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I am submitting herewith a thesis written by Barbara Kocourkova entitled "Effect of Water Stress on Boron Partitioning and Glucosinolate Composition in Two Hydroponically Grown Canola Cultivars". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with major in Plant and Soil Science.

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EFFECT OF WATER STRESS ON BORON PARTITIONING AND GLUCOSINOLATE COMPOSITION IN TWO HYDROPONICALLY GROWN CANOLA CULTIVARS

A Thesis

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Barbara Kocourkova

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To my father

Ing. Jiri Kocourek

(April 4, 1939 - April 25, 1997)

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ABSTRACT

High glucosinolate content in brassica meal is a limiting factor in utilization of rapeseed as livestock feed. In recent years canola cultivars of rapeseed with decreased glucosinolate content have been developed. However, environmental and nutritional factors are also believed to influence glucosinolate content. Two experiments were conducted to determine the relationships among water stress, boron nutrition, and glucosinolate content in cultivars of canola. Two canola cultivars ('Cyclone' and 'American A-112') were grown in a continuously recirculating hydroponic system with modified 'Hoagland solutions'. Boron (B) nutrition varied between experiments. During the Spring 1995 experiment (I) B concentration was 0.6 $mg \cdot l^{-1}$, and during the Spring 1996 experiment (II) 0.3 $mg \cdot l^{-1}$. Water stress was induced gradually (2% per day using polyethylene glycol 8000) starting when plants were four weeks old. Osmotic potential was maintained at -0.1 MPa (high stress treatment), -0.085 MPa (medium stress treatment), and -0.05 MPa (low stress treatment, without PEG). Treatments were arranged in a randomized incomplete block design, with three blocks, four replications, two cultivars, and three treatments. Upper leaves (#15 and up) were collected and analyzed by inductively coupled plasma emission spectrometry for boron content. Total and indole glucosinolate content of seeds were determined colorimeterically and individual glucosinolates were determined by gas chromatography.

For both cultivars, leaf B content decreased as water stress level increased, however, in experiment I, this change was not statistically significant. In experiment II with only

0.3 $mg \cdot l^{-1}$ B available in the solution, leaf B content decreased as in the experiment I and was statistically significant ($P<.001$). The difference between the two stressed treatments was not significant, suggesting that water stress greater then -0.085 MPa does not further decrease B uptake.

Water stress reduced leaf tissue B to about 50 $\mu g \cdot g^{-1}$ dry weight in experiment I but did not alter glucosinolate concentration. In experiment II, total glucosinolate concentration of seeds in "Cyclone" significantly increased ($P<.01$) with medium stress (-.085MPa), but water stress had no significant effect on glucosinolate content of "American-A112" seeds. Linear treatment contrasts showed that further increasing water stress from medium (-0.085 MPa) to high (-0.1 MPa) did not further increase glucosinolate content of seeds. Seed indole glucosinolate concentration was not significantly different among treatments or between cultivars in either experiment.

Both dry weight and yield were reduced as stress level increased ($P<.001$) for both cultivars in both experiments. However, severely stressed plants of "Cyclone" showed greater morphological variability than plants of "American-A112", producing malformed plants with few seeds.

Water stress decreased boron uptake in both cultivars. "Cyclone" was more susceptible to water stress, indicating genetic differences between the cultivars.

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INTRODUCTION

Rapeseed is the third most important source of vegetable oil in the world, after soybean and palm oil. During the past twenty years, it has passed peanut, cottonseed, and most recently, sunflower, in worldwide production (Sovero, 1993). This is mostly due to plant breeding work in Canada and Europe which greatly reduced the levels of two anti-nutritional compounds, erucic acid in the oil and glucosinolates in the meal, creating a high-value oil and protein crop known as canola (in Canada and United States) or double low rapeseed (in Europe).

In the United States the rapeseed marketing chain is not well developed. However, there is a trend toward increasing production, motivated by the granting of GRAS (generally recognized as safe) status to canola oil in 1985 by the US FDA, to meet both domestic and foreign demands (Sovero, 1993).

Although rape is primarily grown for its edible oil, the meal that remains after oil extraction has value as a source of protein for the livestock feed industry. However, the utilization of the meal can be restricted by the presence of high glucosinolates, the breakdown products of which have harmful toxicological effects when fed to animals. Glucosinolate content is an important quality attribute of rapeseed and it may be affected by genetic, cultural, and environmental components. With the introduction of improved double-low varieties glucosinolate levels have decreased from the high levels of 60-80 μmol·g to <20 μmol·g seed. But great variability exists in seed glucosinolates between and within plants, and there are strong genotype by environment interactions. Proper mineral

nutrition, especially nitrogen, sulfur, phosphorus and potassium availability, is crucial to normal rapeseed development. Among micronutrients, boron (B) nutrition is of particular concern, since the range between deficiency and toxicity is very narrow (Holmes, 1990). Seedless plants were observed in fields deficient in B and sulfur (Nuttall et al., 1987). The relationship among B availability and plant morphology and development has been investigated by Kennedy (1994). He found that B deficient plants produced no seeds or few seeds with glucosinolate concentrations above the acceptable canola quality standard. Boron uptake is mostly passive with movement almost entirely confined to the transpiration stream. Therefore, the availability of B is influenced by water regime, temperature and light intensity. It is known that drought at particular stages of growth increases glucosinolate concentrations (Jensen et al., 1996; Milford et al., 1991; Mailer et al., 1987). However, the physiological mechanisms are not fully understood and variations in seed glucosinolates cannot be confidently predicted.

The objectives of this study were to determine the effect of water stress on B uptake and partitioning in two cultivars of rapeseed and to correlate B availability with glucosinolate concentration of seeds.

CHAPTER I

LITERATURE REVIEW

TRANSFORMATION OF RAPESEED TO CANOLA

Rapeseed is the third most important source of vegetable oil in the world, after soybean and palm oil. During the past twenty years, it has passed peanut, cottonseed, and most recently, sunflower, in worldwide production (Sovero, 1993). This is due to plant breeding efforts, initiated in the 1950's in Canada, which resulted in reduced levels of two antinutritional compounds, erucic acid in the oil and glucosinolates in the meal. This work created a new, high - quality oil and protein crop known as canola in the United states and Canada and double low rapeseed in Europe. Canola refers to any rapeseed with less than 2% erucic acid in the oil and less than 20 μmol of the four major aliphatic glucosinolates in a gram of air dry defatted solids. It is a trademark of the Canadian Canola Association. Double low rapeseed, as defined by European community standards, has less than 15$\mu mol \cdot g^{-1}$ of total glucosinolates (Sovero, 1993).

Two species are grown as a rapeseed and used for canola breeding - *Brassica rapa* (turnip rape) and *B. napus* (rape). In some parts of India and China small amounts of *B.juncea* (Indian mustard) and *Sinapis alba* (white mustard) are also grown for rapeseed production. Both *B. rapa* and *B. napus* are either annual or biennial. Intensive breeding work started after World War II in both Europe and Canada. The initial work was supported in order to promote vegetable oils. The breeding was mainly focused on higher

yields and reduction of erucic acid. Prior to these breeding efforts erucic acid was found in far higher concentrations in rape oil than in any other vegetable oils. Experiments conducted during the 1960s on laboratory animals showed an abnormal number of heart lesions caused by erucic acid (Ward, 1985). Such an effect was never observed in humans, and the nutritional danger may have been low. Most recent studies suggest that erucic acid was more of a threat to rats than to men (Shahidi, 1990). However, it was decided that erucic acid should be eliminated from rapeseed oil. Soon successful cultivars were produced, especially in Canada. Low erucic acid cultivars are now defined as; a cultivar with seed in which erucic acid constitutes less than 2% of the measured fatty acids. Such a variety is referred to as a "single low" or LEAR rapeseed. While the presence of erucic acid compromised the nutritional value of oil, goitrogenic glucosinolates limited the possible utilization of defatted meal as a part of livestock rations. A standard of 30$\mu mol \cdot g^{-1}$ was reached in the early 1980s introducing "double low" or canola cultivars. This, however, changed recently. In 1995 a Canadian canola standard was established at 20 $\mu mol \cdot g^{-1}$ and European "double low" standard was set at 15$\mu mol \cdot g^{-1}$ of glucosinolates in meal which brings both definitions closer together (Sovero, 1993). Now canola has to compete with other edible oils, some of which have achieved considerable success.

Potential for expansion and need for canola comes from recent research results which claim that canola oil is an almost perfectly balanced mixture of fatty acids for human nutrition and health. (Ackman, 1990). Recent cardiovascular and biochemical studies have established with four major findings all favorable to canola oil:

1) oleic acid is almost as effective in reducing cardiovascular risk as is linoleic acid

2) linoleic acid , the essential fatty acid found in canola oil (20%) which provides needed daily intake in human nutrition
3) the ideal proportion of linolenic acid to linoleic acid is 1:2
4) content of palmitic acid, the important cardiovascular risk factor, is found in very low concentration relative to most currently available vegetable oils (Ackman, 1990).

All these findings suggest an increase in canola demand in the US. Contemporary society started to pay more attention to canola especially after canola oil was accorded GRAS (generally recognized as safe) status. The estimated domestic need for canola oil in 1995 was one million tons (Sovero, 1993). The domestic production of this much oil would require 1 million to 2 million hectares. Increased interests in canola production has lead to a search for new suitable production regions. Environmental factors play a major role in stable quality high yields of rapeseed. Rapeseed grows best in mild maritime climates. England and The Netherlands are the countries with the highest yields. The optimum range in growth temperatures is between 10° and 30°C with the optimum around 20°C. It is a crop of temperate regions with a cold period required by winter types for vernalization. Heat during blooming time may cause significant losses of yield, oil, and seed quality (Vasak et al., 1991).

Under Northern US environmental conditions canola can be grown as a spring annual, since biennial cultivars cannot survive the extreme winters. The central US provides conditions for biennial cultivars similar to those of Europe. In the Southern portions of the United States canola can be produced as a winter to spring annual, using cultivars with a range of vernalization requirements and cold tolerance. In the southeastern US rainfall is

normally adequate to produce canola. However, the short time water deficits, which may occur during late spring and early summer in the Southeast, could potentially cause yield decreases and higher glucosinolate concentrations. Ward et al. (1985) report that drought stress can reduce plant dry weight by 20%. Therefore, planting dates and production management are critical.

Experiments on the adaptation, management and productivity of winter rapeseed in Tennessee concluded that rapeseed is a suitable crop for this region (Graves, 1991; Latka and Plouy, 1988). Several new cultivars have been developed that have competitive yields in the Southeast. However, inconsistent oil quality has been reported in the region.

Production of canola requires proper mineral nutrition. Sufficient quantities of plant nutrients such as nitrogen, phosphorus, potassium and sulfur are essential. Among micronutrients, boron nutrition is of particular concern, since the range between deficiency and toxicity is very narrow (Holmes, 1980). Boron availability is crucial to the normal ontogeny of oilseed rape. Boron deficiency results in poor seed development, impaired germination, and delayed growth (Gupta, 1985; Murray, 1984). However, the precise range of boron availability for high yields of canola quality oil is not known. Kennedy (1994) investigated the effects of boron deficiency on glucosinolate content of canola. He found that the total glucosinolate concentration of boron deficient plants exceeded canola quality standards and that boron deficiency had a detrimental effect on rapeseed development and yield.

OVERVIEW OF BORON ROLE IN PLANT NUTRITION

Boron (B) is a micronutrient with very low concentrations required by plants. These concentrations are difficult to define since the range between deficiency and toxicity is very narrow. The role of B in plant metabolism is still perhaps the least understood of all the plant nutrients. Boron is unique among essential nutrients since it is not required by microorganisms or animals, and requirements within the plant kingdom differ markedly. Among higher plants, dicots have the highest requirements, especially the *Cruciferae* and *Leguminosae* members (Pilbeam et al., 1983).

H_3BO_3 is the predominant form of B found in agricultural soils. Less than 5% of soil B is available. It is easily leached and less available under drought conditions. The translocation and uptake of B is not very well understood but it seems to be passive most of the time. Under high temperature some active uptake can take place which indicates a direct effect of transpiration on B uptake (Shelp, 1993). Boron is generally thought to undergo limited redistribution after primary translocation in the xylem to transpiring tissues (Shelp, 1988). However, there is evidence from different plant species suggesting that: (1) B is sufficiently mobile in the phloem to support the demands of developing sinks during growth with adequate B; and (2) B partitioning is altered in response to continuous B starvation or to the removal of B after a period of adequate supply (Shelp et al., 1992). They also suggested that the redistribution of B in broccoli is affected by genetic variation among cultivars.

Several possible functions of B in plants are recognized. B is required for cell division and cell elongation, pollen germination and pollen tube formation (Lewis, 1980).

