

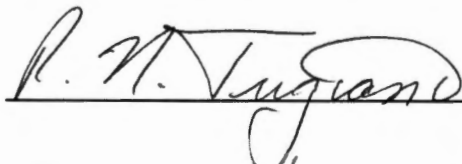
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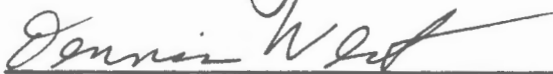
I am submitting herewith a dissertation written by Larry Stone Kennedy entitled "Effects of Boron Deficiency on Morphology, Gas Exchange, and Glucosinolate Composition of Hydroponically Grown Oilseed Rape (*Brassica napus* var. *oleifera*).\" I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.

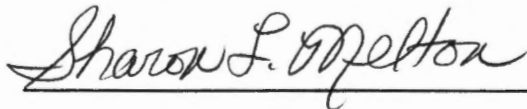


Carl E. Sams, Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the council:



Associate Vice Chancellor
and Dean of the Graduate School

EFFECTS OF BORON DEFICIENCY ON MORPHOLOGY, GAS EXCHANGE, AND
GLUCOSINOLATE COMPOSITION OF HYDROPONICALLY GROWN
OILSEED RAPE (*BRASSICA NAPUS* VAR. *OLEIFERA*)

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Larry Stone Kennedy

May 1994

~~AD-VET-MED~~

Thesis

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To my mother
Julia Evelyn Stone Kennedy
(1913 - 1990)

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ABSTRACT

Oilseed rape (*Brassica napus* var. *oleifera*) was grown in a continuously recirculating hydroponic system designed to accurately and consistently deliver micronutrient concentrations. Perlite was used as the root support medium. Boron (B) treatments ranging from adequate or normal ($0.60 \text{ mg}\cdot\text{L}^{-1}$ added B) to severely deficient ($0.01 \text{ mg}\cdot\text{L}^{-1}$ added B) were applied as modified Hoaglands solutions in three experiments. The effects of B deficiency on plant morphology, seed yield, gas exchange, and seed glucosinolate content were analyzed. Tissue B concentrations were determined by inductively coupled plasma atomic emission spectrometry. Severely B deficient plants were malformed and did not produce seed. Leaf tissue B content was the best indicator of B availability and uptake. Leaves of normal plants contained from 35 to $80 \mu\text{g}\cdot\text{g}^{-1}$, whereas normal appearing but seedless plants averaged $18 \mu\text{g}\cdot\text{g}^{-1}$ B. Histological analysis of stem tissue confirmed that deterioration of vascular tissue and pith cells was directly related to severity of B deficiency. Sporadic cell wall lignification was associated with cellular breakdown in the pith. The best morphological indicators of B deficiency were number of axillary branches and main inflorescence height. An analysis of photosynthesis (Pn) and stomatal conductance (SC) values indicated a minimum leaf tissue B content of $21 \mu\text{g}\cdot\text{g}^{-1}$ was required for normal functioning of leaf Pn and SC. The

stage of plant development was more important than leaf maturity in determining the effect of B on gas exchange capacity. Glucosinolate content was compared by gas chromatography and colorimetry procedures. Gas chromatography proved to be the more accurate and informative method. Seed glucosinolate levels remained stable at leaf tissue B values above $21 \mu\text{g}\cdot\text{g}^{-1}$. Boron deficient plants produced lower quality seed with glucosinolate concentrations significantly above the acceptable canola quality standard of $30 \mu\text{mol}$ per gram of defatted meal. Indole glucosinolates appeared to be less influenced by B availability than aliphatic glucosinolates. The individual glucosinolate composition of seeds was unaffected by B treatment. Tissue B uptake was consistent with treatment B availability throughout all experiments.

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INTRODUCTION

Brassica oilseeds have recently become the third most important source of edible vegetable oils in the world (Downey, 1990). *Brassica napus* and *Brassica rapa* are the predominant oilseed rape species grown around the world. Oilseed rape production greatly increased following the introduction of canola quality rapeseed which must meet reduced erucic acid and glucosinolate requirements (Downey, 1990). Canola oil is highly desirable to health conscious consumers since it contains only 6% saturated fat (Kidd, 1993). In addition, the meal can be used as a component of animal feed.

Although rape production is currently low in the United States, analysts anticipate a steady increase over the coming years to meet both domestic and foreign demand. Plant scientists worldwide are developing cultivars to expand production into new growing regions. Consequently, oilseed rape may be subject to physiological disorders induced by new environmental conditions and cultural requirements.

Mineral nutrition is a major concern of rapeseed producers. The regulation of nitrogen, sulfur, and boron (B) availability is crucial to normal rape ontogeny (Holmes, 1980). Boron nutrition is a particular concern since the range between deficiency and toxicity is very narrow in all the

Brassicas (Gupta et al., 1985). In addition, B deficiency is the most widespread deficiency of the micronutrients (Gupta, 1979). Less than 5% of soil B is available for plant uptake (Evans and Sparks, 1983). Boron is both easily leached and less available under drought conditions. The uptake of B is mostly passive with movement almost entirely confined to the transpiration stream. Therefore, the ability of the plant to absorb B is regulated by the transpiration system, which in turn is influenced by temperature and light intensity (Gupta et al., 1985). At pH values less than 7.0, B is absorbed primarily as boric acid, whereas in solutions of higher pH, borate ions predominate (Evans and Sparks, 1983). Once distributed to the plant cells, B is extremely immobile. Although the morphological effects of B deficiency and toxicity have been well documented (Shorrocks, n.d.), the primary role of B in plant metabolism is still uncertain (Pilbeam and Kirby, 1983). Boron, however, does appear to be closely associated with cell membrane function, which could conceivably affect all aspects of primary plant metabolism (Roldan et al., 1992).

The yield of oilseed rape is clearly the most important consideration to producers. Normal appearing rape plants which produce little or no seed have been noted in fields deficient in B and sulfur (Nuttall et al., 1987). This relationship has not been adequately investigated, nor has the precise range of B availability required to differentiate between normal seed

yielding and normal but seedless rape plants been determined.

B availability is critical to the gas exchange function of plants (Petracek and Sams, 1987). Photosynthesis and stomatal conductance of normal but seedless rape plants, however, have not been evaluated against normal yielding rape plants. Therefore, the degree to which B deficiency impairs gas exchange capacity and ultimately rapeseed production is not known.

The potential of B nutrition to affect glucosinolate biosynthesis in oilseed rape is apparent. Of all the factors controlling secondary metabolism in plants, nutrients are the most important (Barz and Koster, 1981). In addition, seed tissue contains the highest glucosinolate concentration (5 to 10% of dry wt.) of any of the plant tissues (Sang et al., 1984). Since glucosinolates are anionic, a large complement of cations must be present within the seed tissue to maintain the chemical balance (Larsen, 1981). Obviously, the cellular membranes would have to function properly to meet this need.

The objectives of this research were the following: (1) to develop a hydroponic system capable of delivering micronutrients consistently and accurately, (2) to correlate precisely morphological development and seed yield of oilseed rape with B availability, (3) to determine the effects of B availability on gas exchange properties of oilseed rape, and (4) to correlate precisely glucosinolate content and concentration of rapeseed with B availability.

CHAPTER I

LITERATURE REVIEW

BORON NUTRITION IN PLANT METABOLISM

Boron (B) deficiency is the most widespread of any of the micronutrients (Gupta, 1979). Inadequate B nutrition not only impairs plant yield, but also can reduce crop quality. The essentiality of boron to vascular plants was first confirmed in 1923 (Warington, 1923). Among dicotyledons, the requirement for B on a molar basis is higher than for any other micronutrient (Marschner, 1986). Members of the Cruciferae and Leguminosae require the highest levels of B nutrition.

Boric acid (H_3BO_3) is the predominant form of B found in agricultural soils. Most B available to plants comes from soil organic matter and soil surface particles. Less than 5% of soil B is available for plant uptake (Evans and Sparks, 1983). Many soils have insufficient available B because it is both easily leached and less available under drought conditions.

B uptake and translocation in plants is not well understood. Most B is thought to be passively absorbed as undissociated boric acid (Mengel and Kirby, 1982), but some active uptake does occur. Bowen and Nissen (1976; 1977)

determined that low temperature as well as metabolic inhibitors decreased B uptake of excised roots. Gupta et al. (1985) concluded that increasing temperature, light intensity, and photoperiod positively affected B accumulation. In a study utilizing sliced peach leaves, Shu et al. (1991) established that temperature and pH greatly affected B uptake. Active uptake of B by the leaf slices was also found to be greater than passive uptake.

Plant sinks have demonstrated the ability to utilize more B than is normally available or required to produce a healthy plant. The infusion of supplementary B into soybean stems by Schon and Blevins (1987) significantly increased crop yield. Petracek and Sams (1987) found that broccoli plants supplied with levels of B moderately toxic to leaf tissue produced larger heads. Shelp (1988) speculated that, at toxic levels, "B may undergo extensive lateral transfer, probably from xylem to xylem, thereby enhancing the B concentration of developing sinks".

Still, B is relatively immobile in the plant compared to other micronutrients. Long distance transport occurs in the xylem, since B is extremely immobile in the phloem (Mengel and Kirby, 1982). A continuous supply of B is necessary because few plants can redistribute it. Therefore, the transpiration rate has a direct effect on plant B uptake and transport (Halbrooks et al., 1986). Investigators have suspected that small amounts of B were being redistributed from mature to

young tissue when plants are B starved. This was recently confirmed in plants produced under greenhouse (Shelp and Shattuck, 1987) and field conditions (Liu et al., 1993). Genotype differences appear to be crucial in determining how well a plant absorbs and/or transports B in both deficiency (Matoh et al., 1992; Shelp et al., 1992a) and toxicity (Bagheri et al., 1992) studies.

Plant B nutrition is critical because the range between deficiency and toxicity is more narrow than for any other essential element (Epstein, 1972). The morphological consequences of B deficiency and toxicity have been documented for many crop plants (Gupta, 1979; Shorrocks, n.d.). Classic symptoms of B deficiency include inhibition of root growth, breakdown of apical meristems, reproductive system malfunction, and finally cell wall disintegration. Plants subjected to toxic levels of B exhibit marginal and tip chlorosis of leaves that is followed by necrosis.

A definitive primary function for B in the plant is still unknown. This is partly due to the difficulty in separating primary metabolic effects from secondary effects (Pollard et al., 1977). The absence of B quickly inhibits the growth of meristematic tissue and is apparently required for both cell division (Cohen and Lepper, 1977) and cell elongation (Lovatt et al., 1981). Boron is also essential for carbohydrate, nucleic acid, and protein synthesis. RNA production is particularly affected; B deficiency occurs first in

meristematic tissue that requires high levels of RNA synthesis (Mengel and Kirby, 1982).

The effect of insufficient B on plant reproduction is very important to crop producers. A close relationship exists between B availability and the production of copious amounts of viable pollen (Marschner, 1986). In addition, B is required for pollen germination, and is extremely important to pollen tube formation (Lewis, 1980). Reproductive tissue B concentrations required for successful pollination and fertilization are high, and vary considerably among species. Vaughan (1977) determined that silks from B deficient maize plants were non-receptive to viable pollen. Boron nutrition also affects flower morphology and nectar quantity, thereby increasing plant attractiveness to potential pollinators (Marshall et al., 1991).

Lee and Aronoff (1967) proposed that B affects carbohydrate production by regulating the metabolic activity between the glycolytic and pentose phosphate shunt pathways. Under B deficient conditions, the pentose phosphate shunt operates leading to an accumulation of phenolic compounds in the plant. Dugger (1983) stated that lignin develops as a result of this phenolic compound accumulation whereas others disagree (Pilbeam and Kirby, 1983). Ultimately, the formation and accumulation of lignin probably depend on the individual plant species.

The relationships between B nutrition, auxin level, and

lignification are not well understood. Lewis (1980) proposed that lignin biosynthesis and xylem differentiation are primary effects of B nutrition. Pilbeam and Kirby (1983) concluded that B deficiency-induced high auxin concentrations are most likely responsible for the metabolic changes and cell damage seen in B deficient tissues. Marschner (1986), however, proposed that an accumulation of phenolic compounds produced by the pentose phosphate shunt pathway is mainly responsible.

The importance of B to cell wall biosynthesis and stability is well recognized. The cell wall is the largest site of B accumulation in plants (Marschner, 1986). Tanada (1978; 1974) proposed that B was involved in maintaining the structural integrity and bioelectrical potential of plant membranes. Various enzymes associated with membranes are also known to be affected by B availability. Both Parr and Loughman (1983) and Pilbeam and Kirby (1983) concluded that membrane function is the most likely metabolic role of B in plants. Recent studies have supported the positive effects of B on membrane proton transport (Goldbach et al., 1990; Roldan et al., 1992) and membrane potential (Blaser-Grill et al., 1989; Schon et al., 1990).

Any disruption of membrane function would have an immediate and detrimental effect on plant gas exchange properties. Hudak and Herich (1976) noted that B deficiency in sunflowers ultimately resulted in chloroplast destruction, whereas an increase in B availability elevated CO₂

assimilation. Transpiration rate was also determined to be higher in normal than in B deficient plants (Baker et al., 1956). Inadequate B nutrition was found to be responsible for reduced photosynthesis and stomatal conductance levels in broccoli (Petracek and Sams, 1987) and *Mensa arvensis* L. (Srivastava and Luthra, 1992).

HYDROPONIC SYSTEMS IN PLANT NUTRITION RESEARCH

The term hydroponics is credited to Gericke (1940), who demonstrated on a semi-commercial basis that plants could be grown to maturity without soil. Hewitt (1966) traced the scientific basis of modern hydroponics back to the pioneering works of Woodward in 1699 and De Saussure in 1804. Working independently, Sachs and Knop developed the first solution culture systems in the early 1860's. Today, the definition of hydroponics has evolved to cover a variety of scientific and commercial practices, with the commonality being the solution culture of plants.

Hydroponic systems are well suited to the study of plant nutrition. The ability to control completely the root environment, nutrient availability, water supply, and - in greenhouse operations - the light, temperature, humidity, and air composition is essential in modern plant nutrition research (Ellis et al., 1974).

The simplest hydroponic experiments consist of plants

being grown or placed in containers holding static (non-flowing) nutrient solutions (Hewitt, 1966). If plant support is desired, a variety of rooting media is available including sand, gravel, vermiculite, perlite, and peat moss. Nutrient solutions are either changed, renewed, or left intact for the course of the experiment. Adequate aeration of the plant root system is required. Often, for experimental convenience, nutrient solution concentrations are used that greatly exceed normal plant requirements or the amount available in normal soil solutions (Epstein, 1972). This high initial concentration may exceed the toxicity level of certain species sensitive to a particular element (Williams and Vlamis, 1957).

Although still widely used in plant research and for teaching purposes, static solution culture has been surpassed in importance by flowing solution culture. The following two methods are commonly used: (1) a single-pass flow system (open) by which the nutrient solution passes over the plant roots once before being discarded and (2) a continuously recirculating system (closed) in which water and nutrients are added as needed. The operation of closed systems is more economical and environmentally sound, but the initial cost is usually higher. Flowing solution culture is the most practical and flexible system available to study the relationships between plant growth and external nutrient ion concentrations (Asher and Edwards, 1983). The ability to adapt easily the nutrient supply to the changing nutritional needs of the

growing plant using the concept of programmed nutrient addition (Asher and Cowie, 1970) is an important asset to long range plant nutrition studies. In addition, dilute nutrient solutions supplied by flowing solution culture have been shown to induce vigorous growth in plants (Asher and Edwards, 1983). The principle disadvantage of solution culture is the potentially rapid dispersal of soil borne diseases and nematodes to all plants in the system (Jensen and Collins, 1985). Still, the introduction of root infecting pathogens is unlikely if proper sanitation procedures are followed (Jenkins and Averre, 1983). A major requirement of the closed flow system is the need to monitor periodically the recirculating solution nutrient levels. An accumulation of nutrients to toxic levels is possible, but by using dilute concentrations in a programmed nutrient addition approach, this problem is unlikely to occur.

Other minor hydroponic systems that may be useful in plant nutrition studies are floating culture and root mist culture. Floating hydroponics is primarily useful in seed germination studies (Jensen and Collins, 1985), whereas the application of nutrient solution by root misting is very conducive to studies on root system development (Asher and Edwards, 1983). Root misting must be closely monitored, however, because a small interruption (power or mechanical) could lead to irreversible plant damage.

To be used in plant nutrition study, a hydroponic system

must accurately and consistently deliver nutrients to the individual plants. Experimental repeatability must be proven. The system should be constructed as simply and inexpensively as possible while still meeting experimental requirements. The system design should be flexible enough to allow experimental size modifications and should be adaptable to various structures. Although a variety of materials is available to construct hydroponic systems, chemically inert components are crucial to any research involving plant mineral deficiency and toxicity levels. This is especially true when micronutrient deficiency studies are undertaken. The rooting medium also should be chemically inert and sufficiently porous to allow for both adequate aeration and free flow of the nutrient solution (Guevarra et al., 1980). The possibility of outside contamination (chemical or pathological) entering the system must be limited at all levels of the operation.

OVERVIEW OF OILSEED RAPE PRODUCTION

Oilseed rape species collectively belong to the genus *Brassica* (family Cruciferae) that contains many important crop and weed plants. The name rape is derived from the Latin *rapum*, meaning turnip. Oilseed rape is one of the few edible oil crops that can be cultivated in the cooler agricultural regions of the temperate zone (Downey and Robbelen, 1989). Rapeseed also can be grown as a winter crop in areas with

moderate temperatures. Moreover, intensive breeding and selection have considerably enlarged the growing range of rape. Major production regions currently include China, the Indian subcontinent, Canada, and Northern Europe (Downey, 1990). Future crop expansion in the United States, Australia, and South America is likely. Over 60% of the world *Brassica* oilseed production is supplied by Canada, China, and India (Niewiadomski, 1990). Canada, however, is the only major exporter with Japan and the United States being large importers.

On the world market, rapeseed does not derive from one species. *Brassica napus* and *Brassica rapa* are the most important rape oilseeds distributed throughout the world, and are the predominant varieties produced in Europe and North America (Salunkhe and Desai, 1986). In the Indian subcontinent, *Brassica juncea* (rai), *Brassica rapa* var. yellow Sarson, *Brassica rapa* var. brown Sarson, and *Brassica rapa* var. Toria are the dominant varieties grown. *Brassica napus* and *Brassica rapa* are also divided into subspecies: the subspecies *oleifera* is the most significant in agricultural production. These two species, which also occur in annual and biennial forms, differ with respect to cultural requirements (McGregor, 1976; Nuttall et al., 1992), crop yield (Thurling, 1974), and chemical composition (Sang and Salisbury, 1988). Prevailing environmental conditions in the various production regions determine which cultivars are grown.

Rapeseed yields over 40% oil on a dry weight basis and a meal containing 38 to 44% high quality protein (Shahidi, 1990) that is well balanced in amino acid content (Ohlson and Anjou, 1979). Nevertheless, the chemical composition of rapeseed must meet certain standards to allow maximum utilization by the food, chemical, and animal feed industries (Niewiadowski, 1990). The concentration of erucic acid - a long chain (22 C) fatty acid - cannot exceed 2% if the extracted oil is to be used for human consumption. Conversely, a high concentration of erucic acid is desirable if the oil is to be used for industrial purposes. In addition, rapeseed meal must contain no more than 30 μ mol of aliphatic glucosinolates (plant thioglucosides ubiquitous in the Cruciferae) per gram of defatted meal to be used as an animal feed component. Rapeseed that meets these requirements has been designated as canola quality (Shahidi, 1990).

Brassica oilseed crops have become the third most important source of edible vegetable oils in the world, exceeded only by soybean and palm oil (Downey and Robbelen, 1989). From 1983 to 1990, the production of oil from *Brassica* crops increased 53%, which surpassed the rate of growth in palm oil production (Downey, 1990). This increase was due partly to expansion of acreage, improved agronomic practices, and to new cultivars that produced higher seed and oil yields.

