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THE EFFECTS OF SULFUR FERTILIZATION AND WATER STRESS ON THE MORPHOLOGICAL TRAITS OF SPRING CANOLA (*Brassica napus* L.) AND THEIR CONSEQUENCES ON SEED GLUCOSINOLATE, PROTEIN, OIL CONTENT, AND FATTY ACID PROFILE

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Habib Ben Hamza

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By

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ABSTRACT

THE EFFECTS OF SULFUR FERTILIZATION AND WATER STRESS ON THE MORPHOLOGICAL TRAITS OF SPRING CANOLA (*Brassica napus* L.) AND THEIR CONSEQUENCES ON SEED GLUCOSINOLATE, PROTEIN, OIL CONTENT, AND FATTY ACID PROFILE

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Glucosinolates are sulfur-containing compounds of the secondary plant metabolism found typically in Brassica species. Levels of these compounds have become important criteria for canola quality (*Brassica napus* L.) seeds. Although canola cultivars are developed for low glucosinolate content in the seed, soil sulfur levels and water stress have been suggested to increase the concentration of this compound.

Four independent experiments were conducted to study the effects of sulfur fertilization on glucosinolate content of canola seeds under both greenhouse and Michigan field conditions and water stress and both field and controlled conditions. Sulfur fertilization and water stress effects on yield and yield components, protein, oil content, and fatty acid profile were also determined. Suflur fertilization experiments were conducted during 1991 and 1992 at the Michigan State University Agronomy Farm. The experimental design was a randomized complete block with 4 replications, using 3 spring canola cultivars (Bounty, Delta, and Westar), and sulfur rates of 0, 45, and 90 kg S/ha. The sulfur greenhouse experiments were conducted under controlled greenhouse conditions. Delta canola cultivar was grown in five different sulfate concentrations,

0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in modified Hoagland's nutrient solution.

Field water stress experiments were conducted during 1991 and 1992 under a Rainshelter at Kellogg Biological Station near Hickory Corners Michigan. Three cultivars were grown in two different soils (Spinks sand and Kalamazoo loam) under 2 water treatments (control and stressed). Plants were stressed by withholding irrigation water at flowering. Control plots were watered until seed maturity.

Field and greenhouse sulfur treatments sharply increased sulfur content of canola leaves during flowering, indicating a positive response to increasing sulfur applications. Sulfur treatments significantly influenced morphological traits of spring canola under greenhouse conditions but not under field conditions. Sulfur treatments tended to increase seed protein content and decrease its oil content. Sulfur treatments increase seed yield per plant under controlled greenhouse conditions, but yields were not affected under Michigan field conditions. Sulfur treatments increased seed glucosinolate to levels exceeding the canola standard of 30 μmoles of total glucosinolate/g of defatted meal.

Field and greenhouse water stress treatments significantly influenced morphological traits of spring canola, particularly number of pods per plant and seed yield. Water stress treatments influenced protein and oil content and fatty acid profile in the seeds. The ratio of linolenic to linoleic acid was reduced by water stress treatments under field conditions.

Seed glucosinolate levels increased sharply with stress treatment and were above the canola standard of 30 μmoles of total glucosinolate /g of defatted meal, suggesting that in spring canola production areas, warmer and drier season may increase the levels of glucosinolates in the seed.

Dedicated to my parents, my uncle, and my brothers and sisters,
For their sacrifice and continuous encouragement,

And to my beloved wife and daughter,
For their love, patience, motivation, and endless support.

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INTRODUCTION

Over the last two decades, rapeseed/canola (*Brassica napus* L.) has developed as an important world oilseed crop and its production during the past decade has grown faster than any other source of edible oil (Shahidi, 1990). Almost 20% of current global supply of rapeseed comes from within the European Community (FAO, 1991). Canola oil is regarded as a unique edible vegetable oil that is nutritionally well-balanced. It has an almost ideal mixture of fatty acids for human nutrition and health. Canola seeds contain nearly 40% oil and 22% protein, and yield about 38-43% protein in the defatted meal. The proteins in the meal have a favorable composition of essential amino acids.