It affects flower morphology and nectar quality. Boron's major metabolic function in plants seems to be a stabilization of membranes (structural and biochemical), but it is not clear whether the effect is direct or indirect. Pilbeam and Kikby (1983) hypothesized that membrane behavior is influenced by compounds which are themselves affected by B nutrition. Auxins and phenolics are two groups which might play such a role. The phenolic acids are produced as a result of carbohydrate metabolism. The alternative shunt pathway of glucose degradation involves B. Boron is combined with 6-phosphogluconate, the first intermediate in this pathway, to form a 6-phosphogluconate-borate complex. This cannot be further metabolized and the penthose shunt pathway is thereby inhibited. In B deficiency, its operation becomes prominent, and is often accompanied by excessive synthesis of phenolic acids (Epstein, 1972). B has a positive effect on membrane proton transport and membrane potential.

Gupta (1979) described B deficiency symptoms such as slowdown of root extension. This may be a secondary effect of B role in the metabolism, transport, or action of auxin-type hormones. It has been suggested that a continuous supply of B is not essential for all elongation but is required for maintenance of meristematic activity. Deficiency of B results in browning of plant tissues, related to the accumulation of polyphenolic compounds, it also significantly inhibits protein synthesis. B deficiency symptoms are related to reduced activity in the pyrimidine biosynthetic pathway and are not related to a reduction in nucleic acid synthesis. Structural changes in the plant resulting in greater susceptibility are causes of B deficiency.

Shelp et al. (1992) observed inflorescense deformation with occasional formation of brown beads of broccoli plants grown without B. They also noted external signs of stem corkiness and leaf midrib cracking, together with brown discoloration, necrosis, or gap and hollow formation in the central pith.

High levels of B were responsible for reduced photosynthesis in broccoli plants (related to a loss of chlorophyll) but stimulated head development possible due to the high requirement of B for meristematic cell division (Petracek et al., 1987).

Bible et al. (1981) observed that induced B deficiency resulted in increased yield of thiocyanate ion and altered distribution of reducing sugars in radishes (*Raphanus sativa* L.). There has been indirect evidence that B deficiency influenced translocation of glucosinolates and/or their precursors in turnip (Bible et al., 1882). The same phenomenon was observed by Kennedy (1994) in rapeseed plants. This evidence suggested that B nutrition is an important factor influencing glucosinolate content in crucifers.

GLUCOSINOLATES

All members of the Brassicaceae order contain glucosinolates, the hydrophilic, nonvolatile thioglugosides, responsible for a distinctive flavor and odor of many cruciferous crops. Glucosinolates are relatively nontoxic, but their hydrolyses products, formed by myrosinase (thioglucosiase), can produce an organic isothiocyanate (Dawson et al.,1993). These products are responsible of low feed palatability of rapeseed meal in sheep, cattle and pigs. They adversely affect animal growth, reproduction, and performance and cause problems of goitrogenicity in livestock and liver haemorrhage and

abnormalities in internal organs of animals (Mailer and Cornish, 1987; Matthaus and Fiebig, 1996). Myrosinase is stored separately in the cell and hydrolyses take place only when the plant or seed is crushed. Myrosinase also occurs in several microbial species inhabiting the gastrointestinal tract of animals (McDanell et al., 1988).

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

Glucosinolates are sulfur containing secondary metabolites that are derived from amino acids. Over 100 glucosinolates have been identified and all but one possess one structure - a common glycoside moiety - and differ only in the nature of the side chain. In *Brassica* , the glucosinolates can be divided into three major classes: (1) those possessing the side chains, which may have aliphatic alkenyl or hydroxyalkenyl groups; (2) those that have side chains which contain an indolyl group, and (3) those that possess an aralkyl side chain. Glucosinolates possessing aliphatic, indolyl and aralkyl side chains are derived from methionine, tryptophan and phenylalanine respectively (Mithen, 1992). They are all synthesized by a common pathway, with indole glucosinolate being the exception. 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 general pathway for glucosinolate biosynthesis is well understood, factors that control production of the individual glucosinolates are poorly defined (Poulton and Moller, 1993).

Glucosinolates and their breakdown products have both desirable and undesirable

effects on foods and animal feeds. They can reduce the palatability of feeds and impart tanins to food. Although many studies have demonstrated that glucosinolates have toxic effects, few studies have sought to elucidate their role in disease and pests resistance. Mithen et al.(1987) suggested that high levels of alkenyl glucosinolates in the wild form of *Brassica oleracea* were responsible for the partial resistance of these taxa to *Peronospora parasitica* and *Leptosphoeria maculans*. In the past decade there has been increasing evidence that indole glucosinolates and their hydrolyses products have the ability to alter the chemical carcinogenesis (McDanell et al., 1988; Beecher, 1994; Fenwick, 1983).

Glucosinolates in plant tissue are accompanied by the enzyme myrosinase or thioglucosidase. When plant tissue is crushed under moisture conditions, myrosinase hydrolyses glucosinolates yielding D-glucose and an aglucone. Depending on the pH value of the medium, the structure of the glucosinolate, and the presence of compounds which modify the action of the enzyme, other products can be formed (Fenwick at al., 1983). The aglucone, under neutral conditions, gives rise to sulfate and by a Lossen-type rearrangement, an isocyanate. Under weakly acidic conditions or in the presence of ferrous ion, a nitrile and elemental sulfur are formed (Matthaus and Fiebig, 1996). Isocyanates possessing a *B*-hydroxyl spontaneously cyclize to form substituted oxazolidine-2-thiones. These later products have been shown to be the causative agents in brassica seed goitr (McDanell et al., 1988). The isocyanates formed from the indole glucosinolates and p-hydroxybenzylglucosinolates are unstable in neutral or alkaline conditions and give rise to thiocyanate ion. At lower pH, enzymatic fission of 3-indolylmethylglucosinolate leads to the formation of the growth-promoting compound, 3-indolylacetonitrile (Underhill,

1980). It has been suggested that an important function of myrosinase is to generate this auxin and subsequently indole-3-acetic acid (Fenwick et al., 1983). Many investigators have suggested that one of the compounds exerting an activating effect on myrosinase is ascorbic acid. Ohsuru and Hata (1979) have found that ascorbic acid is not itself involved in catalysis but changes the conformation of the active site of the enzyme. They also showed that ascorbic acid reduces the optimum temperature of myrosinase.

Glucosinolate concentration within a plant varies among species and plant parts, and within plant tissues. As a plant matures glucosinolates undergo quantitative, as well as qualitative changes. These may be influenced by genetic, cultural or environmental factors (Fenwick et al., 1983). It has been reported that water stress during the growing period causes an increase in glucosinolate concentration in the seeds of rape (Mailer, 1987; Jensen et al., 1996). Mineral nutrition, particularly sulfur and nitrogen, of *Brassicaceae plants* has an effect on glucosinolate content (Milford et al., 1991; Fenwick et al., 1983). Since glucosinolates contain both nitrogen and sulfur one might expect that deficiencies of these nutrients would reduce glucosinolate production. Low sulfur conditions do cause a decrease in glucosinolate accumulation and other sulfur - containing secondary compounds in a number of vegetable crops, including cabbage, radish, white mustard, garlic and onion, *Brassica nigra* and *Brassica napus*. In contrast to S deficiency, a deficiency of N has been shown to either increase glucosinolate concentration in plants or have no effect on it. This might be due to the fact that glucosinolates contain a much smaller proportion of the total nitrogen in the plant than they do of the total S (Gershenzon, 1984). Shelp et al., (1993) observed that B deficiency increased the

proportion of indolyl glucosinolates in broccoli florets. Results of the study conducted by Kennedy (1994) suggested that boron deficiency increases the seed glucosinolate concentration of oilseed rape.

WATER STRESS

The response and adaptation of a species to water stress are critical for its success in any environmental niche. Numerous physiological responses vary with the severity of the stress. As stress increases, some changes intensify and additional processes become affected in accordance to their relative sensitivity to stress. Reproductive organs and seeds of crops are constructed from resources either recently acquired or previously stored in the vegetative parts. Therefore, any environmental stress that affects vegetative parts may affect productivity, yield and seed composition.

Jensen et al., (1996) observed that drought affected oil, protein, and glucosinolate content of rape seeds. He also suggested that under loss of turgor secondary metabolites accumulate. The leaves and pods loose turgor when water potential (ψ) falls below -1.4 MPa for prolonged periods of time. Stroeher et al. (1995) confirmed in *Brassica napus* that this happens as a result of turgor-responsive gene expression. The gene probably expresses osmoprotective compounds and may thus be responsible for the production of glucosinolates. The time of stress occurrence and the stage of development and plant morphology also plays a major role. Bouchereau et al. (1996) examined the consequence of drought during growth on the main attributes of the biochemical composition of rape seeds. For all the traits related to seed quality in oilseed rape, the flowering period

constituted a water sensitive stage. Major effects of draught were observed in the accumulation of secondary metabolites and changes in fatty acid composition. The glucosinolate metabolism response to water stress was an increased accumulation in the seed, especially if the draught occurred during the early vegetative development.

Water stress reduces plant absorption of inorganic nutrients. A slowing of the transpiration stream alone often reduces uptake by roots as well as movement to the shoots. On the other hand several studies indicate that possible nutritional deficiencies may not be aggravated or induced by water stress in many cases. Bradford et al. (1982) observed that N uptake by *Phaseolus vulgaris* placed in polyethylene glycol solution was inhibited. But in spite of the lower uptake, more nitrogen was accumulated in the stressed tissue as inorganic ions and as soluble nitrogenous compounds. On the other hand, much less N was incorporated into proteins in the stressed plants. These results indicated that stress reduces the demand for nitrogen below the level of supply so that the soluble nitrogen pool was expanded. It is probable that such a model can be implemented for other nutrients such as B. Jensen et al. (1996) hypothesized that drought during vegetative growth restricts primary metabolism and growth but not secondary metabolism and the production of glucosinolate precursors which are utilized later. This theory would explain the accumulation of secondary metabolites in seeds.

CHAPTER II

EFFECT OF WATER STRESS ON BORON PARTITIONING AND GLUCOSINOLATE COMPOSITION IN TWO HYDROPONICALLY GROWN CANOLA CULTIVARS

ABSTRACT

Two experiments were conducted to determine the relationships among water stress, boron nutrition, and glucosinolate content in cultivars of canola. Two canola cultivars ('Cyclone' and 'American-A112') were grown in a continuously recirculating hydroponic system with modified 'Hoagland solutions'. Boron (B) nutrition varied between experiments. During the Spring 1995 experiment (I) B concentration was 0.6 $mg \cdot l^{-1}$, and during the Spring 1996 experiment (II) 0.3 $mg \cdot l^{-1}$. Water stress was induced gradually (2% per day using polyethylene glycol 8000) starting when plants were four weeks old. Osmotic potential was maintained at -0.1 MPa (high stress treatment), -0.085 MPa (medium stress treatment), and -0.05 MPa (low stress treatment, without PEG). Treatments were arranged in a randomized incomplete block design, with three blocks, four replications, two cultivars, and three treatments. Upper leaves (#15 and up) were collected and analyzed by inductively coupled plasma emission spectrometry for boron content. Total and indole glucosinolate content of seeds were determined colorimetrically and individual glucosinolates were determined by gas chromatography.

For both cultivars, leaf B content decreased as water stress level increased, however, in experiment I, this change was not statistically significant. In experiment II with only 0.3

$mg \cdot l^{-1}$ B available in the solution, leaf B content decreased with the same pattern as in the experiment I and was statistically significant ($P<.001$). The difference between the two stressed treatments was not significant, suggesting that water stress greater then -0.085 MPa does not further decrease B uptake.

Gas chromatography revealed that progoitrin was the predominant glucosinolate in both experiments. In experiment 1, 0.6 $mg \cdot l^{-1}$ of B in the nutrient solution provided 50 $\mu g.g^{-1}$ dry weight B in the leaf under the water stress, This amount was sufficient enough to not alter the concentration of progoitrin in either cultivar. Total glucosinolate content was not affected by water stress and 0.6 $mg \cdot l^{-1}$ B concentration in the nutrient solution. The combination of water stress of -0.085 MPa and 0.3 $mg \cdot l^{-1}$ of B available from the nutrient solution did significantly increase the glucosinolate content of "Cyclone" seeds but had no significant effect on the seed glucosinolate content of "American-A112". Seed indole glucosinolate concentrationwas not significantly different among treatments or between cultivars in either experiment.

Both dry weight and yield were reduced as stress level increased ($P<.001$) for both cultivars in both experiments. However, severely stressed plants of "Cyclone" showed greater morphological variability than highly stressed plants of "American-A112", producing malformed plants with few seeds.

Water stress decreased boron uptake in both cultivars. "Cyclone" was more susceptible to water stress, indicating genetic differences between the cultivars.