Today, canola oil is becoming increasingly popular in the U.S. among health conscious consumers. Many nutritionists now

consider this new natural oil to have an almost ideal fatty acid composition for human consumption; it contains only 6% saturated fat, desirable amounts of the polyunsaturated fatty acids linoleic acid (20%) and linolenic acid (9%), with nearly all the remainder being the nutritionally neutral oleic acid (Downey, 1990; Shahidi, 1990). Domestic production of oilseed rape, however, is currently less than 80,000 ha forcing processors to import large amounts of seed from Canada (U.S. Canola Association, Washington, D.C.). Nonetheless, the increased consumer demand coupled with the introduction of cultivars developed for specific growing regions should ensure a steady increase in U.S. production of oilseed rape.

HISTORY OF OILSEED RAPE DEVELOPMENT

The *Brassicas* were among the first plants to be domesticated by man. Indian Sanskrit writing of 1500 to 2000 B.C. refers to the medicinal properties of oilseed rape and mustard - as do Greek, Roman, and Chinese writings of 200 to 500 B.C. In Europe, the cultivation of rape began during the 13th century (Niewiadomski, 1990). Oilseed rape was first commercially produced in Belgium and Holland during the 1600's (Salunkhe and Desai, 1986).

Rapeseed and mustard have been important sources of edible oil in China, Japan, and the Indian subcontinent for thousands of years. In the western world, however, the oil was

used principally as lamp fuel, and later as a lubricant for steam engines. In Europe, the use of rapeseed oil for edible purposes occurred primarily during times of war followed by rapidly declining consumption with the return of peace (Niewiadomski, 1990). Finally, during World War II, western scientists began to recognize the potential of rapeseed as an edible oil source. As rapeseed oil entered western diets in the 40's and 50's, nutritionists noted that the oil differed from other vegetable oils by the presence of significant amounts of the long chain fatty acids eicosenoic acid (20 C) and erucic acid (22 C). Subsequent experiments conducted to determine the effects of these long chain fatty acids on laboratory animals suggested that erucic acid was nutritionally undesirable (Sauer and Kramer, 1983). Downey and Craig (1964) were able to reduce the erucic acid concentration in rapeseed to less than 1% by genetically blocking the biosynthetic pathway that adds 2 C fragments to the carboxyl end of oleic acid to form eicosenoic and erucic acid. Canadian growers converted completely to low erucic acid cultivars by 1974, and European conversion was accomplished by 1978.

Still, the potential of physiologically harmful effects attributable to high glucosinolate content (Duncan, 1991; Pusztai, 1989) reduced rapeseed meal marketability to the animal feed industry. The 1967 discovery of a Polish *Brassica napus* cultivar, Bronowski, that contained only 10% of the usual seed aliphatic glucosinolate content (Finlayson et al.,

1973) led to a significant reduction in rapeseed glucosinolate concentration. A genetic block was found in 'Bronowski' that prevented the biosynthesis of aliphatic glucosinolates from their amino acid precursor methionine (Josefsson, 1973). This trait was successfully transferred to Canadian low erucic acid cultivars resulting in the 1974 release of 'Tower' - the first canola cultivar. By the end of the 1970's, Canada had completely converted to canola cultivars. European conversion is almost complete, and currently, the European Economic Community will only pay support prices for canola quality seed (Shahidi, 1990).

The continuing improvement of *Brassica* oilseed production and canola oil quality is a major objective of both public and private researchers in many countries. In 1992, an estimated \$35 million were spent worldwide on canola breeding and genetics (Kidd, 1993).

Recently, researchers have attempted to modify the fatty acid content of canola oil in order to fill specialized applications. The 8-10% concentration of linolenic acid in traditional canola has been decreased to 3% in the *Brassica napus* cultivar, Stella. This decrease significantly improved oil stability and shelf life (Kay, 1988). The nutritionally desirable oleic acid has been increased to a level > 85% in a *Brassica napus* mutant. Also, a strain of *Brassica rapa* has been developed that contains elevated levels of palmitic and stearic acids, which further the production of superior canola

margarine and shortening. Other research priorities are to produce canola with < 6% saturated fat, and to develop rape plants with an erucic acid content of > 50% for use in the plastics industry (Downey and Bell, 1990).

The production of a canola quality oilseed mustard (*Brassica juncea*) has also become a primary goal of researchers since it is higher yielding than either *Brassica napus* or *Brassica rapa* and can be produced in hotter, more severe climates (Downey, 1990). The discovery of low erucic acid mustard plants (Kirk and Oram, 1981) plus the additional development of plants with low glucosinolate content (Love et al., 1990), ensure that canola quality mustard seed is attainable.

The area of canola research receiving the most attention, however, is the conversion of canola from a non-hybrid into a hybrid (Kidd, 1993). The hybrid seed would be higher yielding, more uniform, and would offer a greater profit margin to the seed companies.

GLUCOSINOLATES IN PLANT METABOLISM

Glucosinolates are sulfur containing secondary metabolites that are derived from amino acids. They are classified as natural toxicants. Almost 100 glucosinolates have been identified (Poulton and Moller, 1993). These compounds occur primarily in the Cruciferae (order

Capparales), but are known to exist in at least 10 other dicotyledonous angiosperm families (Underhill, 1980).

The physiological role of glucosinolates in plant metabolism has not been determined, but is thought to be primarily defensive in nature. Glucosinolates and/or their breakdown products possess antifungal and antibacterial properties, serve as both insect attractants and repellents, and discourage feeding by herbivores (Fenwick et al., 1983).

Although the first glucosinolates were isolated over a century ago, the general structure was not precisely determined until 1956 (Ettlinger and Lundeen, 1956). There is great variation in the chemistry of the glucosinolate aglycones, but all include a common glycoside moiety and a variable side chain.

Glucosinolates are derived from amino acids and most, if not all, are synthesized by a common pathway. Potential modifications of the developing glucosinolate can occur at any time during the biosynthetic process. Although the general pathway for glucosinolate biosynthesis is reasonably well understood, factors that control production of the individual glucosinolates or their interconversions are poorly defined (Poulton and Moller, 1993). During biosynthesis, all glucosinolates share two common features: (1) All receive nitrogen from their precursor amino acid, and (2) a sulfur atom is incorporated. Although the nature of the reduced sulfur donor is not clear, cysteine has been the most

efficient sulfur source of the compounds studied (Underhill, 1980).

Coexisting with glucosinolates in the plant tissue is the enzyme myrosinase or thioglucoside glucohydrolase (EC 3.2.3.1). When plant tissue is crushed in the presence of adequate moisture, myrosinase rapidly hydrolyses the glucosinolate to yield glucose, sulfate, and any one of the following products: isothiocyanates, thiocyanates, nitriles, cyanoepithioalkanes, or oxazolidine-2-thiones (Larsen, 1981). Which product is formed depends on many factors including glucosinolate structure, reaction conditions such as pH, temperature, and duration, the physiological condition of the plant tissue, and the presence of cofactors such as metal ions, ascorbic acid, and specific proteins (McGregor, 1993).

Glucosinolates and their breakdown products have both desirable and undesirable effects on foods and animal feed. Besides being responsible for giving *Brassica* vegetables their distinctive flavor and aroma, glucosinolates and their hydrolysis products can reduce the palatability of feed, and also impart taints to foods. Most importantly, they can make feed or foods goitrogenic or toxic (Duncan, 1991).

Despite the large number of known glucosinolates, most species contain only a few, with one or two usually predominate (Sang et al., 1984). Glucosinolate content may vary among species, cultivars, plant tissue, or even within plant tissue. As the plant develops and matures, glucosinolate

types and concentrations usually undergo radical changes (Clossais-Besnard and Larher, 1991).

Plant glucosinolate biosynthesis can be influenced by genetic, cultural, and environmental components (Fenwick et al., 1983). An increase in the glucosinolate concentration of oilseed rape has been caused by water stress (Mailer and Cornish, 1987), variation in planting date (Sang et al., 1986), and an increase in sulfur availability (Josefsson, 1970). Boron deficiency has been shown to increase glucosinolate content in radish (Bible et al., 1981), turnip (Ju et al., 1982), and broccoli (Shelp et al., 1992b). These results suggest that glucosinolate accumulation is a defensive mechanism apparently induced by plant physiological stress. In the Cruciferae, and particularly the *Brassicacae*, B deficiency would appear to be a potentially significant impetus to glucosinolate formation.

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CHAPTER II

DEVELOPMENT OF A HYDROPONIC SYSTEM TO ACCURATELY DELIVER MICRONUTRIENT CONCENTRATIONS

ABSTRACT

A common problem encountered by researchers concerned with plant micronutrient nutrition is the development of a reliable and affordable experimental system. If nutrient distribution is uneven or subject to outside contamination, then the time and resources dedicated to a project will have been wasted. The purpose of this research was to design and construct a recirculating hydroponic system to use in boron nutrition studies on *Brassica* plants. Boron availability must be precisely regulated since the range between deficiency and toxicity is very narrow. To be successful and practical, the proposed system would have to meet the following requirements: (1) limited possibility of outside contamination, (2) capability of delivering an accurate and consistent nutrient flow to individual plants, (3) adaptability to existing greenhouse structures, (4) simplicity of construction, (5) low

maintenance requirement, and (6) affordability.

The recirculating hydroponic system presented here successfully met these criteria, and several experiments have proven design repeatability. Cost analysis indicated a conservative estimate of \$12.50 per plant for an experimental design consisting of three treatments of 21 replications each. The system is easily expanded, and additional plants can be added at a cost of approximately \$2.00 each. The system design and initial cost are presented here with proposed experimental approaches in using this system.

INTRODUCTION

Continuously circulating solution culture is the most practical method for the study of long-term relationships between plant growth and mineral nutrition (Asher and Edwards, 1983). This technique permits adequate control over the plant root environment that is vital to successful nutrition studies. However, the requirement to maintain consistent minute concentrations of nutrients in long-term solution culture studies (Gutschick and Kay, 1991) is restricted due to the limited sensitivity of available ion-selective electrodes (Bloom, 1989). Potential micronutrient toxicity in susceptible plants is also a concern (Williams and Vlamis, 1957). Researchers have traditionally used nutrient concentrations that are high - sometimes extremely high - in relation to

plant requirements (Ingestad, 1982).

The goal of this research was to design and construct a continuously recirculating hydroponic system to study boron (B) deficiency in *Brassica* plants. This proposed system adequately had to control water volume, nutrient availability, pH, algal growth, and microbial contamination. Other requirements included the following: limited possibility of outside contamination, capability of delivering an accurate and consistent nutrient flow to individual plants, adaptability to existing greenhouse structures, simplicity of construction, low maintenance requirements and affordability.

MATERIALS AND METHODS

The hydroponic system was designed to accommodate an existing greenhouse facility (7.6 m x 7.3 m) containing three benches (5.6 m x 1.1 m x 0.7 m). An important design consideration was the requirement for chemical inertness in all system components. The plants were individually grown in plastic pots (11 liter) with perlite as the root support medium. Each treatment solution was maintained in a separate covered plastic container (110 liter). The solutions were continuously recirculated by a chemical resistant magnetic drive centrifugal pump. Each pump was protected from particulate damage by an inert in-line filter. A check valve was installed in each reservoir to prevent a loss of the pump

prime if an electrical power outage occurred. Standard plastic irrigation tubing and fittings were used for all supply and return lines (Fig. II-1). Each treatment solution was directly applied to the individual pots by 6.4 mm tubes that were supplied by a 12.8 mm primary distribution line. End line emitters (4 liters/h) equally distributed the nutrient solutions to each plant. A ball valve connected the supply and return lines, enabling the pressure to be adjusted as needed. A 12.8 mm tube connected near the base of each pot allowed drainage into 19.2 mm return lines. Excess solution flow and the leachate from the pots was returned via gravity flow to the reservoirs. A nylon mesh screen was placed over the drain fitting in each pot to restrict perlite movement. An opaque plastic cover was placed around the base of each plant to cover the perlite. This served to retard algae growth and limit airborne contamination.

Perlite was selected as the rooting medium since it is physically stable and, for most purposes, chemically inert (Green, 1968). Moreover, perlite exhibits good aeration and free flow qualities that are essential for the growing medium in a recirculating system (Guevarra et al., 1980). The closed cellular structure of perlite results in most of the water being held superficially and released at low moisture tensions (Jackson, 1974).

Nutrient solutions were carefully prepared to minimize any risk of contamination. Double deionized (dd) water was

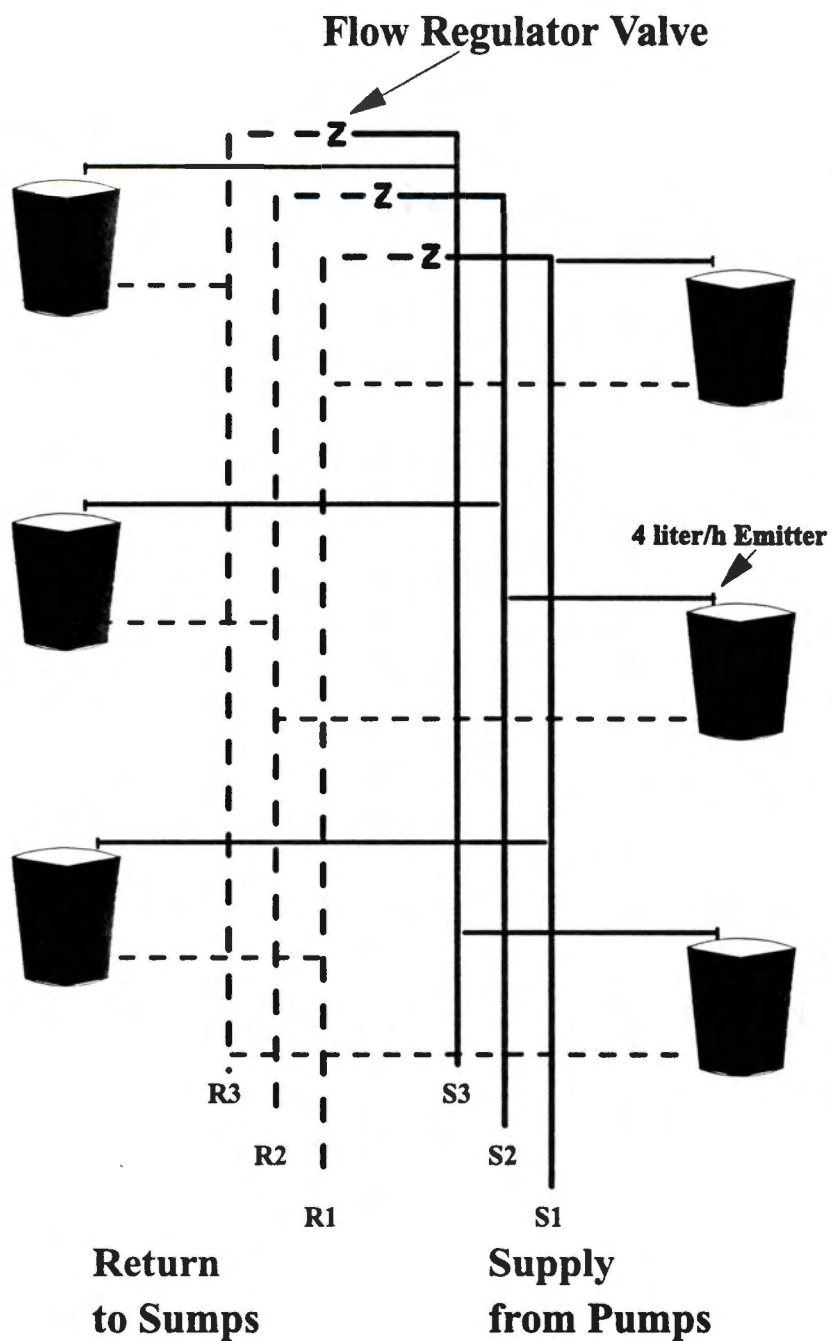


Fig. II-1. Schematic representation of a continuously recirculating hydroponic system with supply (S) and return (R) lines.

used exclusively as were analytical reagent grade chemical components. Nutrient solutions were always mixed and stored in plastic containers to avoid boron contamination from borosilicate glassware. Solutions were kept in darkness to retard the growth of algae. The hydroponic system components were thoroughly cleaned and flushed with dd water between experiments. Perlite was discarded after each experiment.

Seeds could be directly planted or seedlings could be transplanted into the working hydroponic system. Plant stunting due to transplant shock was negligible. Seedlings produced for transplanting were carefully grown to ensure experimental integrity and continuity. Seeds were planted in plastic trays (46 cm x 31 cm x 8 cm) containing perlite. Ample nutrient solution (1:4 dilution) was maintained in each tray to ensure adequate moisture and nutrition for seed germination and seedling growth. *Brassica* seedlings produced in this manner were ready for transplanting in 3 to 4 weeks.

Nutrient solution reservoirs were inspected daily and replenished as needed every 2 to 3 days. ICP analysis (inductively coupled plasma atomic emission spectrometry, Model 61 Thermo Jarrell Ash; Franklin, MA) was conducted on both dd water and freshly prepared nutrient solutions to certify solution quality. Samples periodically taken from the system reservoirs were analyzed to monitor any nutrient accumulation prior to the addition of new solution.

The pH of the recirculating solutions was checked daily

and maintained at $\text{pH } 6.0 \pm 0.5$ by adding HCL (4N) or NaOH (4N) as needed. As a precaution, strongly acidic conditions ($\text{pH } < 5.0$) must be avoided when using perlite as the growth medium. Perlite contains 6.9% aluminum that may become soluble at low pH values (Green, 1968). The release of aluminum into the circulating solution would detrimentally affect plant growth and development.

RESULTS AND DISCUSSION

The interpretation of micronutrient studies on plants is difficult. Although micronutrient deficiency and toxicity levels have been estimated for many plants, the physiological changes that occur incrementally between these extremes are hard to characterize. This is especially true in the study of elements such as B that have a very narrow range between deficiency and toxicity (Gupta, 1979).

The recirculating hydroponic system presented here has proven highly successful in incremental B nutrition studies on *Brassica* plants. Consistent results obtained over three experiments involving oilseed rape (Appendix B) confirmed repeatability. Leaves have been documented as the best and most sensitive indicators of B availability in oilseed rape (Yang et al., 1989). The B concentrations in the lower and upper leaf tissue (Tables A-1, A-2, and A-3) indicate that uptake was consistent with B nutrient solution availability.

This was true even at treatment differences of $0.01 \text{ mg}\cdot\text{L}^{-1}$ added B. The B content in the recirculating nutrient solutions sampled prior to the addition of fresh solution (Figs. A-1, A-2, and A-3) confirmed the consistency of B availability and uptake. The hydroponic system proved to be dependable in operation requiring only minor maintenance. Disposal of system waste material was routine. Both the used perlite and the remaining treatment solutions were environmentally safe and easily disposed of. Outside contamination (chemical or microbial) was not a factor. The affordability (Table II-1) and expandability of the system are major assets to researchers. For the setup described here (63 units), cost analysis suggests a conservative expenditure of \$12.50 per experimental unit. Additional replications could be added for approximately \$2.00 each - lowering the unit cost even further.

Recirculating solution systems, however, do have potential problems and research limitations. Microbial contamination is always a concern. When pathogens are introduced, severe root rot and plant death can quickly occur because all plant root systems are simultaneously exposed. Still, if proper sanitation procedures are followed, the introduction of root infecting plant pathogens is unlikely (Jenkins and Averre, 1983). If microbial contamination was to become a problem, an in-line submicronic filter ($0.22 \text{ }\mu\text{m}$) could easily be installed to deter any future recurrence.

Table II-1. Cost analysis of a chemically inert continuously recirculating hydroponic system consisting of three treatments with 21 replications each.