Despite its nutritive value and economic importance, like most cruciferous plants, canola seeds contain naturally occurring substances called glucosinolates. Glucosinolates are sulfur containing compounds found in the secondary plant metabolism of *Brassica* species. Canola (*B. napus* L.) seeds should normally contain less than 30 μmoles/g glucosinolates of defatted meal and 2% or less erucic acid (22:1n-9) in its oil. These levels have become important criteria for quality. Although, most canola cultivars are genetically fixed for low glucosinolate content, it has been suggested that drought stress, soil sulfur and other factors affect the synthesis and concentration of these compounds by mechanisms not yet fully understood. Water stress has been reported to reduce yield and oil concentration and increase the glucosinolates concentration, while increased soil

sulfur availability causes significant increases in the glucosinolate levels of canola seeds and consequently lowers the protein content of the defatted meal.

The objectives of this research were to:

1. Study the effects of sulfur fertilization rates on the morphological traits, seed glucosinolate concentration, oil and protein content, and fatty acid profile of spring canola grown under Michigan field conditions.
2. Study the effects of five sulfate concentration on the morphological traits, seed glucosinolate concentration, oil and protein content, fatty acid profile, and mineral composition of spring canola grown in modified Hoagland's nutrient solution under controlled greenhouse conditions.
3. Examine the effect of water stress and soil type on the morphological traits, seed glucosinolate concentration, oil and protein content, and fatty acid profile of spring canola under Michigan field conditions.
4. Study the effect of water stress on the morphological traits, seed glucosinolate concentration, oil and protein content, and fatty acid profile of spring canola grown in pots under controlled greenhouse conditions.

LITERATURE REVIEW

Rapeseed History and Origins

The word "rape" in rapeseed originated from the Latin *rapum* meaning turnip. Turnip (*Brassica rapa*), rutabaga (*Brassica napobrassica*), cabbage (*Brassica oleracea var. capitata*), mustard (*Brassica juncea*) and many other well-known vegetables are close relatives of rapeseed/canola (Shahidi, 1990). Early man domesticated rapeseed, among other crops. Ancient civilizations in Asia and along the Mediterranean recorded the use of rapeseed oil for illumination, and later as a cooking oil. In the Indian subcontinent, rapeseed has been cultivated for more than 3,000 years.

Unlike most other oilseeds, rapeseed comes from several species belonging to the genus *Brassica*. These species include *B. napus*, *B. campestris*, and *B. juncea* which are known as rapeseed, turnip rape, and leaf mustard (Downey, 1983). These species are closely related and similar in appearance. The botanical relationships of common rapeseed species are illustrated by the "U triangle" proposed by the Japanese scientist U (1935). There are three basic species, *B. nigra*, *B. oleracea*, and *B. campestris*. By hybridization and chromosome doubling, the three species, *B. carinata*, *B. juncea* and *B. napus* were artificially synthesized.

Canola Development

The traditional varieties and types of rapeseed that are still being produced in Asian countries contain about 22 to 60% erucic acid (C22:1) in their oils. These cultivars are called high erucic acid rapeseed (HEAR). While the presence of erucic acid lowers the nutritional value of the oil, anti-nutritional glucosinolates also affects the feeding value of the rapeseed meal. In Canada during the 1960's, rapeseed species and varieties were genetically modified to decrease the concentration of erucic acid. In 1968, the first low erucic acid rapeseed (LEAR) cultivar, containing less than 5%, was cultivated. This variety was also referred to as "single-low" (Shahidi, 1990), with the low-glucosinolate character obtained from the Polish cultivar Bronowski. Candle, the first *B. campestris* cultivar to be low in both erucic acid and glucosinolates was grown in Canada in 1976 (Shahidi, 1990).

The name "canola" was adopted in 1979 to apply, in Canada, to all "double-low" cultivars. It is the acronym of "**Can**adian **O**il with **L**ow **A**cid" and is a trademark of the Canola Council of Canada. Consequently, canola can be used to describe any rapeseed cultivar with 2 % or less erucic acid (C22:1) in the oil and no more than 30 micromoles per gram of glucosinolates in the defatted meal.

Canola Quality as food and feed

Canola oil is perhaps the only edible vegetable oil that, by today's standards, is considered nutritionally well-balanced. It has an almost perfect mixture of fatty acids for human nutrition and health. The fatty acid composition is uniquely well-balanced. Ackman (1990) reported that oleic acid, the major component fatty acid of canola oil

(60%), is as effective in reducing cardiovascular risk as the polyunsaturated linoleic acid. The second major (20%) fatty acid, linoleic acid, is established as an "essential" fatty acid required in the daily diet of humans. The third major fatty acid, linolenic acid, regarded as the most effective and functional fatty acid in reducing cardiovascular risk, is provided at the ideal ratio of 1:2 relative to the linoleic fatty acid.