INTRODUCTION

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Although rape is primarily grown for its edible oil, the meal that remains after oil extraction has value as a source of protein for the livestock feed industry. However, the utilization of the meal can be restricted by the presence of the high content of glucosinolates, the breakdown products of which have harmful toxicological effects when fed to animals. Glucosinolate content is an important quality attribute of rapeseed and it may be affected by genetic, cultural, and environmental conditions. With the introduction of improved double-low varieties glucosinolate levels have decreased from the high levels of 60-80 $\mu mol \cdot g^{-1}$ to <20 $\mu mol \cdot g^{-1}$ seed. But great variability exists in seed glucosinolates between and within plants, and there are strong genotype by environment interactions. Proper mineral nutrition, especially nitrogen, sulfur, phosphorus and potassium availability, is crucial to normal rapeseed development. Among micronutrients, boron (B) nutrition is of particular concern, since the range between deficiency and toxicity is very narrow (Holmes, 1990). Seedless plants were observed in fields deficient in B and sulfur (Nuttall et al., 1987). The relationship among B availability and plant morphology and

development has been investigated by Kennedy (1994). He found that B deficient plants produced no seeds or few seeds with glucosinolate concentrations above the acceptable canola quality standard. Boron uptake is mostly passive with movement almost entirely confined to the transpiration stream. Therefore, the availability of B is influenced by water regime, temperature and light intensity. It is known that drought at particular stages of growth increases glucosinolate concentrations (Jensen et al., 1996; Milford et al., 1991; Mailer et al., 1987) . However, the physiological mechanisms are not fully understood and variations in seed glucosinolates cannot confidently be predicted.

The objectives of this study were to determine the effect of water stress on B uptake and partitioning in two cultivars of rapeseed and to correlate B availability with glucosinolate concentration of seeds.

MATERIALS AND METHODS

Plant Material

Two genetically diverse canola cultivars "American-A112" and "Cyclone" were germinated in plastic trays containing hydrated perlite. Trays were placed in the greenhouse and watered with 10% basic Hoagland solution without boron (B). After two weeks the plants were transplanted into 11L plastic pots filled with perlite. The pots were than continuously supplied with nutrient solution. Temperatures in the greenhouse were: experiment I low 18.2±1.83°C and high 31.1±2.86°C; experiment II low 14.58±2.57°C and high 30.3±3.13°C. The relative humidity during experiment I was: low 51.5±8.74%

and high 84.7±6.62% and during experiment II: low 40.36±10.24% and high 72.2±13.02%.

Experimental design

The hydroponic system designed by Kennedy (1994) was utilized. The system consisted of 63 11L plastic pots arranged on three benches. Perlite was used as a root supporting medium. Each treatment solution was maintained in a separate container and continuously recirculated by a centrifugal pump. Excess solution drained through pots into return lines and returned via gravity flow to the reservoirs. A black and white plastic cover was placed around the base of each plant to prevent algae growth on the perlite. The experimental design was arranged as a randomized incomplete block design with three blocks, four replications per block, three treatments and two cultivars. The standard analyses of variance procedure (GLM of SAS, 1989) was used to determine significant effects based on linear contrasts.

Nutrient solutions

Modified Hoagland nutrient solutions were prepared with 0.6 $mg \cdot l^{-1}$ and 0.3 $mg \cdot l^{-1}$ of B for experiment I and experiment II, respectively. Standard basic solutions were prepared according to Epstein (1972) and Kennedy (1994). Previous work suggested that 0.6 $mg \cdot l^{-1}$ B is the high range for B deficiency. Since we did not detected symptoms of low B availability on glucosinolate content in experiment I, we lowered the concentration of B by 50% in experiment II. Dilutions of nutrient solutions were used to regulate nutrient

availability according to plant development. Initially, 25% strength of nutrient solution was applied and remained for about three weeks. Then 50 % of strength was applied until the flowering period. During the pot filling stage 100% was used in experiment I and 75% strength in experiment II. The concentrations were then lowered as plants matured slowly to 25%.. Samples were taken weekly before and after addition of a new solution and analyzed by inductively coupled plasma (ICP) atomic emission spectrometry (Model 61 Thermo Jarrell Ash, Franklin,MA) for complete nutrient concentrations (Tables 13-18)* . Plants were allowed to desiccate naturally before seed pods were harvested

Water stress treatments

Polyethylene glycol 8000 (PEG) was used as a water stress inducing agent. Three different stress levels were maintained with osmotic potentials of 0.050 MPa (low stress without PEG), -0.085 MPa (medium stress), or -0.010 MPa (high stress). Water stress was induced gradually (2% per day) starting when plants were four weeks old. Samples were taken frequently and water potential determined on a 5500 Vapor Pressure Osmometer (WESCOR,Inc.;Logan,UT). Modifications in PEG concentrations were made based on actual osmotic potential measurements (Tables 19&20).

Leaf tissue analyses

Upper canopy (nodes #15 and up) were collected, dried for 48h at 70°C and ground with a cyclone sample mill with a 1mm screen. The samples (0.5g) were weighed into

* All tables may be found in the Appendix A.

porcelain crucibles and ashed (24h at 500°C).Samples were then transferred to plastic culture tubes and dissolved in 2N HCl. Boron and other nutrients were determined by ICP.

Dry weight

Upper leaves (nodes #15 and up), lower leaves (nodes #1-14), main stems, side branches, main inflorescence, side inflorescences, pods and seeds from main and side inflorescences were collected separately. All plant parts, except seeds, were dried for 48h at 70°C and dry weight was recorded. Seeds were dried for about 12-24h at 40°C until the moisture content dropped to 8%.

Glucosinolate determination

The colorimetric method was used in experiment I to determine total and indole glucosinolate content of the seeds. The procedure described by McGregor (1990) and modified by Kennedy (1994) was used. Samples were analyzed on a Shimadzu UV-260 Spectrometer (Shimadzu Corporation, Kyoto, Japan). Individual glucosinolates in both experiments were determined by gas chromatography (Raney and McGregor, 1990). A HP 5890 Series II Gas Chromatograph (Hewlett-Packard Company, Avondale, Pa) equipped with a flame ionization detector and a 30m (0.25mm i.d.) Duraband-1 methyl silicone capillary column (J&W Scientific, Folson, CA) was used to separate and quantify the TMS ethers. Benzyl glucosinolate was used as an internal standard.

RESULTS

Boron Uptake

Water stress reduced boron (B) uptake by plants (Table 1&2). Boron availability is correlated with B uptake, with young leaves being the most sensitive and consistent indicator (Yang et al., 1989; Huang et al., 1996). In experiment I, leaf B content decreased significantly under water stress in both cultivars. However, boron uptake by "Cyclone" was more susceptible to water stress. Leaf B content of plants in the high stress treatment decreased by 57% compared to plants in the low stress treatment. The difference between the medium and high stress treatment was not significant. In "American-A112", B content of upper leaves of plants in the medium stress treatment decreased by 28% compared to plants in the low stress treatment. Further increase in water stress did not significantly further decrease the B content of upper leaves (Table 1). These results suggest that "Cyclone" was more susceptible to water stress than "American-A112". In experiment II, with only 0.3 $mg \cdot l^{-1}$ B in the nutrient solution less then half as much B was found in leaf tissue compare to experiment I (Table 2). However, there was no statistically significant difference among treatments in experiment II.

Growth and Development

The reduction of growth and development of plants under water stress was apparent in both experiments (Tables 11&12). Under high water stress "Cyclone" plants were noticeably smaller with leaf margins curled and damaged. "American-A112" plants under high water stress were also smaller compare to nonstressed plants but not as small as

"Cyclone" plants. Under medium stress "Cyclone" plants appeared somewhat smaller with smaller leaves and slight margin cupping. "American-A112" plants in medium stress did not exhibit visual symptoms of water stress (leaves were only slightly more placid). The difference between cultivars was statistically significant under both water stress treatments.

Yield

Water stress resulted in a yield decrease for both cultivars. In experiment I, medium stress "American-A112"plants averaged only 53% and high stress plants only 49% of the nonstressed plants yield. For "Cyclone" the yield was 34% in medium stress and 9.5% in high stress compare to the nonstressed plants (Table 8). In experiment II, with less B available yield was reduced by about 50% compare to experiment I. The differences among treatments and between cultivars followed the same pattern as in experiment I (Table 9).

Glucosinolate content

The use of gas chromatography to quantify the individual glucosinolates revealed that 2-hydroxy-3-butenyl (progoitrin) was the predominant glucosinolate in both cultivars in both experiments. The indole glucosinolate 4-hydroxy-3-indolylmethyl (4-hydroxy-glucobrassicin) was the second most common, and was always found at higher concentration than any of the remaining aliphatic compounds (Table 6&7). In experiment I, with 0.6 B $mg \cdot l^{-1}$ available in the nutrient solution the water stress treatments did not

alter the concentrations of progoitrin in either cultivars. However, the concentration of progoitrin in "American-A112" was significantly higher in all the treatments compared to "Cyclone" (Table 6). Medium stress significantly increased the level of 4-hydroxy-glucobrassicin in "Cyclone". But further increase in osmotic potential decreased the concentration of this particular indole glucosinolate. The reason for this inconsistency was not apparent. The differences among other aliphatic glucosinolates {3-butenyl (gluconapin), 4-penthenyl (glucobrassicanapin), and 2-hydroxy-4-pentenyl (gluconapoleiferin)} were not significant, moreover, these compounds collectively averaged only 53% in "American-A112" and 58% in "Cyclone" of the progoitrin concentrations across all the treatments. In addition, the contribution of 3-indolylmethyl glucosinolate (glucobrassicin) was minor. Traces of a few other glucosinolates occurred, but these six were the most predominant. Indole glucosinolates constituted about 20% of the total glucosinolates present.

The profile of individual glucosinolate concentrations in experiment II was nearly the same as in experiment I, with the medium water stress treatment having a greater effect on glucosinolate content. Progoitrin in the medium stress treatment in "Cyclone" was significantly higher ($P<0.01$) compared to the high stress treatment (Table 7). This pattern was most likely due to decrease of all metabolic functions and drastic reduction of growth in high stress treatment.

Comparison of the glucosinolate totals derived by gas chromatography and colorimetry for experiment I revealed a large discrepancy in the results. Since the same problem was reported earlier (Kennedy, 1994), only gas chromatography was used in experiment II as a

more reliable and efficient method to quantify total and individual glucosinolate concentrations.

DISCUSSION

Water stress effects on the morphological development and yield of both cultivars were apparent. Water stress also decreased the size of seeds. In addition, water stress increased the total glucosinolate concentration in rapeseed. Jensen et al.(1996) reported that seed glucosinolate content increased linearly with seed development and dry weight.
The relatively low glucosinolate content of seeds (below canola quality level) in these experiments might have been partially due to the small seed size produced under water stress (Table 9&10). But it is also known from previous studies of seed glucosinolate accumulation in crops grown in different seasons or harvested using different desiccation practices, that glucosinolates can accumulate independently of seed growth (Milford et al., 1991). Therefore, the change in glucosinolate content observed in these experiments did not arise wholly from the redistribution of a given amount of glucosinolate among more, smaller seeds.

Previous research has suggested that leaf boron deficiency increased seed glucosinolate content and that leaf B concentrations above 35 $\mu g.g^{-1}$ preclude the increase in seed glucosinolates (Kennedy, 1994). The water stress in experiment I lowered the leaf B concentration to 89.58 $\mu g.g^{-1}$ dry weight in "Americane-A112" and 57.20 $\mu g.g^{-1}$ in "Cyclone"(Table 1). In experiment II, these concentrations were 46.48 $\mu g.g^{-1}$ dry weight and 46.48 $\mu g.g^{-1}$ in "American-A112" and "Cyclone" respectively (Table 2). These results

may suggest that 0.6 and 0.3 $mg \cdot l^{-1}$ B available in the nutrient solutions utilized was high enough to provide adequate B concentrations in plants to compensate for the effects of water stress on decreasing B uptake. Water stressed plants did have lower leaf B concentration and higher seed glucosinolate content, but the glucosinolate content did not exceed canola quality standards. Indole glucosinolates constituted about 20% of the total glucosinolates present suggesting, that indole glucosinolates should be considered when determining the canola quality. Currently, only the four aliphatic glucosinolates are used to determine the canola quality rapeseed. Although aliphatic glucosinolate concentration in all the treatments and cultivars did not exceed the maximum canola quality level, the amounts increased by about 50% compare to the glucosinolate concentrations of the seeds used for planting (Table 8). Such differences between seed that is sown and seed that is harvested suggests that environmental conditions may alter the glucosinolate content within a cultivar and thus contribute to inconsistent glucosinolate concentration within that cultivar.

Timing of water stress is also a very important factor. In both experiments plants were under constant water deficiency since they were four weeks old. Previous research (Mailer et al., 1987) suggested that under prolonged and consistent periods of water stress the effects might not be as drastic as in short periods of water stress during or before flowering. Boucherau et al.(1995) found the flowering stage was the most water sensitive stage. This might be another reason why the glucosinolate level did not exceed the 20 $\mu mol \cdot g^{-1}$ dry weight (canola quality level).

In these experiments the whole seeds were used for glucosinolate analyses unlike in several previous studies in which only defated seeds were used. According to Vasak et al. (1991) oil constitutes 45-48% of seed weight, whereas defatted meal contains only 2-10% of oil. This might explain why the amount of glucosinolates in both of these experiments was way below canola standards.