Component	Quantity	Cost (\$)
Little Giant Pump (Model 3-MD-SC)	3	375
Drip Mist In-Line Filter (Model 3A)	3	30
PVC check valve	3	36
Covered plastic can (110 liter)	3	45
Plastic pot (11 liter)	63	75
PVC connector (12.8 mm)	84	24
PVC tubing tee (19.2 x 19.2 x 12.8 mm)	63	45
PVC ball valve (12.8 mm)	9	30
Drip line emitter (4 liters/h)	63	15
Plastic irrigation tubing (19.2 mm)	91 m	33
(12.8 mm)	91 m	24
(6.4 mm)	27 m	3
Miscellaneous fittings		60
Total		795

Schwartzkopt et al. (1987) used this filter and achieved satisfactory results. Attempts to use an in-line ultraviolet sterilization procedure proved less promising due to plant growth inhibition. A common problem in water culture systems occurs because of the comparatively unbuffered status of the nutrient solution (Asher and Edwards, 1983). Large changes in nutrient solution composition can occur quickly as nutrients are depleted. This restricts the usefulness of recirculating solution culture in studying the effects of nutrient ion concentration on nutrient uptake and plant growth. These rapid changes also cause fluctuations in solution pH making precision control difficult, even if automatic pH control is available.

Researchers should apply caution in extrapolating results from recirculating solution culture to soil culture. In solution culture, the plant root environment may be nearly homogeneous, whereas the roots of soil produced plants may have parts located in distinctly different environments. The actual soil root environment may be modified by components not present in solution culture. These include the following: soluble organic compounds, soluble mineral elements not considered essential for plant growth, and microorganisms associated with plant roots. Soil grown plants actually exude many organic compounds into the soil that attract many varied microorganisms (Asher and Edwards, 1983).

Acknowledging these limitations, this hydroponic design

has many potential experimental and educational uses. The most obvious use is in mineral nutrition studies. The morphological and physiological effects as well as the biochemical role of a particular element can be studied. Genotypes could be identified with respect to specific nutritional disorders. The essentiality of an element could be researched. A system of this design would be good in evaluating new biological and chemical control agents. In addition, the effects of root temperature on nutrient uptake and plant growth could be studied using a modification of this design. Another desirable operating feature of this system is the ability to alter the nutrient solution periodically to match more closely the changing nutritional requirements of the plant. Establishing a plan of programmed nutrient addition (Asher and Cowie, 1970) can coordinate nutrient availability with the different nutritional requirements associated with various growth stages of a plant. This information could be manipulated in many experimental approaches utilizing this system. In addition to research, the system could be used in the classroom to demonstrate specific nutrient imbalances.

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CHAPTER III

INFLUENCE OF BORON NUTRITION ON OILSEED RAPE MORPHOLOGY AND YIELD

ABSTRACT

Three greenhouse experiments were conducted to study the effects of Boron (B) nutrition on oilseed rape morphology and yield. A chemically inert continuously recirculating hydroponic system was utilized to deliver nutrient solutions differing only in B availability except for experiment III which had increased KNO_3 . Perlite was used as the root support medium. Severe B deficiency resulted in a malformed and seedless plant, that also exhibited intense sulfur deficiency symptoms. This was thought to occur as a consequence of B deficiency-induced vascular tissue destruction. Deficient B treatments produced normal appearing plants that yielded little or no seed. Histological analysis of rape stem tissue confirmed the detrimental effects of inadequate B availability on vascular tissues and also demonstrated the involvement of lignification in the destruction of pith cells. Tissue B analysis confirmed that rape leaves were the most sensitive indicators of B availability and uptake. Lower leaf (LL)

tissue B concentrations between 35 and 80 $\mu\text{g}\cdot\text{g}^{-1}$ were found in normal rape plants with good seed yields, while LL concentrations of 18 $\mu\text{g}\cdot\text{g}^{-1}$ were found in relatively normal appearing but seedless plants. The range of 0.02 to 0.04 $\text{mg}\cdot\text{L}^{-1}$ added B was found to be critical to normal oilseed rape ontogeny. Excessive nitrogen availability significantly reduced seed yield by apparently limiting the amount of B partitioned to reproductive tissues. The best morphological indicators of B deficiency over all treatments were axillary branch number and main inflorescence height.

INTRODUCTION

Oilseed rape is playing an ever increasing role in the world vegetable oil industry. With the introduction of canola grade rapeseed, *Brassica* oilseed crops have become the worlds third most important source of edible vegetable oils (Downey, 1990). Oilseed rape is adapted worldwide, and is one of the few edible oil bearing plants of the temperate zone (Downey and Robbelen, 1989). Research is continuing to expand rapeseed production into areas that were previously considered unsuitable. Plant nutrition problems will undoubtedly arise as new rape cultivars are introduced into areas with widely divergent soils and environments.

Boron (B) deficiencies occur over a wider range of crops and growing conditions than deficiencies of any of the other

essential micronutrients (Gupta, 1979). Boron is exceptional also due to its narrow range between deficiency and toxicity (Epstein, 1972). Oilseed rape, like other *Brassica* species, is extremely sensitive to B nutrition (Holmes, 1980). Although the primary role and mode of action of B in plants has not been determined (Lewis, 1980; Pilbeam and Kirby, 1983), the morphological consequences of inadequate B availability are well documented (Gupta, 1979; Mengel and Kirby, 1982).

The purpose of this research was to correlate precisely B availability with the morphological development and seed yield of oilseed rape. A further objective was to determine the smallest concentration of available B that would produce a normal seed yield.

MATERIALS AND METHODS

Three greenhouse experiments (I - Fall, 1991; II - Spring, 1992; III - Fall, 1992) were conducted to study the effects of B nutrition on oilseed rape morphology and yield. A spring rapeseed cultivar, Ameri-Can A-112, supplied by Ameri-Can of Memphis, TN was used. The plants were arranged in a randomized complete block design consisting of three blocks. Experiment I had 21 single plant replications, whereas Experiments II and III consisted of 12 replications. A chemically inert continuously recirculating hydroponic system was utilized to deliver three separate concentrations of added

B in each experiment (Appendix B). Standard analysis of variance procedures (GLM procedure of SAS) were used to determine significant effects based on linear contrasts. Greenhouse mean daily temperatures averaged 22.5 ± 1 C for each experiment. Plants were individually grown in 11 liter plastic pots with perlite as the root support medium. Nutrient solutions were carefully prepared using double deionized water and analytical reagent grade chemicals. Precautions were taken to avoid outside chemical contamination. Plastic containers were used exclusively to prevent B contamination from borosilicate glassware. The basic nutrient solution ((Epstein, 1972); Appendix B) remained the same over the three experiments except for the added B treatments. In addition, KNO_3 concentration in experiment III was increased to 4 M. Dilutions of the basic nutrient solutions were utilized in all experiments to regulate nutrient availability (Appendix B). Inductively coupled plasma (ICP) atomic emission spectrometry (Model 61 Thermo Jarrell Ash; Franklin, MA) was used to monitor existing recirculating solutions as well as freshly prepared nutrient solutions. The consistency of B availability and uptake in the recirculating solutions was recorded for all experiments (Figs. A-1, A-2, and A-3).

Seeds were planted in plastic trays containing perlite. Adequate basic nutrient solution (1:4 dilution) was maintained in the trays to prevent seed desiccation and promote plant growth. Approximately four week old seedlings were

transplanted into the working hydroponic system without any apparent transplant shock.

Initially, nutrient solution dilutions of 1:4 were used except for experiment III that began with a 1:2 dilution. Experiment I was switched to a 1:2 dilution 37 days following transplanting whereas experiment II was switched after 23 days. These concentrations remained constant until the experiments ended 70 and 63 days later respectively. The experiment III nutrient solution was changed to a 3:4 dilution 28 days after transplanting. It remained at this level for four weeks until being reduced to a 1:2 dilution. A 1:4 dilution was initiated 26 days later and this remained in effect until the experiment ended four weeks later. Plants were allowed to desiccate naturally before seed pods were harvested. In experiment I, seed pods were further divided into main inflorescence pods and axillary branch pods. The number of pods and seed weight for each individual plant were recorded. Other plant morphological characteristics measured included the following: leaf (node) number, axillary branch number, stem height, main inflorescence height, and plant age at bolting and anthesis.

ICP analysis was used to determine plant tissue B (experiments I, II, and III) and plant tissue sulfur concentrations (experiments II and III). The plant tissue analysis procedure is described in Appendix B. The following plant parts were examined: lower leaves (nodes 1 - 15), upper

leaves (nodes 16+), stem, main inflorescence, axillary branches and roots. In addition, nitrogen (N) analysis (Carlo Erba NA-1500 Nitrogen Analyzer; Milan, Italy) was conducted on the lower and upper leaves in experiments II and III.

A histological analysis of stem tissue was made comparing the three B treatments in experiment II. Samples were obtained when the plants reached anthesis, approximately seven days after bolting. Stem transverse sections (40 μm thick) were collected using a Spenser sliding microtome. The samples were taken approximately 2.5 cm above the first leaf node and immediately placed in FAA fixative (a 10:8:1:1 mixture of ethanol, water, 37% formalin, and glacial acetic acid). A staining procedure (Appendix B) was used to examine the effects of the three B treatments on plant stem internal morphology. Color prints were prepared from Kodachrome slides (ASA 160) taken through a Zeiss MC 80 camera mounted on a Zeiss D-7802 microscope (Carl Zeiss, Oberkochen, Germany).

RESULTS AND DISCUSSION

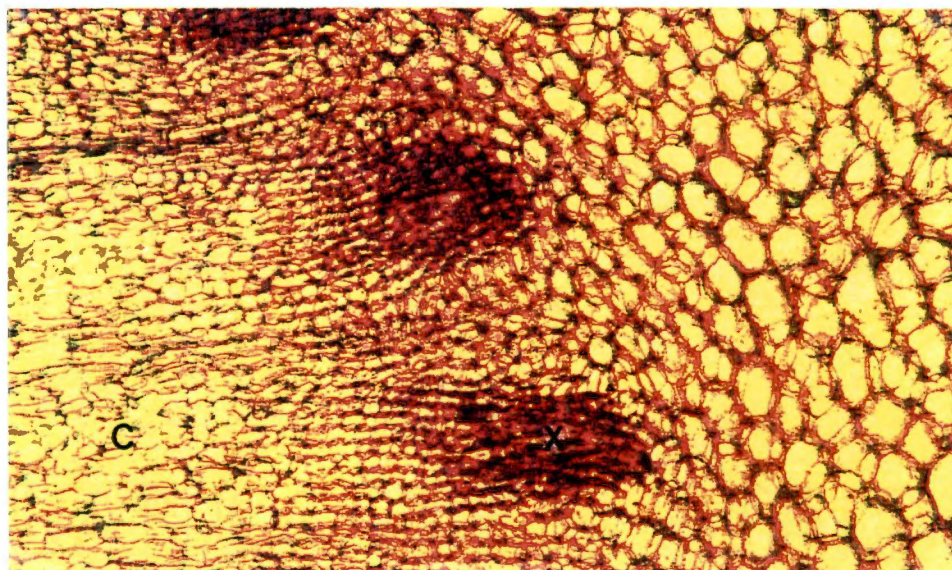
The plants produced in experiment I appeared morphologically indistinguishable (Table A-4). Seed yield (Table A-5) was good and consistent suggesting that all three treatments permitted adequate plant B uptake (Table A-1). The critical level of B availability was apparently less than the low treatment ($0.04 \text{ mg} \cdot \text{L}^{-1}$ added B), which resulted in a lower

leaf (LL) tissue B concentration of $35.1 \mu\text{g}\cdot\text{g}^{-1}$.

Experiment II produced plants that were extremely different morphologically (Table A-6). The severely deficient B treatment ($0.01 \text{ mg}\cdot\text{L}^{-1}$ added B) plants were malformed and seedless attaining a LL tissue B content of only $7.7 \mu\text{g}\cdot\text{g}^{-1}$ (Table A-2). Plant growth and development for all three B treatments appeared consistent and normal until approximately 1 week prior to bolting. At this time, the severely deficient B treatment plants abruptly began to lose vigor leading to a decline in growth and development. As bolting began, these plants did not display stem elongation comparable to the other two treatments. In addition, the younger leaves were small and misshapen with the edges becoming revolute. Ultimately the stems elongated only a few centimeters and often grew crooked and curled as the plant aged. Very few or no flowers appeared; and those that did, died shortly after blooming.

The effect of the three B treatments on stem cellular structure was histologically analyzed as the oilseed rape plants reached anthesis. Although B deficiency is well known to induce drastic cellular changes in plant tissues (Pissarek, 1980; Marschner, 1986), the effect on rape stem development has not been histologically documented. Stem transverse sections taken from severely deficient B treatment plants disclosed impaired xylem differentiation (Figure III-1a), extension of the phloem cells (Figure III-1b), and extreme radial proliferation of cambial cells (Figures III-1a and 1b).

(a)



(b)

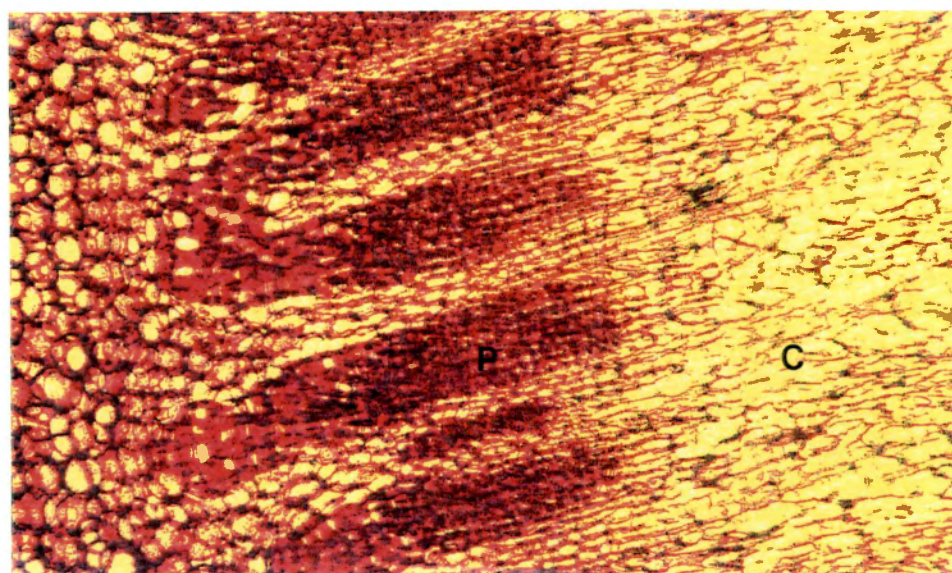


Fig. III-1. Stem transverse section (250x) of oilseed rape produced under severely boron deficient ($0.01 \text{ mg} \cdot \text{L}^{-1}$ added boron) growing conditions. Impaired xylem (X) differentiation (a) and extended phloem (P) cells (b) separated by a proliferation of radially expanding cambial (C) cells are apparent.

Massive cellular disruption within sections of the stem pith tissue (Figure III-2) was apparently caused by uneven cell wall lignification induced by the B deficiency. Kennedy et al., (1992) observed this same phenomenon in B deficient broccoli stems, and used lignin specific stains (Herr, 1992) to support their contention. (The staining series utilized here was proposed by them to elucidate stem pith tissue undergoing this lignification process.) Apparently, some pith parenchyma cells continued to grow and expand vertically (normally), and radially (abnormally) while others became lignified. These conflicting pressures resulted in a separation and tearing apart of the inner stem tissue. This finding was unexpected since lignin biosynthesis was thought to decrease under B deficient conditions (Lewis, 1980). Also, Dutta and McIlrath (1964) had determined that lignin concentration in sunflower stems was reduced under B deficient growing conditions. Apparently, the lack of differentiation and development of stem vascular tissue accounts for this net decrease, even though lignification is increasing in the pith cell walls. The stem transverse section of the deficient B treatment ($0.02 \text{ mg} \cdot \text{L}^{-1}$ added B) plants (Figure III-3) appeared healthy, although lignification was present in the pith (Figure III-4). In contrast, the normal B treatment plants exhibited well developed and differentiated vascular tissue with no indication of lignin in the pith cells (Figure III-5).

As the severely deficient B treatment plants continued to

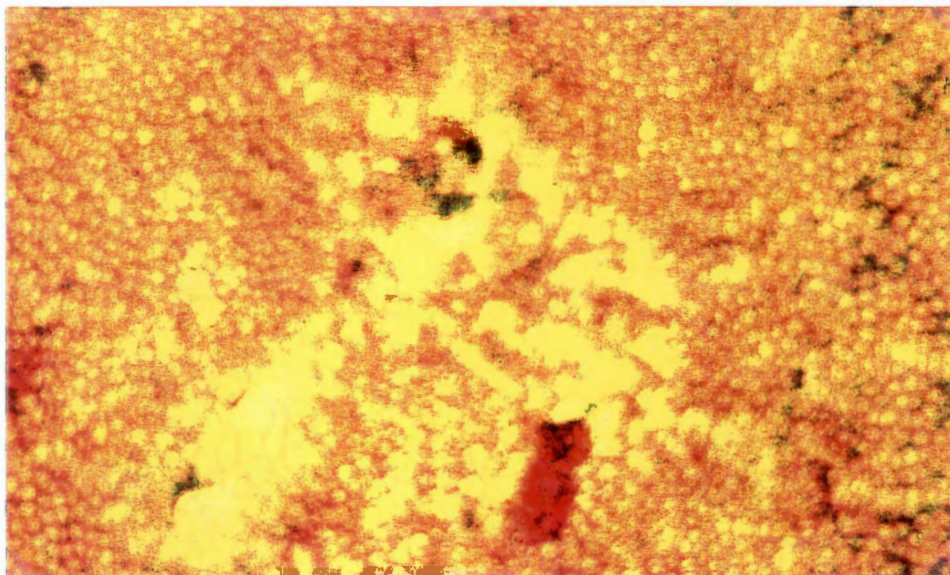


Fig. III-2. Stem transverse section (25x) of oilseed rape produced under severely boron deficient ($0.01 \text{ mg} \cdot \text{L}^{-1}$ added boron) growing conditions. Severe pith cellular deterioration exists due to unequal lignification of cell walls. Red indicates the presence of lignin.

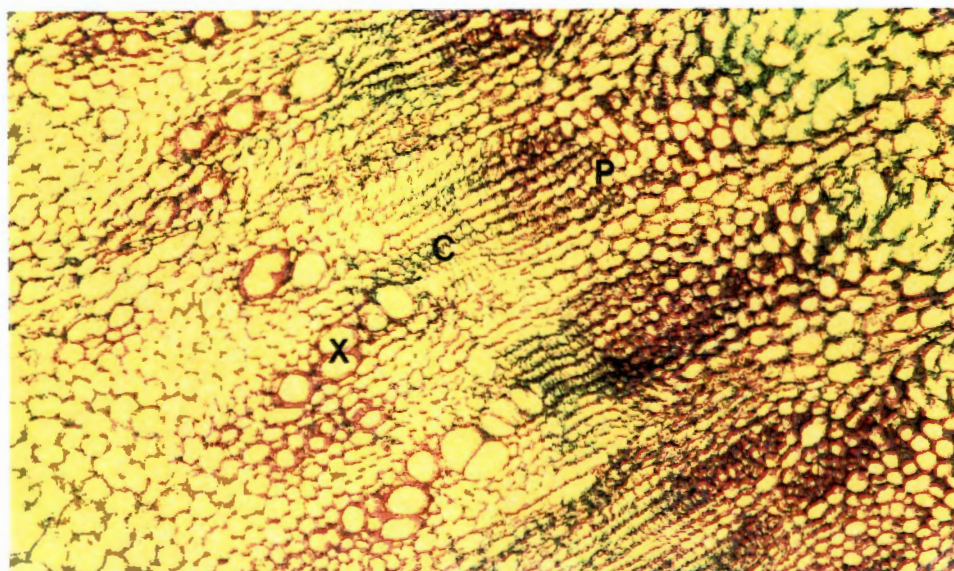


Fig. III-3. Stem transverse section (250x) of oilseed rape produced under boron deficient ($0.02 \text{ mg} \cdot \text{L}^{-1}$ added boron) growing conditions exhibiting apparently healthy xylem (X), phloem (P), and cambial (C) vascular tissues.