Canola seeds contain approximately 40% oil, 22% protein, and yield about 38-43% protein in the defatted meal. The seed coats comprise about 16-18% of the total and about one-third that of the defatted meal. Canola meal constitutes 50-58% of the seed weight on a dry basis. The proteins in the meal have a favorable composition (Ohlson and Anjou, 1979; Sarwar et al., 1984) of essential amino acids, namely lysine, methionine, cysteine, threonine and tryptophane, comparing favorably with that of cereals (Diem and Lenter, 1975; Larsen and Sorensen, 1985). This valuable meal is ideally suited for livestock rations. In China, large amounts of rapeseed meal are used as fertilizers.

Despite its nutritive, economic and industrial value, like most cruciferous plants, canola seeds contain naturally occurring substances called glucosinolates which limit the utilization of canola to its fullest potential. Following is a review of the chemistry, biosynthesis, analysis, and regulatory factors of these secondary metabolites.

GLUCOSINOLATES

Glucosinolates are sulfur-containing compounds produced by the secondary metabolism of certain plant species (Schnug, 1990). Glucosinolates are known to occur in fifteen dicotyledonous families including *Akaniaceae*, *Bataceae*, *Brassicaceae*, *Bretschneideraceae*, *Capparaceae*, *Euphorbiaceae*, *Brassocaceae*, *Gyrostemonaceae*, *Limnanthaceae*, *Moringaceae*, *Pentadiplantdraceae*, *Resedaceae*, *Salvadoraceae*, *Tropaelaceae and Tovariaceae* (Kjaer, 1974; Ettlinger, 1987). All these families are on the same level of evolution without any marked differences in floral morphology (Hofmann, 1987). Therefore the ability to synthesize glucosinolates seems to be the result of parallel evolution (Rodman, 1987).

In the limited context of cultivated crops for human foods and animal feedstuffs, members of the *Brassicaceae* families are very important. They include oilseeds, forage crops, condiments, relishes and vegetables (Crisp, 1976).

Definition and Structure

The glucosinolates are chemically defined as natural plant substances with a side chain (R-group) and D-glucose as ß-thioglucoside attached to the carbon atom in *cis*-N-hydroxime sulfate esters (Olsen and Sorensen, 1981) (Figure 1). X-Ray crystallographic studies (Marsh and Waser, 1970) and direct synthesis (Ettlinger and Lundeen, 1957)

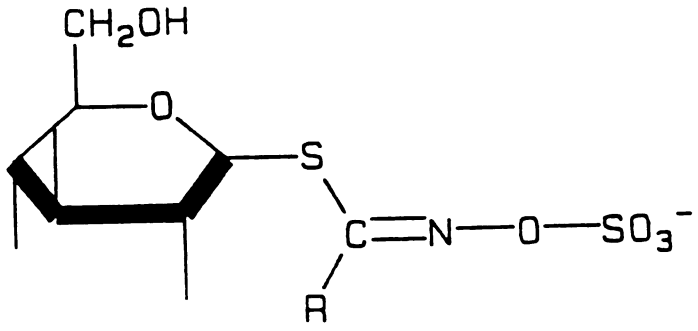

Figure 1. **Principal structure of a glucosinolate molecule. (R is a side chain with structural resemblance to the parent amino acid).**

confirmed their structure and established its main features as a sulfonated oxime grouping shown to be *anti* with the side chain R and *syn* with a thioglycosidic moiety. The sugar, in nearly all cases is a D-glucose. The glucosinolate side chain may comprise aliphatic, aromatic or heteroaromatic groupings which reflect individual properties of each of the glucosinolates (Sorensen, 1990). It also determines the chemical nature of the products of enzyme hydrolysis and hence, their biological effects and potencies (Fenwick et al. 1989). Furthermore, the structural variations among known glucosinolates are due mainly to various side chains that have structural similarity to those of the biosynthetic amino acid precursors (Bjerg et al. 1987b; Eggum and Sorensen, 1989).