CONCLUSIONS

This research shows that water stress decreases the boron content of upper leaves and increases the glucosinolate concentration of seeds in both cultivars. However, the boron concentrations in both experiments were adequate enough to provide enough boron for glucosinolate concentrations to stay below canola quality levels. Decreasing osmotic potential from medium to high stress level did not significantly further increase the glucosinolate content of seeds. These results suggest that the level of water stress is not as important as the occurrence of the phenomenon itself. It is also possible that too much stress affects the rate of metabolism of the whole plant and dramatically reduces growth and development.

"Cyclone" is more susceptible to water stress than "Americane-A112" indicating genetic differences between the cultivars and their susceptibility to environmental conditions.

For future research, smaller amounts of available boron in the nutrient solution should be examined (water potential about -0.10 MPa) to determine the range between sufficient and deficient levels of boron available under the water stress. If this range of B is

established, then the effectiveness of adding more B to prevent the water stress induced increase in glucosinolates can be tested. This possibility is supported by results of previous research (Kennedy, 1994) examining the relationship between boron nutrition and glucosinolate content of rapeseed seeds.

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APPENDICES

APPENDIX A

Data and Statistics

Table 1. Effect of water stress level on the upper leaf nutrient content of two canola cultivars during Spring 1995 (experiment I).

Water stress[Z]	Nutrients (μg/g^{-1} dry weight)[Y]						
	B	Ca	K	Mg	Na	P	S
			AMERICAN A-112				
low	120	45309	38247	4993	3100	4425	2880
medium	86	43879	38013	4811	2422	4718	2508
high	90	45552	43210	4577	2722	4994	2485
Contrasts[X]							
low vs. med.	***	NS	NS	NS	*	NS	NS
med. vs. high	NS	NS	NS	NS	NS	NS	NS
low vs. high	***	NS	NS	NS	NS	NS	NS
			CYCLONE				
low	133	43947	29025	4565	1854	2329	2700
medium	73	39705	20404	4969	1777	3659	3212
high	57	29880	18756	4564	1300	4597	4038
Contrasts[x]							
low vs. med.	***	*	*	NS	NS	**	NS
med. vs. high	*	**	NS	NS	-	-	NS
low vs. high	***	***	*	NS	*	***	*
Contrasts[x]	**American-A112 vs. Cyclone:**						
low	-	NS	NS	NS	**	**	NS
medium	NS	NS	***	NS	-	**	NS
high	**	*	**	NS	**	NS	NS

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's means.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 2. Effect of water stress level on the upper leaf nutrient content of two canola cultivars during Spring 1996 (experiment II).

Water stress[Z]	Nutrients (μg/g^{-1} dry weight)[Y]						
	B	Ca	K	Mg	Na	P	S
			AMERICAN A-112				
low	51	56294	19520	3571	2390	2913	1173
medium	51	41497	19001	3045	2852	4618	879
high	46	32071	26083	2724	1790	4242	828
Contrasts[X]							
low vs. med.	NS	***	NS	**	*	***	**
med. vs. high	NS	***	**	*	***	NS	NS
low vs. high	NS	***	**	***	*	***	**
			CYCLONE				
low	46	41264	17777	2249	1019	1054	1160
medium	45	20392	21932	2538	1004	3786	1908
high	46	17984	26245	2683	613	4379	2831
Contrasts[X]							
low vs. med.	NS	***	*	*	NS	***	-
med. vs. high	NS	-	*	NS	*	*	**
low vs. high	NS	***	***	*	*	***	***
Contrasts[X]	**American-A112 vs. Cyclone:**						
low	*	***	NS	***	***	***	NS
medium	NS	***	NS	**	***	*	*
high	*	***	NS	NS	***	NS	***

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's means.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 3. Colorimetric determination of total and indole glucosinolates of seeds of two canola cultivars ('American A-112', 'Cyclone') grown under different water stress levels during Spring 1995 (experiment I).

Water stress[z]	Cultivar	Glucosinolates (μmol/g^{-1})[Y]	
		Indole	Total
low	A-112	1.02	5.68
	Cyclone	0.90	4.25
medium	A-112	1.15	6.49
	Cyclone	1.00	4.83
high	A-112	1.16	5.61
	Cyclone	1.31	5.20
ANOVA[X]			
Treatment		*	-
Cultivar		NS	***
TxC		NS	NS
Contrasts[X]			
low vs. med	A-112	NS	*
	Cyclone	NS	NS
med. vs. high	A-112	NS	*
	Cyclone	*	NS
low vs. high	A-112	NS	NS
	Cyclone	**	-
Contrasts[X]		**A-112 vs. Cyclone**	
low		NS	***
medium		-	**
high		NS	NS

[z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are lsmeans. Data are corrected for 8% moisture content.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 4. Gas chromatographic determination of total, indole, and aliphatic glucosinolates of seeds of two canola cultivars ('American A-112', 'Cyclone') grown under different water stress levels during Spring 1995 (experiment I).

Water stress[Z]	Cultivar	Glucosinolates (μmol/g^{-1})[Y]		
		Indole	Aliphatic	Total
low	A-112	1.72	6.32	8.58
	Cyclone	1.66	4.84	6.50
medium	A-112	1.76	6.51	8.09
	Cyclone	1.66	4.96	6.65
high	A-112	1.86	5.87	7.72
	Cyclone	1.29	5.19	6.53
ANOVA[X]				
Treatment		NS	NS	NS
Cultivar		*	***	***
TxC		-	NS	NS
Contrasts[X]				
low vs. med	A-112	NS	NS	NS
	Cyclone	NS	NS	NS
med. vs. high	A-112	NS	-	NS
	Cyclone	*	NS	NS
low vs. high	A-112	NS	NS	-
	Cyclone	*	NS	NS
Contrasts[X]		**A-112 vs. Cyclone**		
low		NS	*	**
medium		NS	NS	NS
high		-	NS	NS

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are lsmeans. Data are corrected for 8% moisture content.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 5. Gas chromatographic determination of total, indole, and aliphatic glucosinolates of seeds of two canola cultivars ('American A-112', 'Cyclone') grown under different water stress levels during Spring 1996 (experiment II).

Water stress[Z]	Cultivar	Glucosinolates (μmol/g^{-1})[Y]		
		Indole	Aliphatic	Total
low	A-112	1.80	6.23	8.09
	Cyclone	1.60	5.58	7.60
medium	A-112	1.82	6.18	8.16
	Cyclone	2.02	7.32	9.66
high	A-112	1.82	6.32	8.12
	Cyclone	1.84	5.03	6.77
ANOVA[X]				
Treatment		-	*	*
Cultivar		NS	NS	NS
TxC		NS	**	*
Contrasts[X]				
low vs. med.	A-112	NS	NS	NS
	Cyclone	*	*	*
med. vs. high	A-112	NS	NS	NS
	Cyclone	NS	**	**
low vs. high	A-112	NS	NS	NS
	Cyclone	NS	NS	NS
Contrasts[X]		**A-112 vs. Cyclone**		
low		NS	NS	NS
medium		NS	-	*
high		NS	*	*

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are lsmeans. Data are corrected for 8% moisture content.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 6. Gas chromatographic determination of individual glucosinolate content of seeds of two canola cultivars grown under different water stress levels during Spring 1995 (experiment I).

Water stress[Z]	Glucosinolates (μmol/g^{-1})[Y]					
	Aliphatic				Indole	
	3But	4Pen	2H3B	2H4P	3Ind	4H3I
	AMERICAN A-112					
low	1.01	0.54	4.29	0.38	0.12	1.59
medium	0.97	0.66	4.41	0.66	0.15	1.77
high	0.85	0.57	3.09	0.58	0.15	1.70
Contrasts[X]						
low vs. med.	NS	-	NS	**	NS	NS
med. vs. high	NS	NS	-	NS	NS	NS
low vs. high	NS	NS	NS	-	NS	NS
	CYCLONE					
low	1.34	0.57	2.95	0.08	0.08	1.57
medium	1.05	0.66	3.12	0.13	0.08	1.60
high	1.30	0.57	3.90	0.01	0.07	1.23
Contrasts[X]						
low vs. med.	NS	-	NS	NS	NS	NS
med. vs. high	NS	NS	NS	NS	NS	**
low vs. high	NS	*	NS	NS	NS	**
Contrasts[X]	**Americane-A112 vs. Cyclone**					
low	*	NS	**	**	NS	NS
medium	-	NS	*	**	*	NS
high	-	NS	*	*	**	-

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's lsmeans. Data are corrected for 8% moisture content.
3-Butenyl (Gluconapin); 4-Penthenyl (Glucobrassicanapin); 2-Hydroxy-3-butenyl (Progoitrin); 2-Hydroxy-4-penthenyl (Gluconapoleiferin); 3-indolylmethyl (Glucobrassicin); 4-Hydroxy-3-indolylmethyl.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.1(-).

Table 7. Gas chromatographic determination of individual glucosinolate content of seeds of two canola cultivars grown under different water stress levels during Spring 1996 (experiment II).

Water stress[Z]	Glucosinolates (μmol/g^{-1})[Y]					
	Aliphatic				Indole	
	3But	4Pen	2H3B	2H4P	3Ind	4H3I
	AMERICAN A-112					
low	0.78	0.55	4.41	0.50	0.09	1.71
medium	0.63	0.60	4.22	0.68	0.08	1.73
high	0.59	0.68	4.21	0.77	0.09	1.73
Contrasts[X]						
low vs. med.	***	NS	NS	-	NS	NS
med. vs. high	NS	NS	NS	NS	NS	NS
low vs. high	***	*	NS	**	NS	NS
	CYCLONE					
low	1.43	0.62	3.66	0.07	0.05	1.55
medium	1.81	0.93	4.19	0.10	0.05	1.97
high	1.27	0.64	2.97	0.08	0.06	1.86
Contrasts[X]						
low vs. med.	NS	*	NS	**	NS	*
med. vs. high	-	*	**	NS	NS	NS
low vs. high	NS	NS	*	NS	NS	NS
Contrasts[X]	**American-A112 vs. Cyclone:**					
low	**	NS	NS	***	**	NS
medium	**	*	NS	***	**	NS
high	**	NS	**	***	NS	-

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's means. Data are corrected for 8% moisture content.
3-Butenyl (Gluconapin); 4-Penthenyl (Glucobrassicanapin); 2-Hydroxy-3-butenyl (Progoitrin); 2-Hydroxy-4-penthenyl (Gluconapoleiferin); 3-indolylmethyl (Glucobrassicin); 4-Hydroxy-3-indolylmethyl.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.1(-).

Table 8. Gas chromatographic determination of individual and total glucosinolates of seeds of two canola cultivars used for planting in both experiments.

Glucosinolate[ZY]	*AMERICAN-A112*	*CYCLONE*
3-Butenyl	0.48	0.43
4-Penthenyl	0.11	0.12
2-Hydroxy-3-butenyl	1.43	0.59
2-Hydroxy-4-penthenyl	0.10	0.00
Aliphatic - total	2.11	1.14
3-Indolylmethyl	0.13	0.10
4-Hydroxy-3-indolylmethyl	1.84	2.07
Indole - total	1.97	2.17
TOTAL	**4.08**	**3.50**

[Z] The numbers are $\mu mol.g^{-1}$ dry weight. Data are corrected for 8% moisture content.

[Y] 3-Butenyl (Gluconapin); 4-Penthenyl (Glucobrassicanapin); 2-Hydroxy-3-butenyl (Progoitrin); 2-Hydroxy-4-penthenyl (Gluconapoleiferin); 3-Indolylmethyl (Glucobrassicin); 4-Hydroxy-3-indolylmethyl.

Table 9. Effect of water stress on seed production of two canola cultivars ('American A-112', 'Cyclone') grown under different water stress levels during Spring 1995 (experiment I).

Water stress[Z]	Cultivar	Yield (g)[Y]			Weight (g) of 100 seeds
		Main B	Side B	Total	
low	A-112	2.39	77.08	80.08	0.330
	Cyclone	1.87	43.36	42.78	0.320
medium	A-112	2.04	40.03	42.49	0.325
	Cyclone	2.04	13.21	14.85	0.287
high	A-112	2.79	39.60	39.86	0.344
	Cyclone	1.99	3.77	4.06	0.289
ANOVA[X]					
Treatment		NS	***	***	-
Cultivar		*	***	***	**
TxC		NS	NS	NS	NS
Contrasts[X]					
low vs. med	A-112	NS	***	***	NS
	Cyclone	NS	***	***	NS
med. vs. high	A-112	**	NS	NS	NS
	Cyclone	NS	NS	-	NS
low vs. high	A-112	NS	***	***	NS
	Cyclone	NS	***	***	NS
Contrasts[X]		**A-112 vs. Cyclone**			
low		*	NS	NS	NS
medium		NS	**	**	NS
high		NS	**	**	**

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are lsmeans. Main B = main branch; Side B = side branches.
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 10. Effect of water stress on seed production of two canola cultivars ('AmericanA-112', 'Cyclone') grown under different water stress levels during Spring 1996 (experiment II).