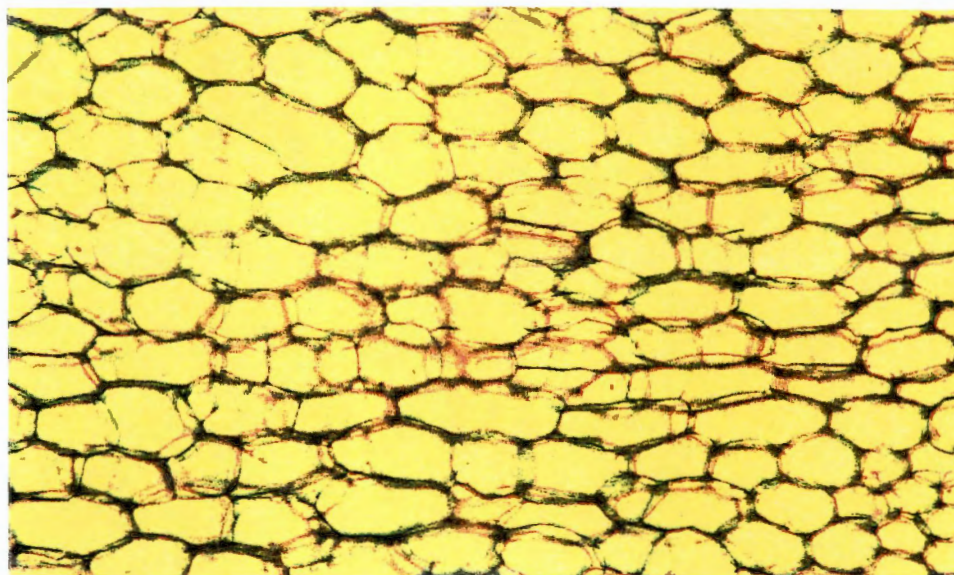


Fig. III-4. Stem transverse section (250x) of oilseed rape produced under boron deficient ($0.02 \text{ mg} \cdot \text{L}^{-1}$ added boron) growing conditions displaying early stages of pith cell wall lignification. Red indicates the presence of lignin.

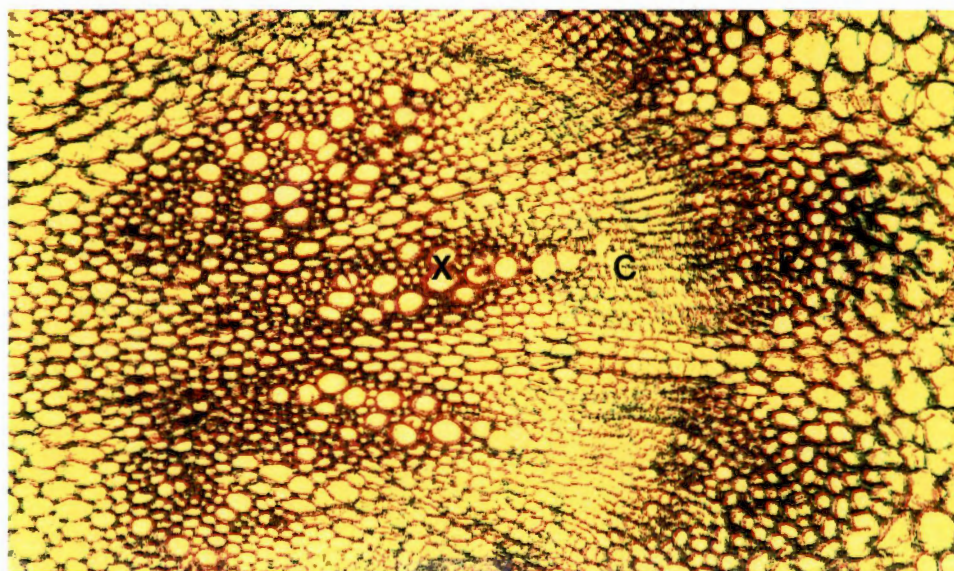


Fig. III-5. Stem transverse section (250x) of oilseed rape produced under normal boron ($0.40 \text{ mg} \cdot \text{L}^{-1}$ added boron) growing conditions displaying healthy xylem (X), phloem (P), and cambial (C) vascular tissues.

age, there was an excessive development of axillary branching. These branches, however, experienced little elongation. The stem base also expanded laterally at an abnormal rate. While the LL appeared to senesce normally, some upper leaves developed regions of interveinal chlorosis. Following the formation of chlorotic spots, a reddish-purple mottling appeared on the abaxial leaf surface. This phenomenon eventually spread to the adaxial leaf margins and ultimately covered the entire adaxial leaf surface. Finally, the discoloration spread to leaves both above and below the area of its initial appearance.

The severely deficient B treatment was terminated approximately 2.5 weeks before the completion of the study. The plants were very compact with many unelongated lateral shoots. The surviving leaf tissue was thick, brittle and broke easily. Profuse cracking and scarring was present on the stem and petioles. The stem base was larger and more fleshy compared to the other two treatments. In addition, the root system was poorly developed.

The first external indication of differences between the deficient B and normal B treatments occurred as flowering and seed production were well advanced. Although pods were forming, the B deficient plants were producing very few seed. Most of this seed developed early during the reproductive stage, but occasionally the main inflorescence or an axillary branch continued seed production sporadically. An additional

disparity eventually developed as the younger leaves of the B deficient plants began to turn yellow.

The mature normal B treatment plants were healthy in appearance and produced an adequate seed yield (Table A-7). Conversely, the deficient B treatment plants were characterized at maturity by poor seed production and excessive branch growth and flowering. The LL tissue B concentrations were $17.9 \mu\text{g}\cdot\text{g}^{-1}$ and $54.5 \mu\text{g}\cdot\text{g}^{-1}$ for the respective deficient and normal B treatments (Table A-2). Plant heights between the 2 treatments appeared roughly equal; however the main inflorescence in the deficient B treatment plants had suffered terminal death and was not dominant. At maturity, these plants were slightly more bush-like in appearance than were the normal plants. These differences became more apparent as the plants underwent desiccation.

The morphological results of experiment III (Table A-8) were comparable to the previous experiments except for the branch and leaf number. These were exaggerated due to the increased N in the nutrient solution. Plant growth was so excessive and intertwined that leaf (node) number was not counted. All three B treatments initially produced normal appearing plants with the leaf growth being larger and more robust than in previous experiments. Rood and Major (1984) reported that leaf growth of oilseed rape was particularly responsive to N fertilization. After the plants passed the bolting stage, however, an excessive amount of branching

(number and size) was soon apparent. The nutrient solution concentration was reduced to repress this growth, but excessive branching, leaf production and flowering continued. The endurance and vitality of many of the leaves throughout seed production was atypical in ordinary rape ontogeny. Although leaves are the primary source of photosynthate during early flowering, the stems and then the pods assume this role midway between flowering and maturity as the leaves die (Chapman et al., 1984). When the experiment was stopped, plants in all treatments were continuing to grow and flower. Although increasing N availability has been shown to promote rapeseed yield (Henry and MacDonald, 1978; Yusuf and Bullock, 1993), the excess N in this experiment was detrimental to seed production (Table A-9 vs. Table A-5). Exposure of oilseed rape to excess N during early plant development apparently promotes irreversible vegetative growth over reproductive growth.

The N concentrations of the lower and upper leaves in experiments II (Table A-10) and III (Table A-11) revealed that significant N only accumulated in the experiment II severely B deficient upper leaves. Boron deficiency has been proposed to reduce the activity of nitrate reductase (NR) in oilseed rape (Shen et al., 1993), thereby increasing N concentration in the tissue. Whether the upper leaf 2.16% N content found in the experiment II severely B deficient treatment (Table A-10) is directly due to NR inhibition or indirectly to the lack of available sinks is unclear. The small N content of oilseed

rape leaves is typical. Due to the indeterminate flowering pattern of rape, much growth occurs after flower initiation, which results in a net dilution of tissue N concentration (Rood et al., 1984).

Tissue B analyses of the three experiments (Tables A-1, A-2, and A-3) indicated that the leaves were the best and most sensitive indicators of B availability. This conclusion agrees with the research of Yang et al. (1989). A comparison of the tissue B concentrations for the normal B treatment plants in experiments II (Table A-2) and III (Table A-3) implied that excess N availability was responsible for poor seed production in experiment III (Table A-9). Since the normal LL tissue B concentrations for experiments I and III were practically identical, the excess N apparently resulted in limited B availability for reproduction. Boron deficiency is well known to affect pollen production, viability, and particularly pollen tube growth (Dugger, 1983). Apparently, extra N coupled with low B availability also detrimentally influenced LL B uptake; the experiment III low B treatment ($0.045 \text{ mg} \cdot \text{L}^{-1}$ added B) resulted in an accumulation of only $20.7 \mu\text{g} \cdot \text{g}^{-1}$, while the experiment I low B treatment ($0.04 \text{ mg} \cdot \text{L}^{-1}$ added B) concentration was $35.1 \mu\text{g} \cdot \text{g}^{-1}$. However, this may be due to plant inability to absorb adequate B quickly enough to meet the demand of N-stimulated sink development.

The phenomenon of normal appearing rape plants that produce little or no seed has previously been observed. In

greenhouse studies, Nyborg and Hoyt (1970) proposed that B deficient soils were responsible for this effect in oilseed rape. While working with rapeseed production on sulfur (S) and B deficient soils, Nuttall et al. (1987) observed rape plants in the field that morphologically resembled both the deficient and severely B deficient plants produced during experiment II. Although their greenhouse research agreed with Nyborg and Hoyt (1970), field experiments indicated that, in some instances, the addition of S alone eliminated the failure of rape to set seed. Still, their best yield results occurred when both S and B were applied to the deficient soils.

Sulfur is essential in the production of oilseed rape since it is used not only for protein but also for glucosinolate synthesis (Holmes, 1980). Sulfur deficiency is very similar to N deficiency except that yellowing first appears on the younger leaves. Tissue yellowing is eventually followed by chlorosis which is sometimes succeeded by the production of anthocyanin pigments (Eaton, 1942). In addition, the leaves are generally small and have a stiff texture. These symptoms are indicative of the plants produced in experiment II. Examination of the tissue S concentrations for experiment II (Table A-12) suggests that the B deficient plant stem, main inflorescence and branches had accumulated an excess of S due to limited seed (sink) production. The severely deficient B treatment plants, however, had a highly significant concentration of S in the stem tissue as an apparent

consequence of no sink (main inflorescence, branch, or seed) development. This insinuates that, in the case of oilseed rape, S deficiency symptoms may be a secondary effect of inadequate B availability. Sulfur is transported within the plant by the xylem and, once absorbed, is fairly immobile (Mengel and Kirby, 1982). The detrimental effect of B deficiency on vascular tissue has been noted. The onset of minor S deficiency symptoms observed in the deficient B treatment plants of experiment II appears to be the result of somewhat late developing vascular damage. The more acute symptoms of S deficiency observed in the severely deficient B treatment plants of experiment II are the result of much earlier vascular destruction. Therefore, it is reasonable to conclude that S fertilization can enhance total seed yield only under conditions of adequate B availability. The tissue S concentrations of experiment III (Table A-13) appear to be more than adequate for normal plant development. Therefore, available S like B, is being devoted to vegetative growth at the expense of reproductive development and seed production.

CONCLUSIONS

Boron nutrition is extremely critical in the production of oilseed rape. Normal appearing but nonproductive rapeseed plants can be the result of B deficiency, S deficiency, or a combination of both. Deformed and grossly underdeveloped rape

plants that exhibit intense S deficiency symptoms occur as the result of a severe B deficiency that has devastated the plant vascular tissue. Excessive N fertilization can produce exorbitantly sized plants that are poor yielding - even if adequate B and S nutrition is available.

The best morphological indicators of B deficiency are axillary branch number and main inflorescence height. In tissue B analysis, rapeseed leaves are the most sensitive indicators of B availability and uptake. Boron deficiency in rape may not be revealed until the reproductive stage is well advanced. A lower leaf tissue B concentration of $35 \mu\text{g}\cdot\text{g}^{-1}$ to $80 \mu\text{g}\cdot\text{g}^{-1}$ is adequate for normal growth and development of oilseed rape. A lower leaf tissue B content between $18 \mu\text{g}\cdot\text{g}^{-1}$ and $35 \mu\text{g}\cdot\text{g}^{-1}$ may result in a normal appearing plant with limited or no seed production.

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CHAPTER IV

INFLUENCE OF BORON NUTRITION ON GAS EXCHANGE OF OILSEED RAPE

ABSTRACT

The effects of boron (B) nutrition on net photosynthesis (Pn) and stomatal conductance (SC) of oilseed rape leaves were analyzed in three greenhouse experiments. A chemically inert continuously recirculating hydroponic system was utilized to deliver nutrient solutions. Perlite was used as the rooting medium. A portable Pn analyzer was utilized to record CO₂ uptake, temperature, humidity, and light intensity values on leaves 8, 12, 16, 20, and 24. Data was collected on clear sunny days at solar noon (± 2 hrs.) on individual leaves (averaging 70 to 90% full expansion) or groups of three successive leaves (with the intermediate leaf averaging 70 to 90% full expansion). Analysis of Pn and SC values indicated a minimum leaf tissue B concentration of approximately 21 $\mu\text{g}\cdot\text{g}^{-1}$ was required for normal gas exchange function. The differential effect of the B treatments on Pn and SC was found to be consistent regardless of leaf age. Gas exchange values were found to decrease as the rape leaves matured. Plant

developmental stage was determined to be more important than leaf age in gas exchange capability.

INTRODUCTION

Although the role of boron (B) in plant metabolism is uncertain (Parr and Loughman, 1983; Pilbeam and Kirby, 1983), the deleterious effects of B deficiency on plant growth and development are well documented (Gupta, 1979; Mengel and Kirby, 1982). Oilseed rape, like other *Brassicas*, is extremely sensitive to B availability (Holmes, 1980).

Inadequate B nutrition has been shown to greatly affect plant gas exchange capacity. Reduced photosynthesis (Pn), stomatal conductance (SC), and transpiration have been observed in broccoli (Petracek and Sams, 1987) and mustard (Sharma and Ramchandra, 1990) produced under B deficient growing conditions. Researchers have documented the detrimental effects of B deficiency on chloroplast structure (Hudak and Herich, 1976), leaf chlorophyll (Petracek and Sams, 1987), and mesophyll cell orientation and vascular tissue differentiation (Pissarek, 1980). In addition, stomatal function has proven very susceptible to B availability. Boron deficient conditions can result in reduced stomatal aperture (Roth-Bejerano and Itai, 1981) and non-functional stomata (Baker et al., 1956).

The efficiency and continuity of Pn in rape is vital to

seed production. All rape plant organs are capable of carbon assimilation since stomata are distributed throughout the plant (Major, 1975). Although leaves are the major photosynthetic organs at anthesis, stems and ultimately pods assume this function as the leaves die (Chapman et al., 1984). The seed are a strong sink for photosynthate produced not only by the pods, but also by the declining upper stem and leaves (Major et al., 1978). Therefore, leaf Pn is critical not only during early plant development but also during early pod and seed production. Tayo and Morgan (1975) estimated that 75% of pods retained until plant maturity formed during the first two weeks following anthesis. Sharma and Ghildiyal (1992) concluded that leaf Pn and pod Pn were equally important in determining seed yield of mustard.

The objectives of this research were to study the chronological effects of B nutrition on Pn and SC of oilseed rape leaves, and to evaluate the relationship between leaf gas exchange efficiency and general plant development and seed yield.

MATERIALS AND METHODS

Three greenhouse experiments (I - Fall, 1991; II - Spring, 1992; III - Fall, 1992) were conducted to examine the influence of B nutrition on gas exchange properties of oilseed rape. A spring rapeseed cultivar, Ameri-Can A-112, was

supplied by Ameri-Can of Memphis, TN for use in these studies. The plants were arranged in a randomized complete block design consisting of three blocks. Experiment I had 21 single plant replications, whereas Experiments II and III consisted of 12 replications. A chemically inert continuously recirculating hydroponic system was designed to deliver three treatments of added B in each experiment (Appendix B). Standard analysis of variance procedures (GLM procedure of SAS) were used to determine significant effects based on linear contrasts. Greenhouse mean daily temperatures averaged 22.5 ± 1 C for each experiment. Plants were individually grown in 11 liter plastic pots with perlite as the rooting medium. Nutrient solutions were carefully prepared using distilled deionized water and analytical reagent grade chemicals. Precautions were taken to avoid outside chemical contamination. Plastic containers were used exclusively to prevent B contamination from borosilicate glassware. Aside from the added B treatments, the basic nutrient solutions ((Epstein, 1972); Appendix B) remained the same except in experiment III where the KNO_3 concentration was increased to 4 M. Dilutions of the basic nutrient solutions were used in all experiments to regulate nutrient availability (Appendix B). Contents of freshly prepared nutrient solutions as well as existing circulating solutions were monitored by inductively coupled plasma (ICP) emission spectrometry (Model 61 Thermo Jarrell Ash; Franklin, MA). The consistency of B availability and

uptake in the recirculating solutions was monitored in all experiments (Figs. A-1, A-2, and A-3).

Seeds were planted in plastic trays containing perlite. In all experiments, adequate basic nutrient solution (1:4 dilution) was maintained in the trays to prevent seed desiccation and promote plant growth. Approximately 4 week old seedlings were transplanted into the working hydroponic system without any apparent transplant shock.

Initially, nutrient solution dilutions of 1:4 were used except for experiment III that began with a 1:2 dilution. Experiment I was switched to a 1:2 dilution 37 days following transplanting while experiment II was switched after 23 days. These concentrations remained constant until the experiments ended after 70 and 63 days respectively. The experiment III nutrient solution was changed to a 3:4 dilution 28 days after transplanting. It remained at this level for 4 weeks until being reduced to a 1:2 dilution. A 1:4 dilution was initiated 26 days later and remained in effect until the experiment ended 4 weeks later. Plants were allowed to desiccate naturally before seed pods were harvested. The number of pods and seed weight for each individual plant were recorded.

Photosynthesis readings were taken at solar noon (± 2 hours) on clear sunny days. An LCA2 portable Pn analyzer (Analytical Development Company, Ltd., Hoddesdon, England) was used to record CO₂ uptake, temperature, humidity, and light intensity. These variables were used to calculate Pn and SC

values (Appendix B). Measurements were taken on postplanting days 58, 76, 86, 97, and 106 (experiment I), and days 50, 70, 78, 88, and 97 (experiment II) on leaf 8, 12, 16, 20, and 24, respectively, when each leaf attained 70 to 90% full expansion. In experiment III, readings were taken on leaf(s) 8, (8 and 12 and 16), (12 and 16 and 20), and (16 and 20 and 24) as leaf 8, 12, 16, and 20, respectively, attained 70 to 90% full expansion. The four measurement dates were 43, 56, 70, and 84 days respectively following the planting date.

Plant tissue analysis (Appendix B) was conducted by ICP spectrometry to determine tissue B concentrations in all experiments. Tissue samples were collected following senescence or after seed harvest. The following plant parts were examined: lower leaves (LL) (nodes 1 - 15), upper leaves (UL) (nodes 16+), stem, main inflorescence, axillary branches and roots.

RESULTS AND DISCUSSION

In experiment I, all B treatments produced healthy plants that were visually identical. There were no significant differences in seed yield among the three B treatments (Table A-5) indicating that the $0.04 \text{ mg} \cdot \text{L}^{-1}$ level of added B allowed sufficient uptake to produce a normal seed yield. The LL tissue B concentration range of 35.1 to $80.5 \mu\text{g} \cdot \text{g}^{-1}$ (Table A-1) was adequate for normal Pn and SC functioning (Table A-14).