Separation and Isolation of Glucosinolates:

Generally, a plant species contains more than one glucosinolate based on the nature of group (R). There are often both qualitative and quantitative differences between the glucosinolate content of the roots, leaves and seeds of a plant (Elliot and Stowe, 1971). Variations in the total glucosinolate content among different genotypes, as well as among individual plants within a genotype have also been noted and provide the basis for breeding programs to eliminate glucosinolate from oil seed crops (Josefsson and Jonsson, 1969). The vast majority of the more than 100 known glucosinolates have not been isolated in the pure state. In recent years, there has been an increasing need for pure glucosinolates in order to assess their anti-nutritional and toxicological properties (Fenwick et al. 1989). The isolation and purification of glucosinolates constitute a challenge because of the difficulty in their extraction (Fenwick et al. 1989). Seed meal is usually preferred, however, it should be handled with extreme care to avoid chemical

and/or enzymatic degradation. The meal should be extracted in boiling alcohol and the glucosinolates separated by column chromatography.

Biosynthesis

Biosynthetic studies have revealed that all glucosinolates are derived from amino acids and that most are formed by a common biosynthetic pathway (Figure 2). Starting with an α-amino (e.g. methionine in the case of alkenyl-, thio-, sulfinyl- and sulfonylglucosinolates; tryptophane in case of indol-glucosinolates), the first stable products in this pathway are hydroxylated amino acids, which are the precursors of aldoximes (Kindl and Underhill, 1968; Underhill, 1980). The aldoxime undergoes oxidation to a nitro compound, the tautomer of which constitutes the site for introduction of thioglycoside-S (Underhill and Wetter, 1973). In studies by Underhill and Wetter (1973) cysteine-S was most readily incorporated to produce a thiohydroxamic acid. UDP-glucose-mediated S-glucosylation produces the desulfoglucosinolate and the final product is obtained following reaction with S3'-phosphoadenosine-5'phosphosulfate (PAPS). Bjerg et al. (1987) suggested that direct incorporation of the amino acid precursors into the corresponding glucosinolate with preservation of the amino acid nitrogen has been shown to occur. While some glucosinolates contain methyl, isopropyl, or 4-hydroxybenzyl side chains are derived from corresponding amino acids (alanine, valine and tyrosine), others require elaboration which commonly involves homologation, elimination or addition and hydroxylation (underhill and Wetter, 1973).