Water stress[Z]	Cultivar	Yeild (g)[Y]			Weight (g) of 100 seeds
		Main B	Side B	Total	
low	A-112	4.13	25.72	29.92	0.245
	Cyclone	2.62	6.53	8.56	0.224
medium	A-112	4.23	19.24	23.41	0.242
	Cyclone	1.66	2.91	4.41	0.248
high	A-112	3.67	19.57	23.14	0.233
	Cyclone	1.51	2.79	4.25	0.231
ANOVA[X]					
Treatment		*	***	***	NS
Cultivar		***	***	***	NS
TxC		NS	NS	NS	NS
Contrasts[X]					
low vs. med.	A-112	NS	**	*	NS
	Cyclone	**	***	**	NS
med. vs high	A-112	NS	NS	NS	NS
	Cyclone	NS	NS	NS	NS
low vs. high	A-112	NS	**	**	NS
	Cyclone	**	***	**	NS
Contrasts[X]		**A-112 vs. Cyclone**			
low		**	***	***	**
medium		***	***	***	NS
high		**	***	***	NS

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are lsmeans. Main B = main branch; Side B = side branche
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 11. Effect of water stress on dry weight of individual plant parts of two canola cultivars during Spring 1995 (experiment I).

Water stress[Z]	Dry weight (g)								
	LL	UL	SL	MI	SI	MS	SS	Pods	Total
				AMERICAN A-112					
low	45.92	7.11	68.80	2.07	67.39	20.75	78.18	88.28	371.98
medium	35.69	5.87	38.96	1.78	40.57	14.55	34.97	50.63	219.69
high	27.12	5.21	35.74	1.40	29.33	9.64	20.11	44.06	171.13
Contrasts[X]									
low vs. med.	**	NS	***	NS	*	***	***	**	***
med. vs. high	-	NS	NS	NS	NS	*	*	NS	NS
low vs. high	***	NS	***	*	**	***	***	**	***
				CYCLONE					
low	31.89	8.21	41.06	1.65	88.55	77.41	105.73	73.85	375.58
medium	19.39	6.78	19.33	1.42	20.83	41.65	17.77	18.39	142.12
high	16.09	4.88	12.24	1.22	8.41	24.01	7.09	11.56	53.66
Contrasts[X]									
low vs. med.	*	NS	***	NS	***	***	***	***	***
med. vs. high	NS	NS	NS	NS	NS	*	NS	NS	-
low vs. high	*	-	***	NS	***	***	***	***	***
Contrasts[X]	**American -A112 vs. Cyclone**								
low	-	NS	***	NS	NS	***	-	NS	NS
medium	**	NS	-	NS	-	**	NS	**	**
high	NS	NS	**	NS	*	**	*	**	**

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's means. LL = lower leaves; UL = upper leaves; SL = side leaves; MS= main stem; SS = side stems; MI= main inflorescence; SI = side inflorescence;
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 12. Effect of water stress on dry weight of individual plant parts of two canola cultivars during Spring 1996 (experiment II).

Water stress[Z]	Dry weight (g)								
	LL	UL	SL	MI	SI	MS	SS	Pods	Total
				AMERICAN A-112					
low	20.12	13.56	17.93	2.09	36.47	15.27	28.86	81.91	246.93
medium	17.81	13.12	13.27	1.91	22.17	10.93	15.25	62.44	166.16
high	13.87	12.20	7.83	1.89	19.66	8.19	9.89	47.00	128.95
Contrasts[X]									
low vs. med.	-	NS	**	*	***	**	***	*	***
med. vs. high	*	NS	*	NS	*	NS	-	-	**
low vs. high	***	NS	***	-	***	***	***	***	***
				CYCLONE					
low	13.76	14.20	35.81	2.38	36.78	44.99	36.57	51.23	249.46
medium	13.41	11.16	18.53	1.92	14.49	23.45	9.73	29.83	125.12
high	11.74	9.25	9.92	1.39	8.58	16.23	5.42	17.98	74.98
Contrasts[X]									
low vs. med.	NS	**	**	NS	***	***	***	**	***
med. vs. high	NS	-	NS	*	**	NS	NS	*	*
low vs. high	NS	***	**	**	***	***	***	***	***
Contrasts[X]	**American-A112 vs. Cyclone:**								
low	***	NS	NS	NS	NS	***	NS	**	NS
medium	***	***	NS	NS	**	**	NS	***	-
high	**	*	NS	-	***		*	***	**

[Z]Low water stress level is -0.05 MPa, medium is -0.085 MPa, and high stress is -0.1 MPa.
[Y]The numbers are the treatment's means. LL = lower leaves; UL = upper leaves; SL = side leaves; MS = main stem; SS =side stems; MI= main inflorescence; SI = side inflorescence;
[X]Linear contrasts are nonsignificant (NS), or significant at P=0.05 (*), 0.01 (**), 0.001 (***), or 0.10 (-).

Table 13. Nutrient availability in solution during Spring 1995 (experiment I); low stress treatment.

	B[z]		Ca[z]		K[z]		Mg[z]		Na[z]		P[z]		S[z]	
Date	old	new	old	new	old	new	old	new	old	new	old	new	old	new
13-Mar	0.23	0.18	48.83	60.85	18.58	47.45	7.84	9.93	43.26	26.29	17.41	22.20	6.05	8.09
21-Mar	0.33	0.35	48.91	58.39	0.96	16.20	7.44	8.23	42.19	40.54	20.03	23.12	1.17	2.04
22-Mar	0.38	0.51	51.50	114.66	0.94	167.56	7.45	17.99	40.85	18.78	21.76	44.32	0.30	12.42
24-Mar	0.50	0.63	49.52	81.31	1.52	66.43	9.98	15.39	35.72	28.75	17.55	29.25	0.12	6.92
27-Mar	0.72	0.70	77.84	109.32	19.40	81.99	15.83	18.80	32.60	22.74	24.85	35.88	0.15	5.87
30-Mar	0.87	0.81	80.50	95.17	38.61	77.33	18.39	20.21	33.18	28.92	24.18	32.07	0.20	4.20
31-Mar	0.88	0.77	69.84	122.38	28.89	150.22	18.02	25.00	33.13	17.52	20.82	42.62	0.10	13.58
1-Apr	1.01	0.86	69.43	155.05	45.67	308.03	21.21	27.73	38.78	13.73	23.09	58.42	0.10	18.09
3-Apr	1.13	0.84	62.10	123.03	98.00	221.24	22.00	23.34	37.58	15.66	19.79	44.21	0.13	12.29
4-Apr	1.12	1.04	54.33	110.89	106.79	228.12	20.19	24.79	39.93	25.81	17.83	39.96	0.10	9.23
5-Apr	1.18	-	58.31	-	133.47	-	21.11	-	33.46	-	20.86	-	0.12	-
7-Apr	1.19	0.91	42.13	63.46	100.16	159.25	18.29	17.34	32.33	27.47	18.41	26.95	0.11	3.67
10-Apr	1.27	0.96	32.67	57.92	46.21	128.59	13.62	14.81	35.89	26.81	17.65	26.45	0.15	4.61
11-Apr	1.39	0.97	29.70	73.51	33.20	128.83	13.92	15.91	44.34	23.11	19.87	32.53	0.13	6.97
13-Apr	1.40	1.01	28.04	62.71	3.11	83.09	12.58	14.46	35.10	21.31	19.45	28.96	0.18	5.76
19-Apr	1.45	1.20	23.97	57.31	0.80	51.43	8.86	12.84	20.58	19.29	19.99	29.24	0.16	5.62
21-Apr	1.57	1.08	18.38	63.06	0.80	67.97	7.59	12.85	20.54	24.62	24.21	32.25	0.15	6.91
23-Apr	1.63	0.83	13.36	72.61	0.80	112.82	6.65	14.49	21.16	30.84	25.71	33.76	0.15	8.91
26-Apr	1.69	0.43	7.74	7.44	0.80	12.55	5.78	1.96	23.84	13.03	30.39	17.84	0.16	1.46
27-Apr	1.64	0.61	9.05	48.75	0.80	131.87	6.89	12.45	24.93	16.73	34.52	33.04	0.20	8.87
30-Apr	1.31	0.68	9.10	17.00	0.80	33.29	2.69	3.61	17.67	10.61	36.46	23.72	0.15	1.98
2-May	1.37	0.63	10.84	22.17	0.80	40.69	1.95	3.59	15.09	6.65	34.82	21.46	0.15	2.49
3-May	1.29	-	12.67	-	0.80	-	2.01	-	11.57	-	32.72	-	0.14	-
4-May	1.37	0.73	18.04	27.09	0.80	30.76	2.13	3.78	7.39	6.35	34.98	23.86	0.14	1.69
5-May	1.23	0.71	15.38	22.16	0.80	27.20	1.70	2.85	7.84	4.08	31.51	22.18	0.17	1.70
9-May	-	0.20	-	15.71	-	30.32	-	2.05	-	1.14	-	8.83	-	1.80
10-May	0.68	0.40	10.44	12.87	0.80	15.37	0.17	1.12	2.42	1.44	16.57	11.86	0.12	0.93
11-May	0.76	0.17	10.94	13.66	0.80	24.70	0.10	1.79	1.77	0.43	16.83	7.54	0.13	1.46
12-May	0.34	0.19	3.99	22.62	0.80	44.32	0.05	3.24	0.50	0.36	5.96	10.41	0.10	2.65
13-May	0.48	0.27	7.47	13.34	0.80	17.63	0.07	1.48	0.66	0.43	10.20	8.67	0.12	1.86
14-May	0.47	0.31	6.98	10.11	0.80	9.78	0.04	0.80	0.76	0.50	9.88	8.21	0.14	1.04
16-May	0.54	0.15	6.65	16.22	0.80	28.10	0.07	2.24	0.44	0.19	8.75	7.36	0.17	2.87
17-May	0.45	0.13	6.25	14.31	0.80	23.86	0.05	1.94	0.32	2.33	7.73	6.31	0.18	2.57
19-May	0.30	0.19	3.31	11.04	0.80	9.58	0.03	1.31	0.21	0.68	3.86	5.14	0.11	1.03
20-May	0.24	0.16	3.77	9.40	0.80	12.97	0.03	1.11	0.21	0.21	4.25	4.65	0.10	1.39
21-May	0.19	0.10	2.53	11.80	0.80	18.73	0.12	1.69	0.24	0.16	2.97	4.75	0.12	2.16
22-May	0.16	0.09	2.57	13.21	0.80	22.37	0.04	1.93	0.10	0.46	2.58	5.18	0.10	2.42
23-May	0.16	0.11	2.27	9.19	0.80	13.80	0.04	1.29	0.11	4.54	2.51	4.16	0.10	1.56
24-May	0.15	0.08	1.50	10.86	0.80	20.51	0.03	1.84	0.13	31.77	1.21	4.18	0.12	2.07
Final	0.13		12.23		20.49		1.71		0.15		5.58		2.14	

[z]The numbers are $mg.l^{-1}$ of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Elements lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 14. Nutrient availability in solution during Spring 1995 (experiment I); medium stress treatment.