Leaf tissue has been proven to be the most sensitive indicator of B availability and uptake (Yang et al., 1989).

Plants produced in experiment II displayed extreme morphological variability. The severely B deficient treatment ($0.01 \text{ mg}\cdot\text{L}^{-1}$ added B) did not result in a viable plant. This is not surprising since the tissue B concentrations of the LL and UL were only 7.7 and $2.3 \mu\text{g}\cdot\text{g}^{-1}$ respectively (Table A-2). The deficient ($0.02 \text{ mg}\cdot\text{L}^{-1}$ added B) and normal ($0.40 \text{ mg}\cdot\text{L}^{-1}$ added B) treatments initially produced morphologically similar plants. When the reproductive stage was well advanced, however, it became apparent that the deficient plants were producing comparatively few seed (Table A-7). Both Pn (Table A-15) and SC (Table A-16) values were not found to be significantly different among treatments until measurements for the sixteenth leaf were recorded. This implies that adequate B was available for normal early plant development until bolting was initiated. By the leaf 16 measurements, the Pn and SC values for the severely deficient plants were significantly less than the other treatments. Apparently, adequate B was still available at this time to meet the increased nutritional demands of the deficient B treatment plants. By the time of the leaf 20 readings, however, all treatment contrasts were highly significant for both Pn and SC values even though seed production was the only apparent morphological difference between the deficient and normal plants. Boron deficiency is well known to adversely affect pollen production, viability,

and especially, pollen tube growth (Dugger, 1983; Lewis, 1980). Prior to the leaf 24 readings, other major differences were becoming apparent. Terminal death of the main inflorescence as well as chlorosis of some younger leaves were occurring on the deficient B treatment plants. The death of apical growing points is a well known consequence of B deficiency (Mengel and Kirby, 1982), whereas chlorosis of immature leaves may indicate a sulfur deficiency (Nuttall et al., 1987) induced by lack of available B. Significant treatment differences among the LL and UL tissue B analyses (Table A-2) support the gas exchange data, and also indicate a good correlation between B availability and plant uptake.

The extra nitrogen added in experiment III resulted in superfluous vegetative growth to the detriment of seed production (Table A-9). All plants were similar in appearance displaying excessive stem growth, leaf production, branching, and flowering. Comparison of Pn (Table A-17) and SC (Table A-18) values revealed no significant differences between the low ($0.045 \text{ mg} \cdot \text{L}^{-1}$ added B) and deficient ($0.03 \text{ mg} \cdot \text{L}^{-1}$ added B) treatments. Furthermore, the only significant treatment contrast occurred between the normal ($0.60 \text{ mg} \cdot \text{L}^{-1}$ added B) and deficient B treatments during the crucial time of bolting and anthesis. Apparently, B uptake from the low treatment was adequate for normal Pn and Sc functioning, but was not sufficient for normal seed production (Table A-9). The excess nitrogen appears to have had a relatively uniform effect, with

seed yield being proportionally reduced in relation to the available B treatments. Tissue B analysis (Table A-3) indicated that both the low and normal treatments were highly significant in contrast to the deficient B treatment. This confirms that B uptake was consistent with B availability. Apparently, the LL and UL tissue B concentration range of 15 to 21 $\mu\text{g}\cdot\text{g}^{-1}$ (Table A-3) is critical to normal Pn and SC function in oilseed rape leaves.

Effects of the experiment III B treatments on the mean gas exchange values of three successive rape leaves ranging from immature to very mature were analyzed as the plants developed. The Pn (Table A-19) and SC (Table A-20) values both increased linearly with B availability, and treatment contrasts generally agreed with those concluded from the individual leaf analyses (Tables A-17 and A-18). These results suggest that when analyzing the effects of B availability, leaf age is not crucial. However, the stage of plant development is important in the study of gas exchange capability.

An analysis irrespective of the three B treatments determined at which general leaf age gas exchange capacities were highest. The Pn (Table A-21) and SC (Table A-22) treatment contrasts indicated leaf age was significant, with the younger leaves having higher values. The decreasing gas exchange capacity of the maturing leaves is in agreement with Paul (1992). He determined that photosynthetic capacity of

oilseed rape leaf tissue quickly peaks when the leaf is at approximately 33% of its mature size.

CONCLUSIONS

The effects of B deficiency on oilseed rape leaf Pn and SC are consistent with plant morphological development and tissue B analysis. Young leaves (less than 40% developed) have a higher rate of gas exchange than more mature leaves. Net photosynthesis appears to be generally consistent with SC except for late developing leaves where SC is usually higher. This inconsistency may be due to the formation of different sinks in the plant, or perhaps is influenced in this case by the constant availability of water. Excessive nitrogen availability during early plant development is extremely detrimental to seed production, but has little effect on rape leaf gas exchange capacity. The range of leaf tissue B concentration from 15 to 21 $\mu\text{g}\cdot\text{g}^{-1}$ appears to be crucial to proper Pn and SC function in oilseed rape. The dissimilar effects of B nutrition on rape leaf tissue Pn and SC appear consistent regardless of leaf maturity.

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CHAPTER V

INFLUENCE OF BORON NUTRITION ON GLUCOSINOLATE CONTENT OF OILSEED RAPE

ABSTRACT

Complete nutrient solutions differing in boron (B) and nitrogen (N) availability were supplied to canola quality oilseed rape plants by a continuously recirculating hydroponic system. Colorimetry and gas chromatography were used to quantify rapeseed glucosinolate content. Plant tissue B concentration and solution B availability were determined by ICP analysis. The predominate glucosinolates in rapeseed were confirmed to be 2-hydroxy-3-butenyl and 4-hydroxy-3-indolylmethyl. Indole glucosinolates were also verified to comprise approximately 40% of the total content. Seed glucosinolate concentration was found to remain stable at a minimum leaf tissue B concentration of $21 \mu\text{g}\cdot\text{g}^{-1}$. The lower leaf tissue B content required for normal seed production was determined to fall between 18 and $35 \mu\text{g}\cdot\text{g}^{-1}$. Excessive N detrimentally affected seed yield, but apparently had little effect on glucosinolate content. Severely deficient B was found to significantly increase seed glucosinolate

concentrations above canola quality levels.

INTRODUCTION

The introduction of canola grade rapeseed (< 2% erucic acid and < 30 μmol of aliphatic glucosinolates per gram defatted meal) has resulted in a dramatic increase in the world production of oilseed rape (Downey, 1990). Canola oil, which contains only 6% saturated fat, has become increasingly desirable to health conscious consumers. In addition, the meal remaining after oil extraction has shown great potential as an animal feed supplement. Rapeseed meal contains a high protein concentration with a good balance of amino acids. Still, the continued, albeit greatly reduced, presence of glucosinolates in rapeseed is a major problem to animal nutritionists. Glucosinolates, and especially their breakdown products, may reduce palatability and nutritional value of animal products when consumed in large amounts as part of their feed (McGregor, 1993). Also, animals fed a diet consisting mostly or entirely of rapeseed meal have experienced nutritional and metabolic disorders affecting the thyroid gland, liver, and kidneys (Pusztai, 1989).

Glucosinolates are secondary metabolites that are found in several orders of dicotyledonous angiosperms. These natural toxicants occur predominantly in the order Capparales and primarily in the Cruciferae family. Their physiological role in the plant has not been determined, but appears to include

storage (Clossais-Besnard and Larher, 1991) and defense (Fenwick et al., 1983). Relative concentrations of individual glucosinolates vary considerably in different organs of the same plant (Larsen, 1981), and change radically as the plant evolves and matures (Booth et al., 1991). The largest concentration of glucosinolates occurs in seed tissue where they may contribute up to 5% of the fresh weight (Sang et al., 1984).

Glucosinolates are always accompanied in plants by the enzyme myrosinase (thioglucoside glucohydrolase (E.C. 3.2.3.1)) that catalyzes the rapid hydrolysis of glucosinolates following plant injury or during processing. Besides glucose and sulfur, other physiologically active products are formed including: isothiocyanates, thiocyanates, organic nitriles, and oxazolidine-2-thiones (Underhill, 1980). These compounds not only contribute to the distinctive flavor and aroma characteristic of the Cruciferae, but also may have undesirable effects in animal feedstuffs due to their pungency and goitrogenic activity (Duncan, 1991; Pusztai, 1989).

Plant glucosinolate biosynthesis and content may be influenced by many factors including variety, environmental conditions, and cultural practices (Fenwick et al., 1983). Mineral nutrition has been proven to alter rapeseed glucosinolate content. Increases in sulfur availability have consistently elevated seed glucosinolate concentrations (Josefsson, 1970; Mailer, 1989; Nuttall et al., 1987). The

effects of micronutrient nutrition on plant glucosinolate formation, however, have not been adequately investigated. Jiracek et al. (1974a;b) determined that copper and zinc inhibited the synthesis of indole glucosinolates in young rape seedlings. Bible et al. (1981) observed significantly higher ionic thiocyanate and reducing sugar concentrations in field grown radishes exhibiting boron deficiency symptoms. Turnip plants with internal, but not external, signs of B deficiency were shown to have high levels of 5-vinyl-oxazolidine-2-thione, isothiocyanates, and thiocyanate ion (Ju et al., 1982). Yang et al. (1989) noted that increasing the potassium and B supply to rape plants resulted in a decreased glucosinolate concentration. Also, B application in conjunction with nitrogen and potassium was found to promote plant growth and development. Shelp et al. (1992) determined that broccoli grown under B deficient conditions experienced increases in glucosinolate concentrations. The detrimental effects of B deficiency on plants are well known (Gupta, 1979; Mengel and Kirby, 1982) though the role of B in plant metabolism has not been established (Marschner, 1986; Pilbeam and Kirby, 1983). Boron is also unique among the essential elements owing to a very narrow range between deficiency and toxicity (Gupta et al., 1985). Although B availability is crucial to normal oilseed rape ontogeny (Holmes, 1980), the effects of B nutrition on seed glucosinolate content have not been investigated. The objective of this research was to

evaluate the influence of B availability on oilseed rape development, yield, and seed glucosinolate content.

MATERIALS AND METHODS

Three greenhouse experiments were conducted (I - Fall, 1991; II - Spring, 1992; III - Fall, 1992) utilizing a canola quality spring rapeseed cultivar, Ameri-Can A-112, supplied by Ameri-Can of Memphis, TN. The plants were arranged in a randomized complete block design consisting of three blocks. Experiment I had 21 single plant replications whereas experiments II and III contained 12 replications. A chemically inert continuously recirculating hydroponic system was designed to deliver three treatments of added B in each experiment (Appendix B). Standard analysis of variance procedures (GLM procedure of SAS) were used to determine significant effects based on linear contrasts. Plants were individually grown in 11 liter plastic pots with perlite as the rooting medium. In all experiments, greenhouse mean daily temperatures averaged 22.5 ± 1 C. Nutrient solutions were carefully prepared using distilled deionized water and analytical reagent grade chemicals. Precautions were taken to avoid outside chemical contamination. Plastic containers were used exclusively to prevent B contamination from borosilicate glassware. Besides the added B treatments, the basic nutrient solution ((Epstein, 1972); Appendix B) remained the same -

except experiment III, where the KNO_3 concentration was increased to 4 M. Dilutions of the basic nutrient solutions were used to regulate nutrient availability (Appendix B). Mineral concentrations in freshly prepared nutrient solutions as well as existing circulating solutions were monitored by inductively coupled plasma (ICP) atomic emission spectrometry (Model 61 Thermo Jarrell Ash, Franklin, MA).

Seeds were planted in plastic trays containing perlite. In all experiments, adequate basic nutrient solution (1:4 dilution) was maintained in the trays to prevent seed desiccation and promote plant growth. Approximately 4 week old seedlings were transplanted into the working hydroponic system without any apparent transplant shock.

Initially, nutrient solution dilutions of 1:4 were used except for experiment III that began with a 1:2 dilution. Experiment I was switched to a 1:2 dilution 37 days following transplanting while experiment II was switched after 23 days. These concentrations remained constant until the experiments ended after 70 and 63 days respectively. The experiment III nutrient solution was changed to a 3:4 dilution 28 days after transplanting. It remained at this level for 4 weeks until being reduced to a 1:2 dilution. A 1:4 dilution was initiated 26 days later and this remained in effect until the experiment ended 4 weeks later. Plants in all experiments were allowed to desiccate naturally before seed pods were harvested. In experiment I, seed pods were further divided into main

inflorescence pods and axillary branch pods. After harvest, seed pods were allowed to dry thoroughly before being shelled and placed into storage.

Two procedures (Appendix B) were used to extract and quantify rapeseed glucosinolate content. A colorimetric technique (McGregor, 1990) was used to determine total glucosinolate and total indole glucosinolate concentrations, whereas a gas chromatographic method (Raney and McGregor, 1990) was used to obtain the concentrations of individual glucosinolates.

For the colorimetric procedure, samples were analyzed on a Shimadzu UV-260 Spectrometer (Shimadzu Corporation, Kyoto, Japan). Glucosinolates were extracted in boiling water and purified by ion exchange chromatography (Thies, 1977). Myrosinase was added to the column to hydrolyze the glucosinolates. This resulted in the release of isothiocyanates and sulfur, which remained bound to the column, and glucose, which was eluted with water. The liberated glucose was quantified against a glucose standard curve to find total glucosinolate content. The indole glucosinolate content was resolved by adding NaOH to the column to convert unstable indole isothiocyanates to inorganic thiocyanate ion, which was then eluted with the NaOH. An ammonium thiocyanate standard curve was used to quantify the indole glucosinolate content.

For gas chromatographic determination, the glucosinolates

were extracted using aqueous methanol, and purified and desulfated on an ion exchange column (Heaney and Fenwick, 1980). The desulfated glucosinolates were eluted with aqueous methanol and derivatized to form volatile trimethylsilyl (TMS) ethers. An HP 5890 Series II Gas Chromatograph (Hewlett-Packard Company, Avondale, PA) equipped with a flame ionization detector and a 30 m (0.25 mm i.d.) Duraband-1 methyl silicone capillary column (J & W Scientific, Folsom, CA) was used to separate and quantify the TMS ethers. Benzyl glucosinolate (glucotropaeolin) was used as the internal standard. Sample size was 5 μ L with the split ratio set at 50%.

ICP spectrometry was utilized to monitor B content in the recirculating nutrient solutions (Figs. A-1, A-2, and A-3) and to determine tissue B concentrations. The following plant parts were examined: lower leaves (nodes 1 - 15), upper leaves (nodes 16+), stem, main inflorescence, axillary branches and roots.

RESULTS AND DISCUSSION

All boron treatments in experiment I produced healthy plants and seed yields that were visually indistinguishable. Experiment II, however, resulted in plants that exhibited extreme morphological variability. The severely deficient B treatment (0.01 mg·L⁻¹ added B) produced a malformed and

seedless plant. The deficient ($0.02 \text{ mg} \cdot \text{L}^{-1}$ added B) and normal ($0.40 \text{ mg} \cdot \text{L}^{-1}$ added B) B treatment plants were morphologically similar except that seed yield of the deficient plants was extremely small - averaging just 6% of the normal B treatment yield. The additional nitrogen in experiment III produced excessive vegetative growth to the detriment of seed production in all three B treatments. The normal B treatment ($0.60 \text{ mg} \cdot \text{L}^{-1}$ added B) produced a small seed yield ($< 50\%$) in comparison to the normal treatments in the two previous experiments. In addition, the low B treatment ($0.045 \text{ mg} \cdot \text{L}^{-1}$ added B) seed yield was 82% lower than the experiment I low B treatment ($0.04 \text{ mg} \cdot \text{L}^{-1}$ added B) yield. Boron availability appeared to be well correlated with B uptake (Tables A-1, A-2, and A-3) with leaf tissue being the most sensitive and consistent indicator (Yang et al., 1989).

The use of gas chromatography to quantify the individual glucosinolates (Tables A-23, A-25, and A-27) revealed that 2-hydroxy-3-butenyl (progoitrin) was the predominant glucosinolate in all 3 experiments. The indole glucosinolate 4-hydroxy-3-indolylmethyl (4-hydroxy-glucobrassicin) was the second most common, and was always found at a higher concentration than any of the remaining aliphatic compounds (Fig. V-1). The aliphatic glucosinolates (3-butenyl (gluconapin), 4-pentenyl (glucobrassicinapin), and 2-hydroxy-4-pentenyl (gluconapoleiferin)) collectively averaged only 30 to 40% of the 2-hydroxy-3-butenyl concentration in all

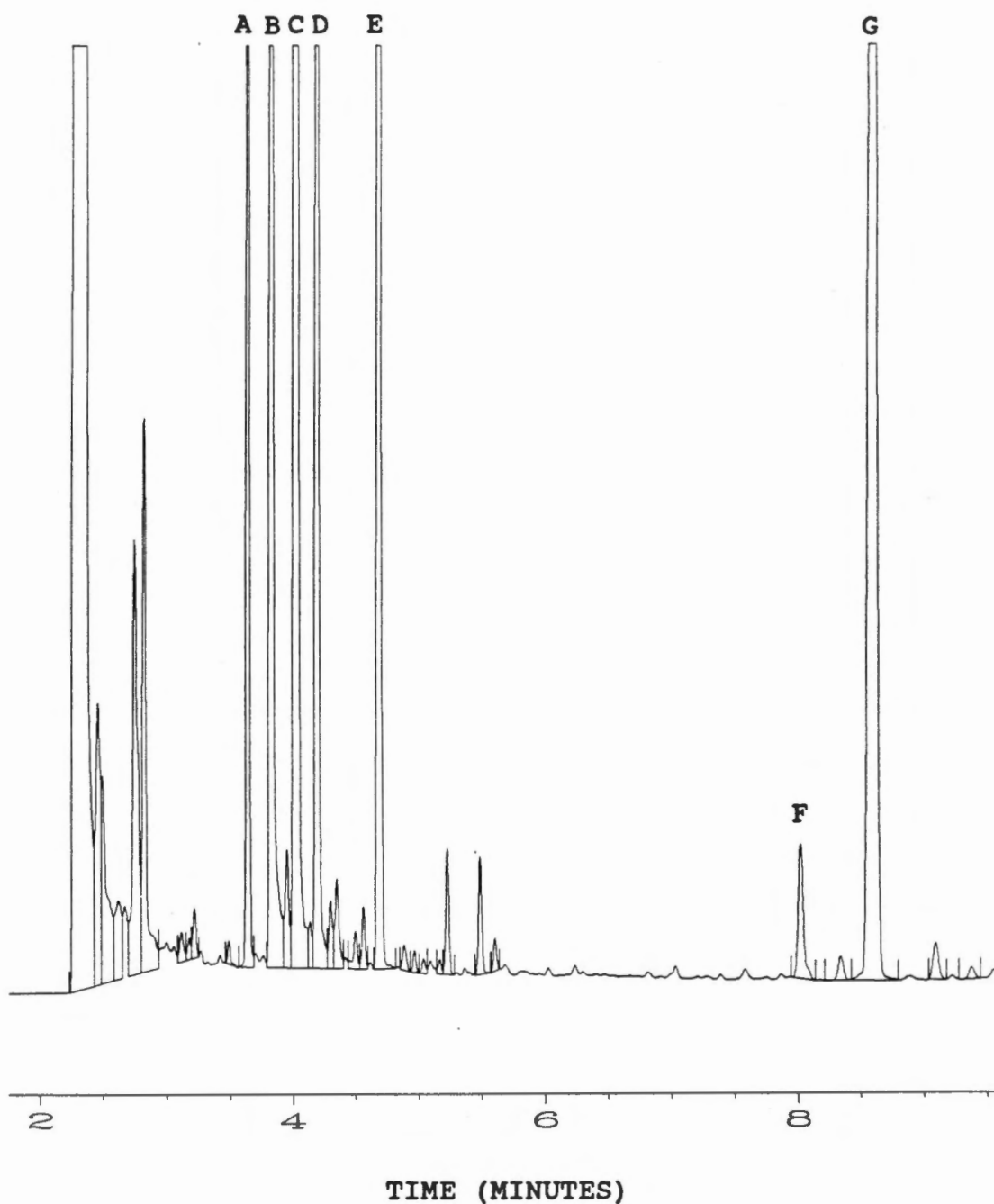


Fig. V-1. Chromatogram of trimethylsilylated desulfoglucosinolates extracted from rapeseed. Peaks: (A) 3-butenyl, (B) 4-pentenyl, (C) 2-hydroxy-3-butenyl (D) 2-hydroxy-4-pentenyl, (E) Benzyl, (F) 3-indolylmethyl, (G) 4-hydroxy-3-indolylmethyl.

experiments. In addition, the contribution of 3-indolylmethyl glucosinolate (glucobrassicin) was minor. These results agree with Sang et al. (1984) who determined that 4-hydroxy-3-indolylmethyl was the major indole glucosinolate in rape seed tissue, while 3-indolylmethyl occurred predominantly in rape leaf tissue. Although a few other glucosinolates do occur in very small quantities in rapeseed, these six are the most important. Presently, only the four aliphatic glucosinolates are used to determine if rapeseed is canola quality. But, as shown in this research and observed by others (Kraling et al., 1990), the indole glucosinolate concentration may contribute nearly 40% of the total glucosinolates present.

