Normal vs Deficient	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-59. Effect of boron treatment concentration on the fresh weight of younger (upper) leaves of broccoli at mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Fresh Weight (g) Mature
0.08	9.24
0.81	9.48
8.10	9.99
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-60. Effect of boron treatment concentration on the fresh weight of older (lower) leaves of broccoli at mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Fresh Weight (g) Mature
0.08	14.37
0.81	15.00
8.10	15.95
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-61. Effect of boron treatment concentration on the leaf area of younger (upper) leaves at mature growth stage of broccoli, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Leaf Area (cm^2) Mature
0.08	105.9
0.81	113.8
8.10	118.7
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-62. Effect of boron treatment concentration on the leaf area of younger (upper) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Leaf Area (cm^2)			
	5L	10L	15L	Mature
0.08	10.3	117.7	223.5	271.7
0.16	10.2	101.6	196.0	201.2
0.32	12.1	107.3	223.2	202.0
0.81	11.6	130.5	220.0	220.5
1.62	12.7	103.6	151.5	208.5
8.10	10.4	124.8	172.0	271.2
Contrasts[ab]				
Toxic vs Deficient	NS	NS	NS	NS
Toxic vs Normal	NS	NS	NS	*
Normal vs Deficient	*	NS	NS	*

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-63. Effect of boron treatment concentration on the leaf area of older (lower) leaves at mature growth stage of broccoli, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Leaf Area (cm^2) Mature
0.08	183.9
0.81	198.4
8.10	214.5
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-64. Effect of boron treatment concentration on the leaf area of older (lower) leaves of broccoli at various growth stages.

Boron Treatment ($mg \cdot L^{-1}$)	Leaf Area (cm^2)		
	10L	15L	Mature
0.08	33.4	170.7	259.5
0.16	42.4	181.7	217.5
0.32	52.0	164.2	237.2
0.81	47.3	163.5	262.0
1.62	40.9	131.0	234.7
8.10	49.2	169.7	224.2
Contrasts[ab]			
Toxic vs Deficient	NS	NS	NS
Toxic vs Normal	NS	NS	NS
Normal vs Deficient	NS	NS	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.16, 0.32, 0.81, and 1.62 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-65. Effect of boron treatment concentration on the ratio of dry extracted cell wall weight to fresh weight of broccoli, mature stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Dry/Fresh Wt ($g \cdot 100\ g^{-1}$ FW)	
	Stalk	Florets
0.08	6.55	7.30
0.81	4.90	6.83
8.10	4.79	6.91
Contrasts[ab]		
Toxic vs Deficient	***	NS
Toxic vs Normal	NS	NS
Normal vs Deficient	***	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatment; and deficient is the 0.08 $mg \cdot L^{-1}$ treatments.

Table B-66. Effect of boron treatment concentration on the calcium content of broccoli stalk cell walls, mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium ($\mu g \cdot g^{-1}$)
0.08	398
0.81	377
8.10	370
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-67. Effect of boron treatment concentration on the calcium content of broccoli floret cell walls, mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Calcium ($\mu g \cdot g^{-1}$)
0.08	314
0.81	268
8.10	265
Contrasts[ab]	
Toxic vs Deficient	**
Toxic vs Normal	NS
Normal vs Deficient	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-68. Effect of boron treatment concentration on the potassium content of stalk cell walls of broccoli, mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium ($\mu g \cdot g^{-1}$)
0.08	421
0.81	334
0.10	349
Contrasts[ab]	
Toxic vs Deficient	*
Toxic vs Normal	NS
Normal vs Deficient	**

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-69. Effect of boron treatment concentration on the potassium content of floret cell walls of broccoli, mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Potassium ($\mu g \cdot g^{-1}$)
0.08	249
0.81	177
8.10	214
Contrasts[ab]	
Toxic vs Deficient	***
Toxic vs Normal	***
Normal vs Deficient	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-70. Effect of boron treatment concentration on the magnesium content of stalk cell walls of broccoli, mature growth stage, experiment one..

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium ($\mu g \cdot g^{-1}$)
0.08	77
0.81	76
8.10	77
Contrasts[ab]	
Toxic vs Deficient	NS
Toxic vs Normal	NS
Normal vs Deficient	NS

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

Table B-71. Effect of boron treatment concentration on the magnesium content of floret cell walls of broccoli, mature growth stage, experiment one.

Boron Treatment ($mg \cdot L^{-1}$)	Magnesium ($\mu g \cdot g^{-1}$)
0.08	64
0.81	43
8.10	54
Contrasts[ab]	
Toxic vs Deficient	**
Toxic vs Normal	***
Normal vs Deficient	***

[a]Linear treatment contrast significant at 0.01 (***), 0.05 (**), 0.10 (*), or nonsignificant (NS).

[b]Toxic is the 8.10 $mg \cdot L^{-1}$ treatment; normal is the 0.81 $mg \cdot L^{-1}$ treatments; and deficient is the 0.08 $mg \cdot L^{-1}$ treatment.

VITA

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