	B^z		Ca^z		Mg^z		K^z		Na^z		P^z		S^z	
Date	old	new	new	old	old	new	old	new	old	new	old	new	old	new
13-Mar	0.26	0.21	37.86	30.35	17.13	31.68	4.83	6.18	32.96	26.37	16.36	19.64	4.38	5.56
17-Mar	0.21	0.23	37.54	29.49	8.58	29.07	4.63	5.29	34.41	31.82	16.93	19.70	3.52	4.06
21-Mar	0.27	0.27	37.63	34.95	12.23	31.94	5.49	5.66	34.14	25.64	20.74	23.77	2.67	4.24
22-Mar	0.29	0.33	51.85	36.93	8.75	34.83	5.60	7.50	38.42	29.44	21.48	27.59	1.75	3.92
24-Mar	0.36	0.33	35.55	44.23	0.80	19.71	5.15	3.53	24.88	16.80	24.52	27.13	0.37	2.47
27-Mar	0.42	0.43	54.22	37.89	6.69	39.83	5.57	8.08	15.50	12.29	25.31	30.43	0.27	3.83
29-Mar	0.45	0.45	53.37	36.73	4.05	53.28	5.51	9.49	15.03	13.31	23.86	31.59	0.18	5.16
31-Mar	0.48	0.50	51.90	31.40	7.44	80.12	5.77	9.42	16.17	13.12	23.09	33.56	0.18	4.98
3-Apr	0.55	0.56	50.19	22.52	14.50	88.85	5.46	8.84	18.62	12.00	21.20	36.71	0.17	5.79
4-Apr	0.55	0.54	43.37	25.60	29.35	84.99	5.45	8.03	19.99	16.41	23.07	32.26	0.21	3.33
9-Apr	0.66	0.47	42.59	15.86	16.41	85.43	4.19	7.74	19.99	11.67	22.51	28.24	0.22	4.79
10-Apr	0.68	-	-	15.90	17.53	-	4.27	-	20.25	-	23.51	-	0.19	-
13-Apr	0.66	0.57	22.62	14.52	13.94	35.96	4.12	5.30	19.96	17.89	22.78	24.62	0.21	1.90
19-Apr	0.72	0.73	10.23	3.05	0.80	3.06	1.42	2.48	13.57	15.49	19.99	24.91	0.22	0.17
21-Apr	0.71	0.59	19.09	3.04	0.80	24.76	1.21	3.83	13.48	16.36	20.46	24.89	0.15	2.63
23-Apr	0.73	0.56	27.94	1.36	0.80	52.07	0.73	5.29	15.60	20.30	22.78	27.19	0.17	4.20
26-Apr	0.72	0.56	10.48	0.89	0.80	26.57	0.52	3.69	17.60	18.24	24.99	24.80	0.25	2.24
27-Apr	0.71	0.57	9.70	0.96	0.80	26.20	0.56	2.35	18.62	18.59	25.94	25.81	0.24	2.14
30-Apr	0.60	0.52	5.77	2.96	1.79	10.73	0.38	0.92	13.56	13.71	23.39	21.77	0.21	0.84
2-May	0.65	0.56	5.20	2.78	0.80	5.75	0.21	0.58	14.69	13.48	23.08	21.22	0.21	0.62
3-May	0.64	0.50	9.31	2.98	0.80	13.84	0.19	1.07	14.01	11.16	22.86	20.17	0.36	1.25
4-May	0.65	0.48	9.79	3.53	0.80	15.37	0.20	1.20	13.60	10.88	22.34	19.68	0.32	1.47
5-May	0.65	0.48	7.68	3.83	0.80	10.51	0.19	0.85	12.95	11.16	22.00	18.16	0.30	1.00
9-May	-	0.37	6.25	-	-	8.22	-	0.63	-	8.77	-	12.12	-	0.82
10-May	0.48	0.38	4.96	3.38	0.80	4.53	0.11	0.39	9.63	8.27	12.96	11.22	0.21	0.44
11-May	0.48	0.36	5.76	2.92	0.80	7.28	0.06	0.52	9.29	7.86	11.86	10.48	0.18	0.64
12-May	0.50	0.26	8.11	2.84	0.80	13.19	0.04	0.91	8.70	5.34	11.11	8.31	0.19	0.95
14-May	0.46	0.39	5.15	2.57	0.80	5.04	0.03	0.39	8.74	7.92	8.84	8.94	0.27	0.83
16-May	0.46	0.35	5.45	2.17	0.80	6.48	0.03	0.50	8.42	6.80	7.44	7.25	0.32	0.99
17-May	0.44	0.28	8.08	2.71	0.80	9.91	0.04	0.76	7.28	4.97	6.46	6.45	0.30	1.34
19-May	-	0.34	4.45	-	-	4.65	-	0.39	-	5.91	-	4.53	-	0.78
20-May	0.40	0.31	5.19	2.54	0.80	5.71	0.05	0.47	6.42	5.30	3.69	4.25	0.31	0.86
22-May	0.43	0.26	8.47	2.64	0.80	12.12	0.03	0.96	5.48	3.66	2.24	4.55	0.36	1.52
23-May	0.38	0.25	5.54	3.30	0.80	5.91	0.06	0.52	3.84	6.91	1.52	2.48	0.31	0.87
24-May	0.37	0.27	7.06	3.13	0.80	7.41	0.04	0.67	4.02	12.54	1.12	2.84	0.30	1.11
Final	0.36			3.63	1.99		0.22		6.87		5.65		0.51	

[z]The numbers are mgl^{-1} of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Element's lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 15. Nutrient availability in solution during Spring 1995 (experiment I); high stress treatment.

	B[z]		Ca[z]		K[z]		Mg[z]		Na[z]		P[z]		S[z]	
Date	old	new	old	new	old	new	old	new	old	new	old	new	old	new
13-Mar	0.16	0.18	21.30	26.18	11.74	19.18	2.90	3.77	22.11	18.94	15.84	18.27	3.38	4.14
17-Mar	0.18	0.19	19.96	22.19	3.91	9.98	2.12	2.54	15.94	16.18	15.66	17.08	2.50	2.79
21-Mar	0.22	0.24	21.47	26.10	3.88	16.73	2.59	3.15	20.99	21.55	17.92	20.93	1.93	2.47
22-Mar	0.23	0.27	21.61	29.41	1.43	16.43	1.93	3.10	16.56	18.04	17.90	23.04	1.13	2.57
24-Mar	-	0.38	-	51.82	-	28.53	-	7.60	-	24.61	-	30.89	-	3.17
27-Mar	0.33	0.37	24.20	39.21	1.92	21.98	2.00	3.61	10.15	8.48	20.18	25.65	0.33	2.83
29-Mar	0.36	0.39	22.37	36.47	0.80	31.66	1.98	3.95	9.77	7.67	18.17	26.58	0.28	3.38
31-Mar	0.39	0.42	18.38	37.47	0.80	55.16	1.82	4.19	9.81	7.70	16.52	28.88	0.37	4.08
1-Apr	0.40	0.52	19.63	49.93	7.88	76.50	1.89	8.97	9.89	12.96	18.33	33.70	0.31	4.48
3-Apr	0.43	0.45	17.15	30.90	9.44	44.46	1.87	3.14	10.74	8.69	18.29	27.16	0.32	3.01
4-Apr	0.42	0.45	16.32	30.45	16.65	53.76	1.88	3.29	13.42	15.66	18.69	26.89	0.27	2.48
5-Apr	0.44	-	17.28	-	20.87	-	1.97	-	15.11	-	20.24	-	0.24	-
9-Apr	0.48	0.42	8.27	19.42	7.95	36.66	1.27	2.56	14.31	11.17	16.75	21.73	0.39	2.24
10-Apr	0.50	-	8.43	-	7.36	-	1.28	-	14.32	-	17.51	-	0.20	-
13-Apr	0.49	0.43	7.91	10.57	3.70	9.59	1.22	1.51	13.36	11.72	16.41	16.57	0.32	0.88
19-Apr	0.53	0.54	2.25	8.92	0.80	4.11	0.42	0.96	10.77	12.32	14.94	19.64	0.36	0.56
21-Apr	1.52	0.50	15.38	9.27	0.80	7.57	7.44	1.24	14.85	14.48	16.31	19.80	0.17	1.04
23-Apr	0.55	0.46	2.79	14.50	0.80	21.22	0.49	2.03	12.40	13.96	16.26	20.84	0.29	2.39
26-Apr	0.53	0.54	2.32	51.05	0.80	122.33	0.59	17.52	13.61	19.33	16.82	30.12	0.39	8.75
30-Apr	0.48	-	2.04	-	0.80	-	0.29	-	11.91	-	14.56	-	0.37	-
3-May	0.47	0.44	2.31	4.41	0.80	4.06	0.21	0.42	11.25	10.37	12.82	13.08	0.41	0.86
5-May	0.50	0.42	2.46	4.29	0.80	4.00	0.13	0.36	11.41	10.39	11.92	11.69	0.37	0.78
10-May	0.45	0.35	2.89	3.68	0.80	2.03	0.05	0.19	9.70	8.41	8.61	7.87	0.45	0.66
11-May	0.44	0.38	2.62	3.37	0.80	1.27	0.04	0.12	9.69	8.60	7.82	7.48	0.45	0.49
12-May	0.46	0.33	2.66	4.50	0.80	3.91	0.03	0.25	11.02	8.35	7.08	6.79	0.67	0.91
13-May	0.42	0.31	2.78	5.38	0.80	5.23	0.03	0.34	10.51	8.13	6.40	6.98	0.56	1.14
16-May	0.41	0.36	2.53	3.51	0.80	1.13	0.03	0.11	10.34	9.20	5.02	5.20	0.52	0.68
17-May	0.39	0.31	2.37	3.59	0.80	2.59	0.03	0.18	10.28	8.53	4.19	4.40	0.49	0.86
19-May	0.37	0.31	2.32	3.26	0.80	1.79	0.03	0.15	9.77	7.96	2.84	3.14	0.50	0.67
22-May	0.28	0.39	5.08	2.34	5.28	0.80	0.36	0.03	7.12	9.68	3.25	1.36	1.09	0.57
23-May	0.37	0.31	2.91	3.87	0.80	2.24	0.05	0.20	8.36	8.30	1.00	1.86	0.44	0.80
24-May	0.37	0.30	3.28	4.41	0.80	2.60	0.04	0.21	8.13	12.15	0.69	1.70	0.49	0.75
Final	0.34		3.21		0.81		0.09		8.84		3.88		0.68	

[z]The numbers are mgl^{-1} of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Elements lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 16. Nutrient availability in solution during Spring 1996 (experiment II); low stress treatment.

	B[z]		Ca[z]		K[z]		Mg[z]		Na[z]		P[z]		S[z]	
Date	old	new	old	new	old	new	old	new	old	new	old	new	old	new
4-Mar	0.08	0.08	18.25	20.53	12.65	19.10	2.99	3.25	23.96	21.23	5.95	6.65	3.67	3.96
8-Mar	0.04	0.05	20.35	25.53	16.98	23.01	3.44	5.65	19.15	17.10	6.26	8.76	3.70	3.98
25-Mar	0.05	0.07	5.20	24.76	0.80	39.65	1.34	4.20	14.92	10.73	7.81	13.20	0.10	3.75
27-Mar	0.21	0.27	7.18	21.86	0.80	30.03	1.24	3.29	15.67	12.47	9.02	12.93	0.10	2.99
29-Mar	0.21	0.27	4.97	21.85	0.80	33.23	0.83	3.35	13.66	10.20	7.47	12.50	0.10	3.39
8-Apr	0.04	0.16	4.23	26.31	0.80	40.58	0.40	3.78	1.79	2.04	5.63	8.06	0.10	4.51
9-Apr	0.01	0.09	12.02	48.78	0.80	70.61	1.41	7.27	4.21	2.42	6.84	11.14	0.16	11.43
12-Apr	0.02	0.09	20.37	50.52	0.80	69.00	2.24	7.17	1.77	1.35	9.80	20.75	0.15	11.35
13-Apr	0.01	0.15	9.06	66.00	0.80	114.31	0.84	9.18	1.00	0.85	4.68	25.35	0.10	11.51
15-Apr	0.04	0.06	20.62	30.27	0.80	17.49	2.37	3.87	2.27	3.15	10.17	13.63	0.10	2.07
17-Apr	0.02	0.07	22.83	41.99	0.80	47.72	2.98	5.88	1.84	1.52	11.22	17.82	0.11	5.05
18-Apr	0.02	0.14	15.13	74.83	0.80	140.49	1.85	11.26	0.77	0.68	8.26	28.44	0.12	14.47
19-Apr	0.02	0.04	23.83	30.21	0.80	22.81	3.30	4.29	1.59	1.25	12.11	13.73	0.10	2.48
21-Apr	0.01	0.08	7.71	37.73	0.80	66.92	1.36	5.90	1.28	0.71	2.39	13.75	0.10	7.41
24-Apr	0.01	0.05	3.81	22.33	0.95	40.11	0.68	3.45	0.87	7.30	3.76	9.15	0.12	4.55
28-Apr	0.01	0.03	2.24	13.10	0.80	23.19	0.13	1.89	1.34	8.51	3.20	5.39	0.15	2.62
29-Apr	0.01	0.04	2.28	16.79	0.80	31.56	0.12	2.66	1.75	17.11	3.34	6.98	0.18	3.63
30-Apr	0.01	0.03	1.57	10.71	0.80	21.02	0.09	1.73	5.26	21.91	3.30	5.09	0.10	2.16
1-May	0.01	0.02	1.49	10.64	0.80	19.26	0.06	1.50	9.00	9.74	3.34	5.35	0.10	2.09
2-May	0.01	0.02	1.83	9.58	0.80	16.61	0.03	1.32	5.94	5.92	3.33	5.05	0.13	1.80
6-May	0.01	0.01	0.38	1.65	0.80	0.80	0.03	0.03	1.61	2.07	0.15	0.01	0.11	0.10
8-May	0.01	0.01	0.03	0.33	0.80	0.80	0.03	0.03	2.41	0.60	0.01	0.01	0.11	0.10
10-May	0.02	0.01	10.59	0.11	0.80	0.80	0.03	0.03	21.87	1.10	0.30	0.01	0.10	0.10
13-May	0.01	0.01	0.31	0.20	0.84	0.84	0.03	0.03	0.60	0.11	0.01	0.01	0.10	0.10
15-May	0.01	0.01	0.19	0.15	0.80	0.80	0.03	0.03	0.25	0.12	0.01	0.03	0.10	0.10
Final	0.01		0.05		0.80		0.03		4.47		0.12		0.34	

[z]The numbers are mgl^{-1} of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Elements lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 17. Nutrient availability in solution during Spring 1996 (experiment II): medium stress treatment.