The aliphatic glucosinolates in experiment I (Tables A-23 and A-24) appear to have been affected more by the intermediate than by the normal or low B treatments. The reason for this inconsistency was not apparent, but more importantly, the low vs. normal treatment contrasts were not significant. Conversely, the treatment contrasts for the aliphatic glucosinolates in experiment II (Tables A-25 and A-26) were highly significant. However, 4-hydroxy-3-indolylmethyl was significant only at 5%, and 3-indolylmethyl was not significant. This suggests that the biosynthetic pathways of the indole glucosinolates are less sensitive to B nutrition than are the biosynthetic pathways of the aliphatics. In experiment III (Tables A-27 and A-28), the low vs. normal treatment contrast was found nonsignificant for all

glucosinolates. The results from experiments I and III suggest that a leaf tissue B concentration of $21 \mu\text{g}\cdot\text{g}^{-1}$ (Table A-3) was sufficient to produce normal seed glucosinolate content. However, the LL tissue B concentration required to ensure normal seed yield could only be approximated between 18 (Table A-2) and $35 \mu\text{g}\cdot\text{g}^{-1}$ (Table A-1). A notable effect of the added nitrogen in experiment III was the significant treatment contrasts seen for 3-indolylmethyl (Table A-27). Apparently, the excess nitrogen either accentuates the B deficiency here, or perhaps has an entirely separate impact on biosynthesis.

Comparison of the glucosinolate totals derived by gas chromatography and colorimetry for the three experiments (Tables A-24, A-26, and A-28) revealed a large discrepancy in the results. Since the colorimetric procedure results were consistently lower, the two most plausible experimental problems would appear to be the following: (1) failure to inactivate completely the endogenous myrosinase enzyme or (2) poor digestion due to inferior quality exogenous myrosinase. The most significant results were found in experiment II (Table A-26) where the deficient B treatment resulted in an increase of the total glucosinolate concentration above canola quality standards. Furthermore, all added B treatments in experiment III (Table A-28) produced a total glucosinolate content slightly above canola quality levels. The extra sulfur availability in the 3:4 dilution of basic nutrient solution was probably responsible for this increase since elevated

nitrogen availability does not significantly raise rapeseed glucosinolate concentration (Josefsson, 1970).

A comparison of glucosinolate concentrations between main inflorescence seed and branch seed in experiment I (data not shown) revealed no significant differences. This finding agrees with the results of Clossais-Besnard and Larher (1991). They determined that 3-butenyl and 2-hydroxy-3-butenyl were more concentrated in seed located on the proximal part of the branches, while 4-hydroxy-3-indolylmethyl was primarily found in seed produced in the distal area. Yet the total remained constant for each branch due to differential distribution of glucosinolates within each branch.

CONCLUSIONS

Of the two procedures attempted, gas chromatography is the easier and more reliable method to quantify total and individual glucosinolate concentrations. Boron deficiency has a profound detrimental effect on rapeseed production and yield even when plant morphological differences are not readily apparent. In addition, B deficiency can increase rapeseed glucosinolate totals above the level required for canola quality certification. A leaf tissue B concentration of 21 $\mu\text{g}\cdot\text{g}^{-1}$ is apparently sufficient to preclude an increase in seed glucosinolate content, but may not be adequate to ensure normal seed yield. Indole glucosinolates may contribute up to

40% of the total glucosinolates present in rapeseed, and should be considered when determining quality. Apparently, the synthesis of indole glucosinolates is less affected than the aliphatic glucosinolates by B nutrition.

Excessive nitrogen availability greatly increases plant vegetative growth at the expense of seed production. Sulfur, however, is more important in elevating seed glucosinolate totals provided that adequate nitrogen is available.

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APPENDIXES

APPENDIX A
DATA AND STATISTICS

Table A-1. Effect of boron nutrient solution concentrations on the tissue boron content of hydroponically grown oilseed rape plants during the Fall of 1991 (experiment I).

Boron treatment ^a (mg·L ⁻¹)	Boron content (μg·g ⁻¹)					
	Lower leaves	Upper leaves	Stem	Main infl ^b .	Branches	Roots
0.04	35.1	31.9	50.2	88.4	46.2	60.3
0.20	70.5	85.1	55.4	89.2	46.3	52.0
0.40	80.5	145.4	63.4	100.1	59.0	65.4
Contrasts ^c						
0.04 vs 0.20	**	**	NS	NS	NS	NS
0.20 vs 0.40	**	**	*	NS	**	NS
0.04 vs 0.40	**	**	**	NS	**	NS

^aNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

^bMain inflorescence.

^cLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-2. Effect of boron nutrient solution concentrations on the tissue boron content of hydroponically grown oilseed rape plants during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Boron content (μg·g ⁻¹)					
	Lower leaves	Upper leaves	Stem	Main infl ^b .	Branches	Roots
0.01	7.7	2.3	19.4	NA ^c	NA	38.0
0.02	17.9	10.8	18.1	74.0	18.9	21.5
0.40	54.5	83.4	29.1	72.1	30.1	30.4
Contrasts ^d						
0.01 vs 0.02	**	NS	NS	NE	NE	**
0.02 vs 0.40	**	**	**	NS	**	*
0.01 vs 0.40	**	**	**	NE	NE	*

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bMain inflorescence.

^cNot available (insufficient plant development).

^dLinear treatment contrast significant at 0.01 (**), 0.05 (*), nonsignificant (NS), or non-estimable (NE).

Table A-3. Effect of boron nutrient solution concentrations on the tissue boron content of hydroponically grown oilseed rape plants during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Boron content (μg·g ⁻¹)					
	Lower leaves	Upper leaves	Stem	Main infl ^b .	Branches	Roots
0.03	15.7	14.3	11.1	22.6	7.1	22.3
0.045	20.7	21.1	11.4	22.8	7.8	11.5
0.60	82.5	148.5	36.6	37.4	38.5	31.7
Contrasts ^c						
0.03 vs 0.045	NS	NS	NS	NS	NS	**
0.045 vs 0.60	**	**	**	**	**	**
0.03 vs 0.60	**	**	**	**	**	**

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bMain inflorescence.

^cLinear treatment contrast significant at 0.01 (**), or nonsignificant (NS).

Table A-4. Effect of boron nutrient solution treatments on morphological development of hydroponically grown oilseed rape plants during the Fall of 1991 (experiment I).

Boron treatment ^a (mg·L ⁻¹)	Leaves (nodes)	Branches	Stht ^b (cm)	Miht ^c (cm)	Bolting (days)	Anthesis (days)
0.04	30.8	7.0	10.6	33.8	92.5	98.4
0.20	31.4	6.3	11.1	33.6	95.8	105.3
0.40	31.4	6.1	10.7	33.3	94.4	102.6
Contrasts ^d						
0.04 vs 0.20	NS	NS	NS	NS	NS	*
0.20 vs 0.40	NS	NS	NS	NS	NS	NS
0.04 vs 0.40	NS	*	NS	NS	NS	NS

^aNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

^bStem height (distance between the lowest and uppermost nodes).

^cMain inflorescence height (length of dominant flowering branch initiated at uppermost node).

^dLinear treatment contrast significant at 0.05 (*), or nonsignificant (NS).

Table A-5. Effect of boron nutrient solution treatments on seed production and yield of hydroponically grown oilseed rape plants during the Fall of 1991 (experiment I).

Boron treatment ^a (mg·L ⁻¹)	Per plant					
	Pods			Seed wt (g)		
	MI ^b	Branch	Total	MI	Branch	Total
0.04	86.2	147.6	233.8	4.3	5.7	10.0
0.20	91.3	145.4	236.7	4.1	5.4	9.5
0.40	90.8	142.4	233.7	4.0	5.1	9.1
Contrasts ^c						
0.04 vs 0.20	NS	NS	NS	NS	NS	NS
0.20 vs 0.40	NS	NS	NS	NS	NS	NS
0.04 vs 0.40	NS	NS	NS	NS	NS	NS

^aNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

^bMain inflorescence.

^cLinear treatment contrasts nonsignificant (NS).

Table A-6. Effect of boron nutrient solution treatments on morphological development of hydroponically grown oilseed rape plants during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Leaves (nodes)	Branches	Stht ^b (cm)	Miht ^c (cm)	Bolting (days)	Anthesis (days)
0.01	NA ^d	NA	NA	NA	77.1	83.3
0.02	29.6	10.4	36.1	81.9	75.2	82.0
0.40	30.1	7.9	36.4	103.2	77.4	83.5
Contrasts ^e						
0.01 vs 0.02	NE	NE	NE	NE	NS	NS
0.02 vs 0.40	NS	**	NS	**	NS	NS
0.01 vs 0.40	NE	NE	NE	NE	NS	NS

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bStem height (distance between the lowest and uppermost nodes).

^cMain inflorescence height (length of dominant flowering branch initiated at uppermost node).

^dNot available (insufficient plant development).

^eLinear treatment contrast significant at 0.01 (**), nonsignificant (NS) or non-estimable (NE).

Table A-7. Effect of boron nutrient solution treatments on seed production and yield of hydroponically grown oilseed rape plants during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Per plant	
	Pods	Seed wt (g)
0.01	NA ^b	NA
0.02	69.2	1.0
0.40	429.1	15.8
Contrasts ^c		
0.01 vs 0.02	NE	NE
0.02 vs 0.40	**	**
0.01 vs 0.40	NE	NE

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bNot available (insufficient plant development).

^cLinear treatment contrasts significant at 0.01 (**), or not estimable (NE).

Table A-8. Effect of boron nutrient solution treatments on morphological development of hydroponically grown oilseed rape plants during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Leaves (nodes)	Branches	Stht ^b (cm)	Miht ^c (cm)	Bolting (days)	Anthesis (days)
0.03	NA ^d	15.9	35.4	72.2	64.6	73.4
0.045	NA	14.8	36.1	107.2	64.9	74.0
0.60	NA	13.8	35.4	120.4	65.5	73.8
Contrasts ^e						
0.03 vs 0.045		*	NS	**	NS	NS
0.045 vs 0.60		NS	NS	NS	NS	NS
0.03 vs 0.60		**	NS	**	NS	NS

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bStem height (distance between the lowest and uppermost nodes).

^cMain inflorescence height (length of dominant flowering branch initiated at uppermost node).

^dNot Available (data not collected).

^eLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-9. Effect of boron nutrient solution treatments on seed production and yield of hydroponically grown oilseed rape plants during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Per plant	
	Pods	Seed weight (g)
0.03	23.9	0.3
0.045	109.5	1.8
0.60	322.4	4.8
Contrasts ^b		
0.03 vs 0.045	NS	NS
0.045 vs 0.60	**	**
0.03 vs 0.60	**	**

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bLinear treatment contrasts significant at 0.01 (**), or nonsignificant (NS).

Table A-10. Effect of boron nutrient solution concentrations on tissue nitrogen content of hydroponically grown oilseed rape plants during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Nitrogen content (%)	
	Lower leaves	Upper leaves
0.01	0.71	2.16
0.02	0.59	1.02
0.40	0.51	0.81
Contrasts ^b		
0.01 vs 0.02	NS	**
0.02 vs 0.40	NS	NS
0.01 vs 0.40	NS	**

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bLinear treatment contrast significant at 0.01 (**), or nonsignificant (NS).

Table A-11. Effect of boron nutrient solution concentrations on tissue nitrogen content of hydroponically grown oilseed rape plants during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Nitrogen content (%)	
	Lower leaves	Upper leaves
0.03	0.75	0.65
0.045	0.68	0.60
0.60	0.79	0.57
Contrasts ^b		
0.03 vs 0.045	NS	NS
0.045 vs 0.60	NS	NS
0.03 vs 0.60	NS	*

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bLinear treatment contrast significant at 0.05 (*), or nonsignificant (NS).

Table A-12. Effect of boron nutrient solution concentrations on tissue sulfur content of hydroponically grown oilseed rape plants during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Sulfur Content (μg·g ⁻¹)					
	Lower leaves	Upper leaves	Stem	Main infl ^b .	Branches	Roots
0.01	5496	3873	4690	NA ^c	NA	3729
0.02	7686	5588	1735	3678	2326	2088
0.40	6843	6712	1287	1687	1410	1506
Contrasts ^d						
0.01 vs 0.02	**	**	**	NE	NE	**
0.02 vs 0.40	NS	**	NS	**	**	NS
0.01 vs 0.40	**	**	**	NE	NE	**

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bMain inflorescence.

^cNot available (insufficient plant development).

^dLinear treatment contrast significant at 0.01 (**), nonsignificant (NS), or non-estimable (NE).

Table A-13. Effect of boron nutrient solution concentrations on tissue sulfur content of hydroponically grown oilseed rape plants during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Sulfur Content (μg·g ⁻¹)					
	Lower leaves	Upper leaves	Stem	Main infl ^b .	Branches	Roots
0.03	7422	6654	2926	5402	2303	3954
0.045	7140	6395	1647	5028	2153	2727
0.60	6697	6423	1822	5147	1960	2305
Contrasts ^c						
0.03 vs 0.045	NS	NS	**	NS	NS	**
0.045 vs 0.60	NS	NS	NS	NS	NS	**
0.03 vs 0.60	*	NS	**	NS	**	**

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bMain inflorescence.

^cLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-14. Effect of boron nutrient solution treatments on net photosynthesis and stomatal conductance of 70 to 90% fully expanded oilseed rape leaves hydroponically grown during the Fall of 1991 (experiment I)^a.

Boron treatment ^b (mg·L ⁻¹)	Plant leaf				
	8	12	16	20	24
Photosynthesis ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$)					
0.04	19.8	14.2	21.7	13.8	18.4
0.20	22.0	13.5	22.8	14.7	13.0
0.40	19.7	13.5	21.9	18.1	14.8
Stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$)					
0.40	1501.8	693.1	1964.7	624.6	919.8
0.20	1504.0	643.2	2255.2	658.3	796.3
0.40	1400.1	585.3	1981.8	711.0	842.7

^aAll treatment contrasts are nonsignificant.

^bNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

Table A-15. Effect of boron nutrient solution treatments on net photosynthesis of 70 to 90% fully expanded oilseed rape leaves hydroponically grown during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Plant leaf				
	8	12	16	20	24
Photosynthesis (μmol CO ₂ ·m ⁻² ·sec ⁻¹)					
0.01	22.6	18.9	16.6	6.4	NA ^b
0.02	25.2	20.6	22.2	12.4	9.2
0.40	20.0	20.3	22.2	15.7	12.8
Contrasts ^c					
0.01 vs 0.02	NS	NS	**	**	NE
0.02 vs 0.40	*	NS	NS	**	**
0.01 vs 0.40	NS	NS	**	**	NE

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bNot available (insufficient plant development).

^cLinear treatment contrast significant at 0.01 (**), 0.05 (*), nonsignificant (NS), or non-estimable (NE).

Table A-16. Effect of boron nutrient solution treatments on stomatal conductance of 70 to 90% fully expanded oilseed rape leaves hydroponically grown during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Plant leaf				
	8	12	16	20	24
Stomatal conductance (mmol·m ⁻² ·sec ⁻¹)					
0.01	662.1	449.3	566.8	189.0	NA ^b
0.02	740.0	531.3	790.3	475.1	679.5
0.40	605.6	509.9	809.2	688.4	712.2
Contrasts ^c					
0.01 vs 0.02	NS	NS	**	**	NE
0.02 vs 0.40	*	NS	NS	**	NS
0.01 vs 0.40	NS	NS	**	**	NE

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bNot available (insufficient plant development).

^cLinear treatment contrast significant at 0.01 (**), 0.05 (*), nonsignificant (NS), or non-estimable (NE).

Table A-17. Effect of boron nutrient solution treatments on net photosynthesis of 70 to 90% fully expanded oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Plant leaf			
	8	12	16	20
Photosynthesis (μmol CO ₂ ·m ⁻² ·sec ⁻¹)				
0.03	13.0	10.0	12.0	16.8
0.045	12.4	11.0	13.8	19.4
0.60	13.3	12.8	17.2	18.8
Contrasts ^b				
0.03 vs 0.045	NS	NS	NS	NS
0.045 vs 0.60	NS	NS	NS	NS
0.03 vs 0.60	NS	*	*	NS

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bLinear treatment contrast significant at 0.05 (*), or nonsignificant (NS).

Table A-18. Effect of boron nutrient solution treatments on stomatal conductance of 70 to 90% fully expanded oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Plant leaf			
	8	12	16	20
Stomatal conductance (mmol·m ⁻² ·sec ⁻¹)				
0.03	925.5	661.3	644.6	778.6
0.045	982.6	668.8	837.5	891.8
0.60	998.2	802.6	873.8	885.8
Contrasts ^b				
0.03 vs 0.045	NS	NS	NS	NS
0.045 vs 0.60	NS	NS	NS	NS
0.03 vs 0.60	NS	NS	*	NS

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bLinear treatment contrast significant at 0.05 (*), or nonsignificant (NS).

Table A-19. Effect of boron nutrient solution treatments on mean net photosynthesis of successive oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Plant leaves		
	(8,12,16) ^b	(12,16,20) ^c	(16,20,24) ^d
Photosynthesis (μmol CO ₂ ·m ⁻² ·sec ⁻¹)			
0.03	9.3	12.5	16.9
0.045	10.4	13.8	18.0
0.60	11.3	15.0	19.9
Contrasts ^e			
0.03 vs 0.045	NS	NS	NS
0.045 vs 0.60	NS	NS	NS
0.03 vs 0.60	**	**	*

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^b8 is very mature; 12 is mature (70 to 90% fully expanded); 16 is immature.

^c12 is very mature; 16 is mature (70 to 90% fully expanded); 20 is immature.

^d16 is very mature; 20 is mature (70 to 90% fully expanded); 24 is immature.

^eLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-20. Effect of boron nutrient solution treatments on mean stomatal conductance of successive oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Plant leaves		
	(8,12,16) ^b	(12,16,20) ^c	(16,20,24) ^d
Stomatal conductance (mmol·m ⁻² ·sec ⁻¹)			
0.03	782.4	652.1	743.8
0.045	758.0	740.3	863.4
0.60	860.4	746.4	876.1
Contrasts ^e			
0.03 vs 0.045	NS	NS	*
0.045 vs 0.60	NS	NS	NS
0.03 vs 0.60	NS	*	**

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^b8 is very mature; 12 is mature (70 to 90% fully expanded); 16 is immature.

^c12 is very mature; 16 is mature (70 to 90% fully expanded); 20 is immature.

^d16 is very mature; 20 is mature (70 to 90% fully expanded); 24 is immature.

^eLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-21. Mean effect of boron nutrient solution treatments (0.03, 0.045, and 0.60 mg·L⁻¹ added boron) on net photosynthesis of successive oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Leaf (node)	Measurement ^a		
	2 ^b	3 ^c	4 ^d
Photosynthesis (μmol CO ₂ ·m ⁻² ·sec ⁻¹)			
8	7.3		
12	11.3	7.0	
16	12.5	14.4	14.6
20		19.8	18.4
24			21.7
Contrasts ^e			
8 vs 12	**		
12 vs 16	NS	**	
8 vs 16	**		
16 vs 20		**	*
12 vs 20		**	
20 vs 24			*
16 vs 24			**

^aMeasurements 2, 3, and 4 were taken on days 56, 70, and 84 respectively following the planting date. (Measurement 1 recorded leaf 8 only).

^bLeaf 8 is very mature; leaf 12 is mature (70 to 90% fully expanded); leaf 16 is immature.

^cLeaf 12 is very mature; leaf 16 is mature (70 to 90% fully expanded); leaf 20 is immature.

^dLeaf 16 is very mature; leaf 20 is mature (70 to 90% fully expanded); leaf 24 is immature.

^eLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-22. Mean effect of boron nutrient solution treatments (0.03, 0.045, and 0.60 mg·L⁻¹ added boron) on stomatal conductance of successive oilseed rape leaves hydroponically grown during the Fall of 1992 (experiment III).

Leaf (node)	Measurement ^a		
	2 ^b	3 ^c	4 ^d
Stomatal conductance (mmol·m ⁻² ·sec ⁻¹)			
8	574.0		
12	710.9	413.1	
16	1144.6	785.3	740.9
20		931.6	852.1
24			890.2
Contrasts ^e			
8 vs 12	**		
12 vs 16	**	**	
8 vs 16	**		
16 vs 20		**	*
12 vs 20		**	
20 vs 24			NS
16 vs 24			**

^aMeasurements 2, 3, and 4 were taken on days 56, 70, and 84 respectively following the planting date. (Measurement 1 recorded leaf 8 only).

^bLeaf 8 is very mature; leaf 12 is mature (70 to 90% fully expanded); leaf 16 is immature.

^cLeaf 12 is very mature; leaf 16 is mature (70 to 90% fully expanded); leaf 20 is immature.

^dLeaf 16 is very mature; leaf 20 is mature (70 to 90% fully expanded); leaf 24 is immature.

^eLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-23. Gas chromatographic determination of aliphatic and indole glucosinolate content of rapeseed hydroponically grown in different boron nutrient solutions during the Fall of 1991 (experiment I).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Aliphatic				Indole	
	3But ^b	4Pen ^c	2H3B ^d	2H4P ^e	3Ind ^f	4H3I ^g
0.04	2.28	3.76	12.63	1.87	0.25	5.37
0.20	2.57	4.41	13.27	2.15	0.21	5.07
0.40	2.17	3.66	12.03	1.83	0.26	4.97
Contrasts ^h						
0.04 vs 0.20	NS	*	NS	*	NS	NS
0.20 vs 0.40	*	**	NS	*	NS	NS
0.04 vs 0.40	NS	NS	NS	NS	NS	NS

^aNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

^b3-Butenyl.

^c4-Pentenyl.

^d2-Hydroxy-3-butenyl.

^e2-Hydroxy-4-pentenyl.

^f3-Indolylmethyl.

^g4-Hydroxy-3-indolylmethyl.

^hLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-24. Total glucosinolate content, determined by gas chromatography and colorimetry, of rapeseed hydroponically grown in different boron nutrient solutions during the Fall of 1991 (experiment I).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Gas chromatography			Colorimetry		
	Alip ^b	Ind ^c	Total	Nonind ^d	Ind	Total
0.04	20.55	5.62	26.17	13.70	1.79	15.49
0.20	22.40	5.28	27.68	14.70	1.79	16.49
0.40	19.70	5.22	24.92	13.64	1.78	15.42
Contrasts ^e						
0.04 vs 0.20	NS	NS	NS	NS	NS	NS
0.20 vs 0.40	*	NS	*	NS	NS	NS
0.04 vs 0.40	NS	NS	NS	NS	NS	NS

^aNormal is 0.40 mg·L⁻¹ added boron; intermediate is 0.20 mg·L⁻¹ added boron; low is 0.04 mg·L⁻¹ added boron.

^bAliphatic.

^cIndole.

^dNon-indole.

^eLinear treatment contrast significant at 0.05 (*), or nonsignificant (NS).

Table A-25. Gas chromatographic determination of aliphatic and indole glucosinolate content of rapeseed hydroponically grown in different boron nutrient solutions during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Aliphatic				Indole	
	3But ^b	4Pen ^c	2H3B ^d	2H4P ^e	3Ind ^f	4H3I ^g
0.01	NA ^h	NA	NA	NA	NA	NA
0.02	3.51	4.07	25.45	2.86	0.15	5.23
0.40	2.53	2.15	14.39	1.40	0.16	3.98
Contrasts ⁱ						
0.01 vs 0.02	NE	NE	NE	NE	NE	NE
0.02 vs 0.40	**	**	**	**	NS	*
0.01 vs 0.40	NE	NE	NE	NE	NE	NE

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^b3-Butenyl.

^c4-Pentenyl.

^d2-Hydroxy-3-butenyl.

^e2-Hydroxy-4-pentenyl.

^f3-Indolylmethyl.

^g4-Hydroxy-3-indolylmethyl.

^hNot available (insufficient plant development).

ⁱLinear treatment contrast significant at 0.01 (**), 0.05 (*), nonsignificant (NS), or non-estimable (NE).

Table A-26. Total glucosinolate content, determined by gas chromatography and colorimetry, of rapeseed hydroponically grown in different boron nutrient solutions during the Spring of 1992 (experiment II).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Gas chromatography			Colorimetry		
	Alip ^b	Ind ^c	Total	Nonind ^d	Ind	Total
0.01	NA ^e	NA	NA	NA	NA	NA
0.02	35.89	5.38	41.27	18.45	1.50	19.95
0.40	20.50	4.15	24.61	11.58	1.73	13.30
Contrasts ^f						
0.01 vs 0.02	NE	NE	NE	NE	NE	NE
0.02 vs 0.40	**	*	**	**	NS	**
0.01 vs 0.40	NE	NE	NE	NE	NE	NE

^aNormal is 0.40 mg·L⁻¹ added boron; deficient is 0.02 mg·L⁻¹ added boron; severely deficient is 0.01 mg·L⁻¹ added boron.

^bAliphatic.

^cIndole.

^dNon-indole.

^eNot available (insufficient plant development).

^fLinear treatment contrast significant at 0.01 (**), 0.05 (*), nonsignificant (NS), or non-estimable (NE).

Table A-27. Gas chromatographic determination of aliphatic and indole glucosinolate content of rapeseed hydroponically grown in different boron nutrient solutions during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Aliphatic				Indole	
	3But ^b	4Pen ^c	2H3B ^d	2H4P ^e	3Ind ^f	4H3I ^g
0.03	1.54	3.77	17.12	2.72	0.20	4.71
0.045	1.99	4.20	19.69	2.60	0.11	4.39
0.60	1.94	3.62	20.31	2.49	0.15	3.68
Contrasts ^h						
0.03 vs 0.045	**	NS	NS	NS	**	NS
0.045 vs 0.60	NS	NS	NS	NS	NS	NS
0.03 vs 0.60	*	NS	*	NS	*	NS

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^b3-Butenyl.

^c4-Pentenyl.

^d2-Hydroxy-3-butenyl.

^e2-Hydroxy-4-pentenyl.

^f3-Indolylmethyl.

^g4-Hydroxy-3-indolylmethyl.

^hLinear treatment contrast significant at 0.01 (**), 0.05 (*), or nonsignificant (NS).

Table A-28. Total glucosinolate content, determined by gas chromatography and colorimetry, of rapeseed hydroponically grown in different boron nutrient solutions during the Fall of 1992 (experiment III).

Boron treatment ^a (mg·L ⁻¹)	Glucosinolates (μmoles·g ⁻¹)					
	Gas chromatography			Colorimetry		
	Alip ^b	Ind ^c	Total	Nonind ^d	Ind	Total
0.03	25.15	4.91	30.06	23.17	1.82	24.99
0.045	28.48	4.50	32.99	19.92	1.75	21.68
0.60	28.36	3.83	32.19	19.61	1.57	21.17
Contrasts ^e						
0.03 vs 0.045	NS	NS	NS	**	NS	**
0.045 vs 0.60	NS	NS	NS	NS	NS	NS
0.03 vs 0.60	NS	NS	NS	**	NS	**

^aNormal is 0.60 mg·L⁻¹ added boron; low is 0.045 mg·L⁻¹ added boron; deficient is 0.03 mg·L⁻¹ added boron.

^bAliphatic.

^cIndole.

^dNon-indole.

^eLinear treatment contrast significant at 0.01 (**), or nonsignificant (NS).

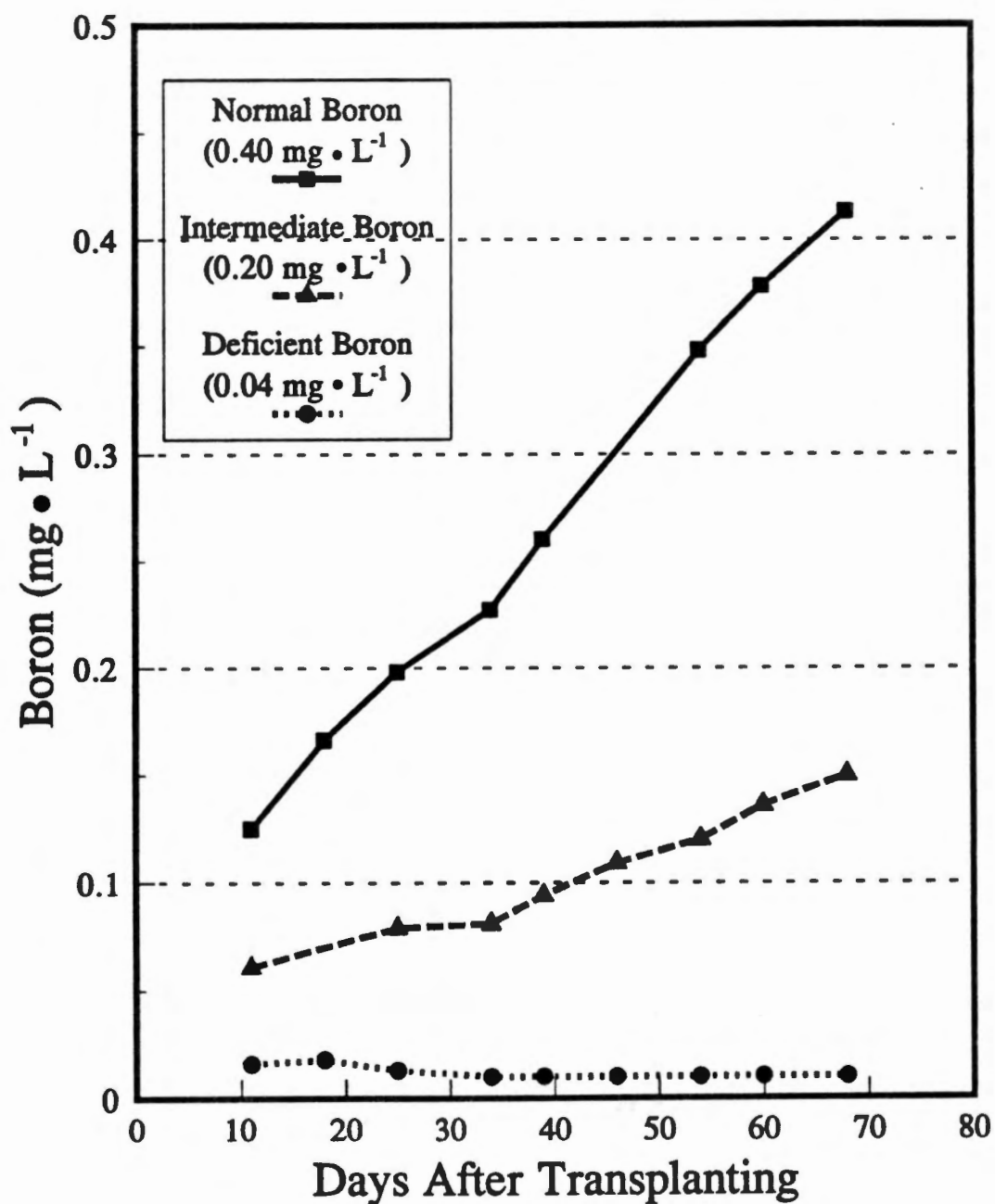


Fig. A-1. Availability and uptake of boron during the Fall 1991 (experiment I) hydroponic study growing oilseed rape. The recirculating nutrient solution was sampled prior to nutrient additions.

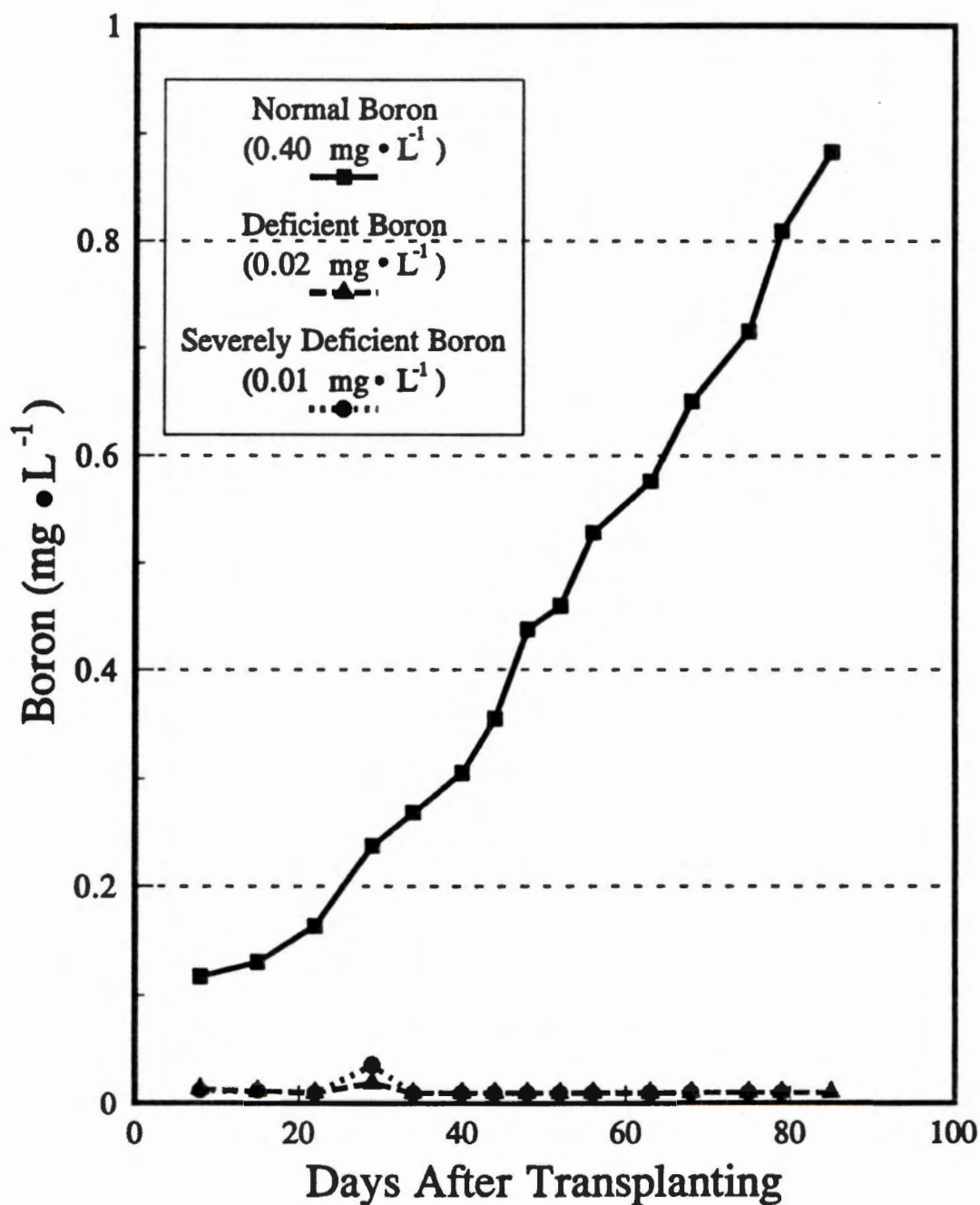


Fig. A-2. Availability and uptake of boron during the Spring 1992 (experiment II) hydroponic study growing oilseed rape. The recirculating nutrient solution was sampled prior to nutrient additions.

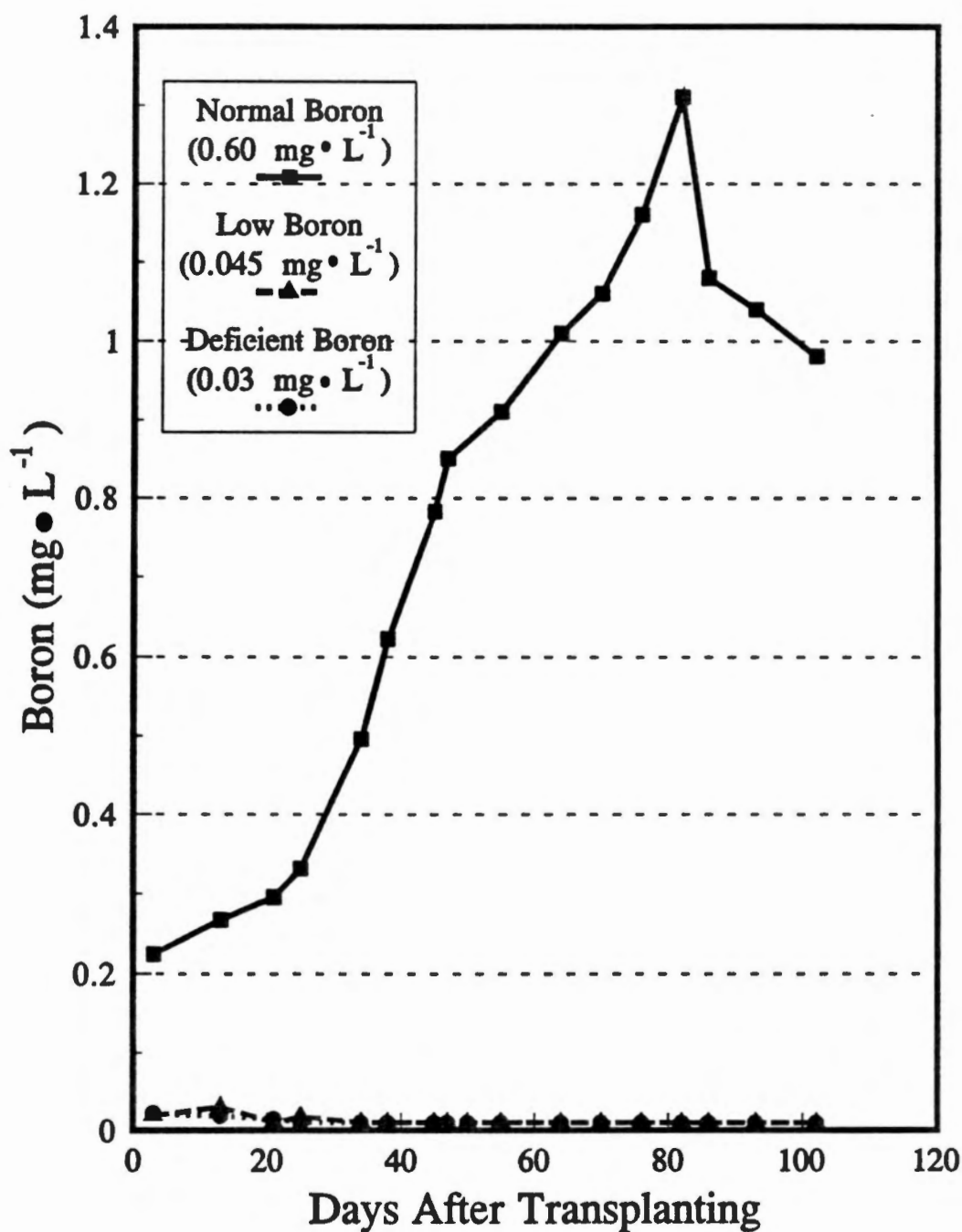


Fig. A-3. Availability and uptake of boron during the Fall 1992 (experiment III) hydroponic study growing oilseed rape. The recirculating nutrient solution was sampled prior to nutrient additions.