	B[z]		Ca[z]		K[z]		Mg[z]		Na[z]		P[z]		S[z]	
Date	old	new	old	new	old	new	old	new	old	new	old	new	old	new
4-Mar	0.09	0.09	19.43	20.94	13.52	19.24	3.13	3.33	26.58	23.74	6.24	6.82	4.11	4.29
8-Mar	0.04	0.05	20.51	23.62	17.96	26.20	3.47	3.89	20.81	18.90	6.48	7.62	4.05	4.51
25-Mar	0.05	0.06	7.05	13.23	1.42	14.23	1.08	1.86	14.46	13.05	9.78	12.30	0.10	1.10
27-Mar	0.22	0.24	5.13	9.55	0.80	9.23	0.76	1.17	15.27	13.33	10.17	10.60	0.10	0.71
29-Mar	0.21	0.22	3.06	8.56	0.80	10.60	0.54	1.14	16.31	13.67	10.22	11.17	0.10	0.90
8-Apr	0.09	0.15	1.40	5.60	0.80	5.55	0.06	0.57	8.38	8.58	6.72	6.29	0.10	0.67
9-Apr	0.03	0.05	4.31	15.48	0.80	18.46	0.36	1.81	11.05	9.18	6.56	10.22	0.10	2.97
12-Apr	0.03	0.05	10.90	21.90	0.80	22.61	0.82	2.39	10.21	8.57	9.54	14.32	0.10	2.26
13-Apr	0.02	0.06	7.35	25.88	9.54	33.75	0.71	2.88	8.21	7.56	6.55	15.36	0.10	3.45
15-Apr	0.04	0.04	10.55	13.28	0.80	3.42	0.91	1.24	9.92	9.93	9.15	8.94	0.10	0.16
17-Apr	0.02	0.05	10.61	21.25	0.80	22.62	0.92	2.42	9.50	8.34	7.88	12.94	0.10	2.29
18-Apr	0.02	0.09	9.02	40.98	0.80	64.83	0.72	5.35	9.73	7.21	6.91	20.87	0.10	6.69
19-Apr	0.03	0.06	13.51	25.37	0.80	27.19	1.32	3.26	10.07	7.88	8.89	14.06	0.10	3.05
21-Apr	0.02	0.05	9.91	20.32	0.80	25.10	1.13	2.81	11.23	7.70	4.88	9.80	0.10	2.80
24-Apr	0.02	0.05	9.54	19.71	0.80	27.19	0.96	2.60	11.56	13.80	2.84	7.32	0.10	2.82
28-Apr	0.01	0.03	8.76	12.48	0.80	11.22	0.31	1.20	11.50	14.33	0.77	2.61	0.10	1.20
29-Apr	0.01	0.02	7.95	9.85	0.80	7.95	0.26	0.73	12.52	17.29	0.66	1.39	0.10	0.72
30-Apr	0.01	0.01	6.01	6.64	0.80	2.30	0.12	0.32	13.46	16.18	1.01	0.60	0.10	0.17
1-May	0.01	0.01	5.39	8.58	0.80	8.48	0.05	0.73	14.87	9.37	1.05	2.00	0.10	0.83
2-May	0.01	0.02	5.51	7.68	0.80	6.36	0.06	0.51	13.70	11.09	1.10	1.12	0.10	0.53
3-May	0.02	0.01	5.72	7.82	0.80	5.66	0.03	0.47	13.31	9.32	1.04	1.51	0.10	0.53
6-May	0.02	0.01	4.32	5.67	0.80	0.80	0.03	0.03	13.22	10.11	0.85	0.70	0.10	0.10
8-May	0.01	0.01	4.86	4.23	0.80	0.80	0.03	0.03	12.42	11.78	0.24	0.08	0.10	0.10
10-May	0.01	0.01	5.46	4.22	0.80	0.80	0.03	0.03	12.70	11.12	0.17	0.07	0.10	0.10
13-May	0.01	0.01	4.52	3.61	0.80	0.80	0.03	0.03	10.05	8.92	0.27	0.23	0.10	0.10
15-May	0.01	0.01	3.92	2.74	0.80	0.80	0.03	0.03	9.29	8.39	0.22	0.12	0.10	0.10
Final	0.01		2.63		0.80		0.06		11.44		0.27		0.10	

[z]The numbers are mgl^{-1} of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Elements lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 18. Nutrient availability in solution during Spring 1996 (experiment II): high stress treatment.

	B^z		Ca^z		K^z		Mg^z		Na^z		P^z		S^z	
Date	old	new	old	new	old	new	old	new	old	new	old	new	old	new
4-Mar	0.09	0.09	18.59	22.84	16.77	31.17	3.02	3.58	23.69	17.36	6.25	7.73	3.92	4.55
8-Mar	0.04	0.04	21.03	24.64	22.67	30.57	3.46	3.92	18.67	16.11	6.55	7.84	3.96	4.54
25-Mar	0.05	0.22	9.94	11.75	7.51	12.21	0.90	1.03	9.06	9.87	11.04	12.24	0.10	0.10
27-Mar	0.18	0.20	7.41	9.55	3.49	8.20	0.68	0.89	10.42	9.60	10.72	11.21	0.10	0.10
29-Mar	0.20	0.22	4.73	9.76	0.80	11.14	0.53	1.03	10.24	8.97	9.62	12.00	0.10	0.70
8-Apr	0.10	0.13	7.47	9.52	0.80	2.16	0.48	0.63	8.12	7.48	5.65	5.01	0.10	0.10
9-Apr	0.02	0.04	12.23	17.48	0.80	7.89	0.88	1.46	11.60	11.66	4.84	6.90	0.10	0.75
12-Apr	0.02	0.03	18.56	13.88	0.80	17.14	1.28	1.28	12.42	4.70	8.75	5.40	0.10	0.58
13-Apr	0.03	0.06	15.00	26.26	6.18	18.73	1.13	2.15	9.75	10.69	5.61	12.27	0.10	1.44
15-Apr	0.04	0.05	17.98	21.07	0.80	3.80	1.28	1.56	12.25	11.92	7.26	7.77	0.10	0.10
17-Apr	0.03	0.05	20.14	26.89	0.80	15.02	1.37	2.26	11.79	10.70	6.83	11.03	0.10	1.24
18-Apr	0.03	0.06	19.79	27.60	0.80	17.49	1.30	2.30	12.43	10.15	6.62	10.09	0.10	1.40
19-Apr	0.03	0.03	19.84	21.85	0.80	4.56	1.33	1.68	11.75	10.70	6.07	7.44	0.10	0.12
21-Apr	0.02	0.03	19.97	20.50	0.80	5.51	1.19	1.50	13.05	11.23	3.53	4.88	0.10	0.20
24-Apr	0.02	0.04	19.83	21.81	0.80	13.50	1.03	1.80	13.96	15.67	2.04	5.19	0.10	0.95
28-Apr	0.02	0.03	16.83	18.32	0.80	3.61	0.69	0.93	16.03	19.14	1.16	1.51	0.10	0.10
29-Apr	0.01	0.02	13.43	16.18	0.80	4.24	0.52	0.84	17.12	21.61	0.83	1.58	0.10	0.13
30-Apr	0.01	0.02	13.37	13.96	0.80	2.12	0.40	0.65	18.38	20.04	1.29	1.64	0.10	0.10
1-May	0.02	0.02	11.31	12.28	0.80	3.18	0.34	0.61	20.54	19.48	1.72	1.81	0.10	0.10
3-May	0.03	0.03	11.66	12.25	0.80	0.80	0.19	0.32	21.54	19.07	1.86	2.20	0.10	0.10
8-May	0.01	0.01	10.73	9.60	0.80	0.80	0.03	0.03	21.43	20.56	0.34	0.23	0.10	0.10
10-May	0.01	0.02	0.22	9.17	0.80	0.80	0.03	0.03	1.25	20.71	0.01	0.41	0.10	0.10
13-May	0.01	0.02	9.83	8.77	0.80	0.80	0.03	0.05	20.11	22.36	0.42	0.36	0.10	0.10
15-May	0.01	0.02	9.17	7.53	0.80	0.80	0.03	0.03	21.55	21.81	0.44	0.25	0.10	0.10
Final	0.02		8.88		0.80		0.04		26.38		0.35		0.10	

[z]The numbers are mgl^{-1} of each nutrient determined by ICP before (in) and after (out) addition of new nutrient solution.

Elements lowest detection limits are (mgl^{-1}): B - 0.1; Ca - 0.02; K - 10; Mg - 0.03; Na - 0.5; P - 0.1; S - 0.1. The nutrient solutions were mixed with the intention of 100% strength. However, nutrient solution concentration was intentionally varied at different growth stages and dilutions were used as explained in text.

Table 19. Water stress level measurements during Spring 1995 (experiment I).

	Treatment (MPa)		
Date	**high stress**	**medium stress**	**low stress**
13-Mar	-0.053	-0.053	-0.053
14-Mar	-0.053	-0.052	-0.05
15-Mar	-0.068	-0.054	-0.053
17-Mar	-0.083	-0.058	-0.049
20-Mar	-0.095	-0.056	-0.05
24-Mar	-0.111	-0.06	-0.05
27-Mar	-0.107	-0.07	-0.056
29-Mar	-0.091	-0.067	-0.056
30-Mar	-0.089	-0.061	-0.053
3-Apr	-0.084	-0.055	-0.055
4-Apr	-0.097	-0.065	-0.051
7-Apr	-0.097	-0.06	-0.048
10-Apr	-0.1	-0.066	-0.043
24-Apr	-0.093	-0.063	-0.039
27-Apr	-0.095	-0.06	-0.035
1-May	-0.096	-0.061	-0.039
4-May	-0.098	-0.062	-0.043
8-May	-0.099	-0.062	-0.045
10-May	-0.099	-0.06	-0.049
17-May	-0.098	-0.059	-0.051
24-May	-0.099	-0.06	-0.05
30-May	-0.097	-0.06	-0.054

Table 20. Water stress measurements during Spring 1996 (experiment II).

	Treatment (MPa)		
Date	**high stress**	**medium stress**	**low stress**
10-Mar	-0.00708	-0.0092	-0.0123
11-Mar	-0.0123	-0.0097	-0.0097
13-Mar	-0.02011	-0.01751	-0.0097
14-Mar	-0.03313	-0.01751	-0.0123
16-Mar	-0.05396	-0.02792	-
21-Mar	-0.05656	-0.0123	-0.0149
22-Mar	-0.05624	-0.02189	-0.01397
24-Mar	-0.0536	-0.01925	-0.0034
25-Mar	-0.06152	-0.01397	0.001885
27-Mar	-0.05096	-0.01661	0.004527
29-Mar	-0.04831	-0.01397	-0.0034
8-Apr	-0.06523	-0.02797	-0.012
9-Apr	-0.0494	-0.00425	-
10-Apr	-0.05205	-0.01221	-
12-Apr	-0.05471	-0.00956	-
15-Apr	-0.0494	-0.00956	-
17-Apr	-0.04143	-0.00956	0.006379
18-Apr	-0.03288	0.003591	-
19-Apr	-0.02767	0.008801	-
21-Apr	-0.02767	0.008801	-
22-Apr	-0.03288	0.021827	0.027038
24-Apr	-0.03549	0.011406	0.029643
28-Apr	-0.03809	0.003591	0.024432
29-Apr	-0.0433	0.014012	0.008801
1-May	-0.07596	-0.02284	0.006379
2-May	-0.06268	-0.01753	0.011692
3-May	-0.12476	-0.05233	-
4-May	-0.06268	-0.02905	-
6-May	-0.12476	-0.06527	-0.03681
8-May	-0.08066	-0.04641	-0.0069
10-May	-0.09647	-0.05432	-0.0148
13-May	-0.11437	-0.06945	-
15-May	-0.11965	-0.07738	-0.05624
22-May	-0.13286	-0.06945	-0.05096

Table 21. Greenhouse weekly temperature and relative humidity measurements.

	Temperature (°C)		Relative Humidity (%)	
Date[z]	low	high	low	high
Spring 1995 (experiment I)				
4-Apr	17.5	30.7	33.6	70.9
11-Apr	17.8	29.3	50.2	79.3
18-Apr	18.3	29.8	59.8	88.5
25-Apr	16.3	27	55.2	86.6
2-May	16.4	29	41	85.9
9-May	18.3	31.3	53.6	90.9
16-May	17	30.6	59.1	89.6
23-May	19.6	32.7	57	77.7
30-May	18.3	33.3	59	90.8
6-Jun	22.6	37.4	46.6	87.2
Average	**18.2±1.83**	**31.1±2.86**	**51.5±8.74**	**84.7±6.62**
Spring 1996 (experiment II)				
22-Feb	17	34.8	29.5	70.8
Feb-29	12.8	30	25	51
7-Mar	10.3	33.16	41	53
14-Mar	13.7	29.3	44.6	70.5
21-Mar	13	26.7	26.4	56.3
28-Mar	16.3	28.4	37.8	73.7
4-Apr	12.4	29	34.5	69.6
12-Apr	14.3	24.6	37.9	72.6
19-Apr	14.8	28.4	47.2	82.2
26-Apr	14.9	29.9	39	80.6
3-May	18.1	32.6	54	90
10-May	12.5	31.5	49.3	74.8
19-May	19.5	35.5	58.5	93.5
Average	**14.58±2.57**	**30.3±3.13**	**40.36±10.24**	**72.2±13.02**

[z]Week averages starting on date shown.

APPENDIX B

Procedures

NUTRIENT SOLUTION

Chemicals	FW	Use(g)	Molarity	ml used
KNO_3	101.11	202.2	2	60
$NH_4H_2PO_3$	115.03	230.06	2	15
$Ca(NO_3)_2 . 4H_2O$	236.16	236.16	1	60
$MgSO_4 . 7H_2O$	246.48	246.48	1	15
KCl	74.56	74.55	0.5	15
H_3BO_3	61.84	4.638	0.075	
11.25^Z				
$MnSO_4 . H_2O$	169.01	0.338	0.002	15
$ZnSO_4 . 7H_2O$	287.55	0.575	0.002	15
$CuSO_4 . 5H_2O$	249.71	0.125	0.5 mM	15
$Na_2MoO_4 . 2H_2O$	241.95	0.121	0.5 mM	15
$FeSO_4 .7H_2O$	278.01	5.560	0.02	*
Na_2EDTA	372.24	7.414	0.02	*

Preparation

*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. Take 15 ml of the solution and mix with other stock solutions.

[z]A 0.042 M was utilized in experiment II (use 2.573g of H_3BO_3; use 10 ml for 15 L of final basic nutrient solution).

1. Dissolve each of the chemicals in separate 1000 ml solutions to make working stock solutions.
2. Mix given amount of each stock solution in 1000 ml volumetric flask and bring to volume with distilled H_2O .
3. Mix in 15 l plastic container with another 14 l of distilled H_2O to get final basic nutrient solution.
4. Adjust pH of final mixture with NaOH or HCl to 6.0-6.5.

Reference

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

LEAF TISSUE ANALYSES

1. Dry leaf tissue in paper bags in drying oven at 70°C for 2 days or until at constant weight.
2. Grind the samples on 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 ≅ 0.5 g of the ground tissue into a porcelain crucible and record the sample weight. Ash the sample at 500°C for 24 hours.
4. Dilute the ashed sample with 2N HCl (selected AR) and record the dilution factor. Place diluted sample into plastic test tube for analysis by inductively coupled plasma emission spectrometry).
5. Calculate element concentration:

$$\mu g.g^{-1} \text{ dry weight of element} = \frac{\text{ICP value for nutrient} * \text{dilution factor}}{\text{sample tissue weight}}$$

GREENHOUSE WATER STRESS INDUCTION USING PEG

Desired stress levels: 1 = low stress; -0.05 MPa (nutrient solution without PEG)

2 = medium stress; -0.10 MPa (PEG at 42 g/L)

3 = high stress; -0.20 MPa (PEG at 96 g/L)

Stress was imposed gradually, at approximately 2% per day by adding given amounts of PEG into 50 L of nutrient solutions.

PEG addition schedule:

Spring 1995 (experiment I):

-0.2 MPa

Date	Conc	Amt to add	Estimated potential
3/5	20 g/L	1000g	-0.075 MPa
3/6	40 g/L	1000g	-0.085 MPa
3/7	60 g/L	1000g	-0.125 MPa
3/8	80 g/L	1000g	-0.170 MPa
3/9	96 g/L	800g	-0.20 MPa

-0.1 MPa

Date	Conc	Amt to add	Estimated potential
3/8	20 g/L	1000g	-0.075 MPa
3/9	22 g/L	1100g	-0.10 MPa

Spring 1996 (experiment II)

-0.12 MPa

Date	Conc	Amt to add	Estimated potential
3/10	25.5 g/L	1275g	-0.024 MPa
3/11	50 g/L	1275g	-0.048 MPa
3/12	75.5 g/L	1275g	-0.072 MPa
3/13	100 g/L	1275g	-0.096 MPa
3/14	125.5 g/L	1275g	-0.120 MPa

-038 MPa

Date	Conc	Amt to add	Estimated potential
3/10	20 g/L	850g	-0.016 MPa
3/11	40 g/L	850g	-0.032 MPa
3/12	60 g/L	850g	-0.048 MPa
3/13	80 g/L	850g	-0.064 MPa
3/14	100 g/L	850g	-0.08 MPa

PEG (polyethylene glycole 800) was added each day at about 3:00 p.m. Samples were taken the following morning (9:00 am) and water potential determined on 5000 Vapor Pressure Osmometer (WESCOR, Inc., Logan,UT). If necessary, modifications in PEG concentrations were made based on actual osmotic potential measurement. The added amounts in experiment II were based on results of water potential measurements and plant reactions to stress in the experiment I.

References:

The water stress regime was designed and calculated by Dr. Norman K. Lownds, Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88007

Krizek, D.T., 1985. Methods of inducing water stress in plants. HortScience, Vol.20(6):1028-1036.

DETERMINATION OF RAPESEED GLUCOSINOLATE - GAS CHROMATOGRAPHIC PROCEDURE

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

Benzyl glucosinolate (1mM): Weigh 24.1 mg of benzyl glucosinolate, tetreamethyl ammonium salt, into a 50 ml volumetric flask and make to volume with water.

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 ml) and glacial acetic acid (1.5 ml) to a 1 liter 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 ml of water and swirl gently (minimizing foam formation) to dissolve sulfatase.
3. Add 3 ml of absolute ethanol and mix.
4. Centrifuge at 2000 g for 20minutes.
5. Transfer supernatant into second test tube and discard precipitate.
6. Add 9 ml of absolute ethanol to the supernatant, mix and centrifuge again. For 15 minutes.
7. Discard the supernatant and dissolve the precipitate in 2.5 ml 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 ml of 100% 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 ml of supernatant onto a 0.2 ml DEAE Sephadex A-25 column (column consits of 1 ml plastic syringe with porous polyethylne (1.6 mm, 35 μm pore size) retainer).
7. Wash column with : 1.8 ml of 67% methanol, 1.8 ml 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 over night.
9. Elute column twice with 0.45 ml of 60% methanol into a 1 ml 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 ml of water).
10. Place vial in block heater (60°C), and take to dryness by passing nitrogen over the sample.
11. Prepare derivatization mixture immediately before use: aceton, DMF, TMCS, and 1-methyimidazole (40:20:20:2:1).
12. Add 390 µl of derivatization mixture to dryed sample vial and cap 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)	290
Detector temperature (°C)	300
Initial column temperature (°C)	270
Initial time (minute)	6
Program rate	15
Final temperature	290
Final time	5

Calculation of individual glucosinolate content

$$\frac{\text{Area unknown}}{\text{Area benzyl}} * RF * 0.86 * \frac{1000}{200} = \mu mol/g \text{ of seed}$$

Benzyl glucosinolate = internal standard.

RF = response factor.

Individual glucosinolate response factors:

Allyl	1.16
3-butenyl	1.12
4-Pentenyl	1.07
2-hudroxy-3-betanyl	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 Center, Ottawa.

DETERMINATION OF RAPESEED GLUCOSINOLATE CONTENT - COLORIMETRIC PROCEDURE

Chemicals

Acetic Acid (glacial).

4-Aminoantipyrene.

Ammomium thiocyanate standard solution (10 N).

Barium Acetate

Ferric nitrate.

β-D-glucose.

Glucose oxidase.

Imidazole.

Lead acetate.

Mercuric chloride.

Myrosinase (prepared from yellow mustard (*Sinapis alba*) seeds).

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) and glacial acetic acid (15 ml) 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 (4.45g or 4.24 ml) to a 1L volumetric flask and make to volume with water.

Color reagent I: Add glucose oxidase (14.4 mg) and 4-aminoantipyrine (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 II together.

Ferrric nitrate reagent: Add ferric nitrate (6 g) to a 25 ml volumetric flask and make to volume with 1.5 N nitric acid (5 ml). (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.

Sample preparation

1. Dry seed sample (about 300 g) at 45oC for 1 hour.
2. Prepare columns with 0.2 ml DEAE Sephadex A-25 (columns consists of 1 ml plastic syringe with porous polyethylene (1.6 mm, 35 µm pore size) retainer).

3. Grind samples as fine as possible, then place 200 mg into appropriate test tube.
4. Place tube into water bath with boiling water for 1 minute.
5. Add 1 ml of hot water (>90°C), shake tube and place back in boiling water for 3 minutes.
6. Add 0.5 ml barium lead acetate - lead acetate solution. Vortex.
7. Centrifuge at 2000 g for 10 minutes and transfer supernatant to a volumetric test tube.
8. Add 1.5 ml water to a test tube, vortex and centrifuge. Transfer supernatant to the volumetric tube. Repeat.
9. With a pasteur pipette, add water to a volumetric tube to bring to 5 ml. Vortex.
10. Place 2.5 µml extract on each column.
11. Wash column (plus blank column) twice with 0.5 ml 4 M glacial acetic acid, then twice with 1 ml water.
12. Add 0.1 ml fresh thioglucosidase (myrosinase) solution (3 mg/ml) to a column. Cover and let sit over night.

Determination of glucose standard

1. Prepare glucose standards if there are none less than 24 hours old. Prepare half serial dilution: 1µmol/ml, 0.5, 0.25, 0.125, 0.0625. To make 1 µmol/ml, use 1.186 g β-D(+) glucose and make up to mark with water in a 1 liter volumetric flask.
2. Prepare fresh glucose oxidase-peroxidase reagent (equal volumes of color reagent I and II).

3. Place 0.2 ml of each concentration of glucose standard into test tubes.
4. Add 0.6 ml water and 0.4 ml fresh glucose oxidase-peroxidase reagent to each glucose standard test tube.
5. Prepare a reference sample. Add 0.8ml water and 0.4ml fresh oxidase-peroxidase ragent to a test tube.
6. Vortex tubes with glucose standards and let stand 1 hour.
7. Record the absorbance of the standards at 505 ηm.

Determination of thiocyanate ion standard

1. Prepare NH_4SCN standards if there are none less then 24 hours old. Prepare half serial dilutions: 1 μmol/ml, use 0.076 ammonium thiocyanate and make up to mark with water in a 1 liter volumetric flask.
2. Prepare fresh ferric nitrate (weigh 6 g ferric nitrate in 25 ml volumetric flask and make to volume with 1.5 N nitric acid.
3. Place 0.2 ml of each concentration NH_4SCN standard into test tubes.
4. Add 0.6 ml water and 0.4 ml fresh ferric nitrate reagent to each NH_4SCN standard test tube.
5. Prepare a reference sample. Add 0.8 ml water and 0.4 ml fresh ferric nitrate reagent to the test tube.
6. Vortex the tubes and let stand in dark for 15 minutes.
7. Read the absorbance of the standards at 470 ηm.

Determination of total glucosinolate content

1. Set up sample vials under columns (samlpes and blank).
2. Elute each column with 1 ml water.
3. To each of the sample test tubes and blank, add 0.2 ml elute from corresponding vial, 0.6 ml water, and 0.4 ml fresh glucose oxidase-peroxidase reagent.
4. Vortex and let stand for 1 hour.
5. Record the absorbance at 505 ηm.

Determination of indole glucosinolate content

1. Set up sample vials under columns.
2. Place 1 ml of 0.5 N NaOH into each column and let stand for 15 minutes.
3. Elute into vials.
4. Place 1 ml of 0.5 N nitric acid into each column and elute.
5. To each of the test tubes add 0.2 ml elute, 0.6 ml water, and 0.4 ml fresh ferric nitrate reagent.
6. Prepare reference sample. Add 0.8 ml water, 0.4 ml fresh ferric nitrate reagent, and 0.02 ml 6% mercuric chloride.
7. Vortex the samples and let stand for 15 minutes in the dark.
8. Record the absorbance at 470 ηm for the first samples using the reference without mercuric chloride. Then add 0.02 ml mercuric chloride to the sample, insert the reference with mercuric chloride and record the absorbance at 470ηm.
9. Repeat for the rest of the samlpes.

Calculation of total glucosinolate content:

$$A * \frac{1}{m} * 1.1 * \frac{5.0}{2.5} * \frac{1000}{200} = \mu mol/g \text{ of seed}$$

A = sample absorbance (minus blank)

m = slope of plot of glucose standards

Calculation of indole glucosinolate content:

$$A * \frac{1}{m} * 1.1 * \frac{5.0}{2.5} * \frac{1000}{200} = \mu mol/g \text{ of seed}$$

A = 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. Proceeding of the oil crops network Brassica sub-network workshop held in Shanghai, China April 21-23, 1990. International Development Research Centre, Ottawa.

VITA

Barbara Kocourkova was born in 1971 in Chrudim, The Czech Republic. She grew up and attended high school in Prague. In 1989 she was accepted to the Agriculture University of Prague, College of Agronomy. After the student revolution of Fall , 1989, in which she took part, she took advantage of open borders and new opportunities and traveled extensively to the USA, Great Britain, France, Germany ,and The Netherlands. In 1992 she received a scholarship in business and public policy from the Northwood University in Midland, MI, for nondegree training during two semesters. In 1994 she graduated from the Agriculture University in Prague with Master of Science equivalent diploma in Horticulture.

During Fall, 1994 she met with Dr. Carl E. Sams who offered her a graduate research assistantship at the Plant and Soil Science Department, at the University of Tennessee, Knoxville, to work toward a MS degree in crop physiology and nutrition.