APPENDIX B
PROCEDURES

EXPERIMENTAL BORON TREATMENTS

Experiment date	Boron concentration (mg·L ⁻¹)		Minimum dilution factor
	Basic	Treatment ^a	
Fall, 1991 (Exp. I)	0.08	0.04	1:2
	0.40	0.20	1:2
	0.80	0.40	1:2
Spring, 1992 (Exp. II)	0.01 ^b	0.01	1:2
	0.04	0.02	1:2
	0.80	0.40	1:2
Fall, 1992 (Exp. III)	0.04	0.03	3:4
	0.06	0.045	3:4
	0.80	0.60	3:4

^aExperiment I boron treatments were selected based on previous boron deficiency studies of *Brassica* plants. Experiment II boron treatments were selected based on nonsignificance of experiment I treatment results. Experiment III boron treatments were selected to further define the narrow range between deficient and normal boron.

^b0.01 mg·L⁻¹ is the limit of sensitivity for inductively coupled plasma emission spectrometry analysis of boron.

NUTRIENT SOLUTION

Chemicals

KNO_3	FW 101.11 use 202.22 grams	(2M) ^a
$\text{NH}_4\text{H}_2\text{PO}_4$	FW 115.03 use 230.06 grams	(2M)
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	FW 236.16 use 236.16 grams	(1M)
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	FW 246.48 use 246.48 grams	(1M)
KCl	FW 74.56 use 3.728 grams	(.05M)
H_3BO_3	FW 61.84 use 4.638 grams	(.075M)
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	FW 169.01 use 0.338 grams	(.002M)
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	FW 287.55 use 0.575 grams	(.002M)
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	FW 249.71 use 0.125 grams	(.5mM)
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	FW 241.95 use 0.121 grams	(.5mM)
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	FW 278.01 use 5.560 grams	(.02M)
Na_2EDTA	FW 372.24 use 7.414 grams	(.02M)

^aA 4 M concentration was utilized in experiment III to address apparent nitrogen deficiency symptoms.

Procedure

1. Dissolve Na_2EDTA in 400 ml of heated water. Dissolve FeSO_4 in another 400 ml of water. Add Na_2EDTA solution to FeSO_4 solution and bring to 1000 ml volume.
2. Dissolve each of the other chemicals in separate 1000 ml solutions to make working stock solutions.
3. Allow 1 ml of each stock solution per liter of final basic nutrient solution.
4. Adjust final nutrient mixture with NaOH to pH 6.0.

Reference

Epstein, E. 1972. The media of plant nutrition, p. 29-49. In: Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Inc., New York.

PLANT TISSUE ANALYSIS

Procedure

1. Heat plant tissue samples in drying oven (70⁰ C) for 2 to 3 days until at constant weight.
2. Grind the sample plant material thoroughly using a cyclone sample mill (Udy Corporation, Fort Collins, CO) equipped with 1 mm screen (18 mesh), and store desiccated in sterile plastic bags.
3. Weigh out approximately 0.5 g of the ground tissue into a porcelain crucible and record the sample weight. Ash the sample at 500⁰ C for 24 to 36 hours.
4. Dilute the ashed sample with 2N HCL (Select AR) and record the dilution factor. Place diluted sample into plastic test tube for analysis (inductively coupled plasma emission spectrometry).
5. Determine element concentration by using this formula:

$$\mu\text{g}\cdot\text{g}^{-1} \text{ Element} = \frac{\text{ICP value for nutrient} * \text{dilution factor}}{\text{sample tissue weight}}$$

HISTOLOGY OF OILSEED RAPE STEM TRANSVERSE SECTION

Chemicals

Hematoxylin stain.

Safranin-O stain.

Alcian blue 8GX stain.

Isopropyl alcohol (IPA).

Tertiary butyl alcohol (TBA).

AmeriClear[®] histology clearing solvent.

Sodium bicarbonate.

Magnesium sulfate.

Reagent

Scott's water: Add NaHCO_3 (2 g) and MgSO_4 (20 g) to 1 L volumetric flask and make to volume with water.

Procedure

1. Remove cross section tissue sample from FAA fixative and rehydrate by running through IPA series:

35% IPA - 10 min.

20% IPA - 10 min.

10% IPA - 10 min.

Distilled water - 10 min.

2. Place tissue section in hematoxylin stain for 1 hour.
3. Rinse tissue with water and place in Scott's water for 10 minutes.
4. Rinse tissue with water and place in safranin-O stain (0.01%) overnight.

5. Rinse tissue with water and place in alcian blue 8GX stain (0.01%) for 30 minutes.
6. Dehydrate tissue sample by quick rinsing through Johansen's TBA series:
 - 50% - 5 sec.
 - 70% - 5 sec.
 - 85% - 10 sec.
 - 95% - 20 sec.
 - 100% - 30 sec.
7. Rinse tissue in a 1:1 mixture of Johansen's 100% TBA and Americlear[®].
8. Store stained tissue specimen in Americlear[®] until ready to mount on slide.

References

- Graham, E.T. 1991. A quick-mixed aluminum hematoxylin stain. *Biotechnic and Histochemistry* 66:279-291.
- Johansen, D.A. 1935. Dehydration and infiltration. *Science* 82:253-254.

CALCULATION OF NET PHOTOSYNTHESIS

Procedure

$$1. P_n \text{ (g} \cdot \text{cm}^{-2} \cdot \text{sec}^{-1}) = \frac{\Delta C}{A} * \frac{J * 44}{22414} * \frac{273}{T} * \frac{P}{1013}$$

$$\Delta C = \frac{(\quad) \text{ cm}^3}{1,000,000 \text{ cm}^3} \quad (\text{CO}_2 \text{ uptake in PPM})$$

$$A = 6.4516 \text{ cm}^2 \quad (\text{Leaf area})$$

$$J = 8.33 \text{ cm}^3 \cdot \text{sec}^{-1} \quad (\text{Air flow rate})$$

$$T = ^\circ\text{C} + 273.15 \quad (\text{Temperature in } ^\circ\text{K})$$

$$P = 1013 \text{ mb} \quad (\text{Std. atmospheric pressure})$$

2. Conversion to $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$

$$1 \text{ ngCO}_2 \cdot \text{cm}^{-2} \cdot \text{sec}^{-1} = 0.227 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$$

Reference

Catsky, J., J. Janac, and P.G. Jarvis. 1971. General principles of using IRGA for measuring CO₂ exchange rates, p. 161-167. In: Z. Sestak, J. Catsky, and P.G. Jarvis (eds.). Plant photosynthetic production manual of methods. Dr. W. Junk N.V., Publishers, The Hague, Netherlands.

CALCULATION OF STOMATAL CONDUCTANCE

Procedure

$$1. \text{ SC (cm} \cdot \text{sec}^{-1}) = \frac{1}{(1/h - 1) * (s/f) - r_s}$$

$h = ()\%$ (Relative humidity)

$s = 6.4516 \text{ cm}^3$ (Leaf area)

$f = 8.33 \text{ cm}^3 \cdot \text{sec}^{-1}$ (Air flow rate)

$r_s = 0$ (Assumed) (Boundary layer resistance)

2. Conversion to $\text{mmol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$:

$$1 \text{ cm} \cdot \text{sec}^{-1} = 441.688 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$$

Reference

Beadle, C.L., M.M. Ludlow and J.L. Honeysett. 1985. Water relations, p. 50-61. In: J. Coombs, D. O. Hall, S. P. Long and J. M. O. Scurlock (eds.). Techniques in bioproductivity and photosynthesis. Pergamon, New York.

DETERMINATION OF RAPESEED GLUCOSINOLATE CONTENT
(COLORIMETRIC PROCEDURE)

Chemicals

Acetic acid (glacial).

4-Aminoantipyrene.

Ammonium thiocyanate standard solution (10 N).

Barium acetate.

Ferric nitrate.

β -D-Glucose.

Glucose oxidase.

Imidazole.

Lead acetate.

Mercuric chloride.

Myrosinase (prepared from yellow mustard (*Sinapsis alba*) seed.

Nitric acid.

Peroxidase.

Phenol.

Pyridine.

Sephadex (DEAE A-25).

Sodium Azide.

Sodium hydroxide.

Reagents

Barium acetate (0.6 M)/lead acetate (0.6 M) (1:1 v/v). Add 3.07 g of barium acetate to 100 ml volumetric flask and make to volume with water. Add 4.55 g of lead acetate to 100 ml flask and make to volume with water. Combine equal volumes.

Pyridine-acetate (0.5 M): Add pyridine (19.8 mls) and glacial acetic acid (15 mls) to 500 ml volumetric flask and make to volume with water.

Imidazole buffer: Add imidazole (9.82 g), sodium azide (0.29 g), and glacial acetic acid (4.45 g or 4.24 L) to a 1 L volumetric flask and make to volume with water.

Color reagent I: Add glucose oxidase (14.4 mg) and 4-aminoantipyrene (28.8 mg) to 25 ml volumetric flask and make to volume with imidazole buffer. (This solution is stable up to 14 days when stored at 4° C).

Color reagent II: Add peroxidase (2.2 mg) and phenol (144.3 mg) to 100 ml volumetric flask and make to volume with imidazole buffer. (This solution is stable up to 14 days when stored at 4° C).

Glucose oxidase-peroxidase reagent: Add equal volumes of color reagent I and color reagent II together.

Ferric nitrate reagent: Add ferric nitrate (6 g) to a 25 ml volumetric flask and make to volume with 1.5 N nitric acid (5 mls). (This solution must be freshly prepared).

Mercuric chloride (6%): Add mercuric chloride (1.5 g) to a 25 ml volumetric flask and make to volume with 1.5 N nitric acid.

Preparation of Sephadex

1. Place 10 g of DEAE Sephadex A-25 into beaker, add 150 mls of water and gently swirl overnight.
2. Slurry onto a 20 x 400 mm column.
3. Pass 500 mls of 0.5 N NaOH through the column.
4. Wash column with 250 mls of water to remove excess NaOH. Check to ensure the pH has dropped to neutral.
5. Pass 400 mls of 0.5 M pyridine-acetate through the column.
6. Wash with 250 mls of water and slurry into flask for storage.

Procedure

1. Dry seed sample at 45⁰ C for 1 hour.
2. Grind seed sample and place 200 mg into a 16 x 25 culture tube.
3. Place tube into boiling water for 1 minute.
4. Add 1 ml of hot (>90⁰ C) water, mix quickly and continue heating for 3 minutes.
5. Add 0.5 ml of 0.06 M barium acetate-lead acetate solution and mix.
6. Centrifuge at 2000 g for 10 minutes and transfer supernatant to a 5 ml volumetric container.
7. Wash pellet twice with 1.5 mls of water, pool the supernatants, and make to volume.
8. Place 2.5 mls of extract on 0.2 ml DEAE Sephadex A-25 column. (Column consists of 1 ml plastic syringe with porous polyethylene (1.6 mm, 35 μ m pore size) retainer).
9. Wash column (plus blank column) twice with 0.5 ml of 4 M glacial acetic acid and twice with 1 ml of water.
10. Add 0.1 ml of fresh myrosinase solution (3 mg/ml) to each column. Cover columns and let sit overnight.

Determination of total glucosinolate content:

1. Elute column with 1 ml water into a vial.
2. Mix 0.2 ml of eluate, 0.6 ml water, and 0.4 ml fresh glucose oxidase-peroxidase reagent.
3. Let stand for 1 hour, place in 1.5 ml cuvette and record absorbance at 505 nm.
4. Subtract blank reading to obtain total glucosinolate content.

Determination of indole glucosinolate content:

1. Place 1 ml of 0.5 N NaOH into column and let stand for 15 minutes before eluting into a 4 ml vial.

2. Add 1 ml of 0.5 N nitric acid to column and pool eluate in the 4 ml vial.
3. Mix 0.2 ml eluate, 0.6 ml water, and 0.4 ml ferric nitrate reagent.
4. Let sit in dark for 15 minutes, place in 1.5 ml cuvette, and record absorbance at 470 nm.
5. Add 0.020 ml of 6% mercuric chloride to cuvette, mix and read at 470 nm.
6. Subtract difference to obtain indole glucosinolate content.

Determination of glucose standard:

1. To each of 5 vials, add 0.2 ml of a $1.0 \mu\text{mole}\cdot\text{ml}^{-1}$ solution of glucose and half serial dilutions (0.5, 0.25, 0.125, and $0.0625 \mu\text{mole}\cdot\text{ml}^{-1}$ respectively).
2. Add 0.6 ml of water and 0.4 ml of glucose oxidase-peroxidase reagent.
3. Mix and let stand for 1 hour.
4. Record the absorbance at 505 nm.

Determination of thiocyanate ion standard:

1. To each of 5 vials, add 0.2 ml of a $1.0 \mu\text{mole}\cdot\text{ml}^{-1}$ solution of ammonium thiocyanate and half serial dilutions (0.5, 0.25, 0.125, and $0.0625 \mu\text{mole}\cdot\text{ml}^{-1}$ respectively).
2. Add 0.6 ml of water and 0.4 ml of ferric nitrate reagent.
3. Mix and let stand in dark for 15 minutes.
4. Record the absorbance at 470 nm.

Calculation of total glucosinolate content:

$$\Delta A * \frac{1}{m} * 1.1 * \frac{5.0}{2.5} * \frac{1000}{200} = \mu\text{moles per gram of seed}$$

ΔA = Sample absorbance (minus blank).

m = Slope of plot of glucose standards.

Calculation of indole glucosinolate content:

$$\Delta A * \frac{1}{m} * 2.0 * \frac{5.0}{2.5} * \frac{1000}{200} = \mu\text{moles per gram of seed}$$

ΔA = Sample absorbance (minus background).

m = Slope of plot of ammonium thiocyanate standards.

REFERENCE

McGregor, D.I. 1990. Determination of total glucosinolate and total indole glucosinolate content of rapeseed/canola using glucose oxidase to measure glucose and ferric nitrate to measure free thiocyanate ion, D. I. McGregor (ed.). Selected methods for glucosinolate analysis. Proceedings of the oil crops network Brassica sub-network workshop held in Shanghai, China April 21-23, 1990. International Development Research Centre, Ottawa.

DETERMINATION OF RAPESEED GLUCOSINOLATE CONTENT
(GAS CHROMATOGRAPHY METHOD)

Chemicals

Acetone.

Barium acetate.

Benzyl glucosinolate.

Dimethylformamide (DMF).

Ethanol (absolute).

Glacial acetic acid.

Hydrochloric acid.

Lead acetate.

Methanol.

1-Methyl imidazole.

N,O-bis(trimethylsilyl)tetrafluoroacetamide (BSTFA).

Pyridine.

Sephadex (DEAE A-25).

Sinigrin (allyl glucosinolate).

Sodium acetate.

Sodium Hydroxide.

Sulfatase (type H-1).

Trimethylsilylchlorosilane (TMCS).

Reagents

Barium lead acetate (0.3 M): Add barium acetate (7.66 g), lead acetate (11.38 g), and glacial acetic acid (0.29 ml) to a 100 ml volumetric flask and make to volume with water.

Pyridine acetate (0.02 M): Add pyridine (2 mls) and glacial acetic acid (1.5 mls) to a 1 L volumetric flask and make to volume with water.

Sodium acetate (1 M): Add sodium acetate (8.2 g) to a 100 ml volumetric flask and make to volume with water.

Purification of Sulfatase

1. Place 70 mg of sulfatase into a centrifuge tube.
2. Add 3 mls of water and swirl gently (minimizing foam formation) to dissolve sulfatase.
3. Add 3 mls of absolute ethanol and mix.
4. Centrifuge at 2000 *g* for 20 minutes.
5. Decant supernatant into second test tube and discard precipitate.
6. Add 9 mls of absolute ethanol to the supernatant, mix and centrifuge at 2000 *g* for 15 minutes.
7. Discard the supernatant and dissolve the precipitate in 2.5 mls of water.
8. Store frozen (-40° C) and thaw just before use.

Procedure

1. Dry seed sample at 45° C for 1 hour.
2. Grind seed sample and place 200 mg into a 4 ml vial .
3. Add (in this order): 2 mls of methanol, 1 ml of 1 mM benzyl glucosinolate (internal standard), and 0.1 ml of 0.3 M barium lead acetate.
4. Shake vial (approximately 200 strokes per minute) for 1 hour.
5. Centrifuge at 2000 *g* for 10 minutes.
6. Place 1.5 mls of supernatant onto a 0.2 ml DEAE Sephadex A-25 column. (Column consists of 1 ml plastic syringe with porous polyethylene (1.6 mm, 35 μ m pore size) retainer).

7. Wash column with: 1.8 mls of 67 % methanol, 1.8 mls of water, and 1.0 ml of 0.02 M pyridine acetate.
8. Add 0.05 ml of purified sulfatase onto column, cap and let stand overnight at room temperature.
9. Elute column twice with 0.45 ml of 60% methanol into a 1ml autosampler vial. (Column may be regenerated by washing with 1 ml of 1 M sodium acetate, 1 ml of 0.5 M NaOH, 1 ml of 0.5 M HCL, and 4 mls of water.
10. Place vial in block heater (60⁰ C), and take to dryness by passing nitrogen over the sample.
11. Prepare derivatization mixture immediately before use: acetone, DMF, BSTFA, TMCS, and 1-methylimidazole (40:20:20:2:1).
12. Add 400 μ l of derivatization mixture to dried sample vial and cap vial immediately.
13. Let stand 15 minutes before reading.

Chromatographic conditions for capillary column:

Carrier gas (helium) (ml·min ⁻¹)	optimized
Air (ml·min ⁻¹)	optimized
Hydrogen (ml·min ⁻¹)	optimized
Detector attenuation	0
Injector temperature (°C)	300
Detector temperature (°C)	300
Initial column temperature (°C)	245
Initial time (minute)	1
Program rate (°C·min ⁻¹)	13
Final temperature (°C)	281
Final time (minute)	8.2

Calculation of individual glucosinolate content:

$$\frac{\text{Area unknown}}{\text{Area benzyl}} * \text{RF} * 0.86 * \frac{1000}{200} = \mu\text{moles per gram of seed}$$

Benzyl glucosinolate = internal standard.

RF = response factor.

Table of Response Factors:

Glucosinolate	RF
Allyl	1.16
3-Butenyl	1.12
4-Pentenyl	1.07
2-Hydroxy-3-butenyl	1.00
2-Hydroxy-4-pentenyl	0.97
Benzyl	1.00
3-Indolylmethyl	0.94
4-Hydroxy-3-indolylmethyl	0.85

REFERENCE

Raney, J.P. and D.I. McGregor. 1990. Determination of glucosinolate content by gas liquid chromatography of trimethylsilyl derivatives of desulphated glucosinolates, D. I. McGregor (ed.). Selected methods for glucosinolate analysis. Proceedings of the oil crops network Brassica sub-network workshop held in Shanghai, China April 21-23, 1990. International Development Research Centre, Ottawa.

VITA

Larry Stone Kennedy was born on August 26, 1954 in Louisville, Mississippi. He graduated from Louisville High School in May 1972. In December 1976, he received a Bachelor of Science degree in Horticulture from Mississippi State University. Returning to MSU in August 1987, he earned a Master of Science degree in Horticulture, graduating in May 1989. In January 1990, he accepted a graduate research assistantship at The University of Tennessee at Knoxville. The author is a member of Gamma Sigma Delta, American Society for Horticultural Science, and American Society of Plant Physiologists.