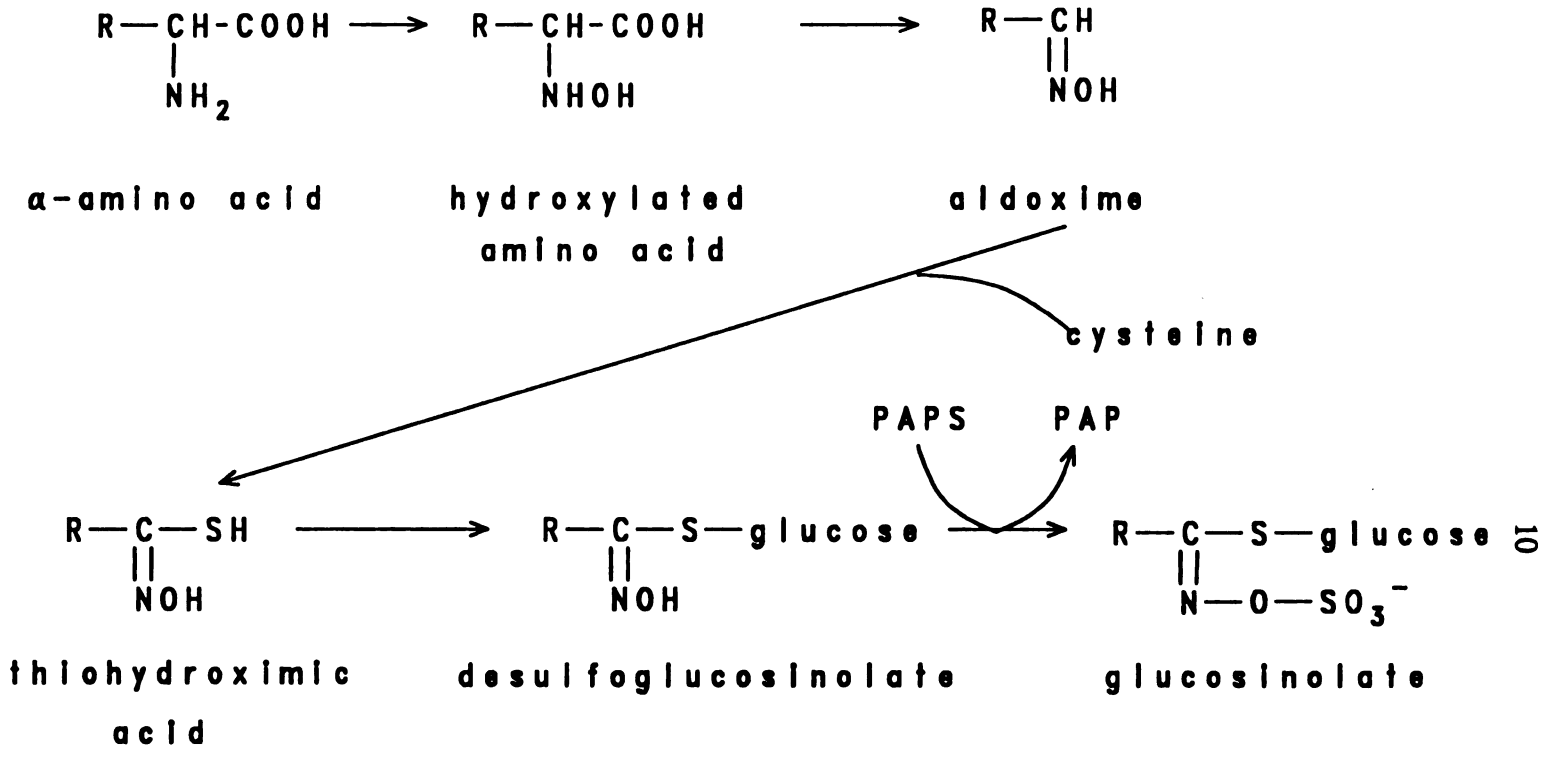

Figure 2. **Biosynthesis of glucosinolates.**

Metabolism

Glucosinolates are always accompanied within the same plant by the hydrolytic enzyme, thioglucosid-glucohydrolase myrosinase. Myrosinase always occurs in plant tissues, either isolated in idioblasts, stomatal guard cells or inside cells associated to cisterns of the endoplasmic reticulum and mitochondria, respectively (Joergensen, 1987 and Pihakaski and Iversen 1976). When the plant tissues are crushed or during a mechanical injury, myrosinase hydrolyses the ß-glucosidic bond between glucose and reduced sulfur (Bjorkman, 1976), splitting the glucosinolate molecule to glucose and aglucone. Under neutral conditions, the aglucone decomposes to sulfate ion, and by a Lossen-type rearrangement, produces an isothiocyanate (Schnug, 1990). Glucosinolate side chains are extremely varied, as are the properties and functions of glucosinolates and their degradation products, including isothiocyanates, oxazolidine-2-thiones, nitriles, thiocyanates, thiocyanate ions and amines (Sorensen, 1985).

Determination of Glucosinolates

Numerous methods have been reported for the quantitative and qualitative analysis of glucosinolates. The comparison of different analytical methods has always been a problem in glucosinolate research (Schnug, 1989). Chromatographic, colorimetric, enzymatic, and instrumental methods of glucosinolate analysis have produced different results in identical samples (Wagstaffe, 1989).

With the development and introduction of the new OO-varieties containing low amounts of glucosinolates, high performance liquid chromatography (HPLC) became the preferred method for determination of individual glucosinolates (Whatelet, 1986; Bjerg

et al., 1987c) whereas X-ray fluorescence spectroscopy seem to be the most reliable method for total glucosinolate analysis (Schnug and Haneklaus, 1988). The HPLC analysis technique can be advantageous in detecting both intact or desulfated individual glucosinolates (Moller, 1984a).

Intact glucosinolates can be easily extracted in boiling methanol water solutions, homogenized, isolated, purified, concentrated on DEAE-Sephadex A-25 micro-columns, and eluted in HPLC vials for analysis (Moller et al., 1985).

Isolation of desulfoglucosinolates can also be used in HPLC analysis of crude canola seed extracts. This technique involves similar preparation steps: extraction, homogenization, isolation, purification, concentration of desulfated glucosinolates on micro-columns, and their elution in HPLC vials. Desulfation of glucosinolates is obtained by a solution of sulfatase which is not known to occur in glucosinolate-containing plants.

The determination of the total glucosinolate content by X-ray fluorescence is a relatively new method (Schnug and Haneklaus, 1988) . It is based on the close association between total sulfur and total glucosinolate content in rapeseed and does not require lengthy, precise, chemical processes. However, the factors that influence the relationship between seed sulfur content and glucosinolates affects the precision and reproducibility of results.

Antinutritional Effects of Glucosinolates

Many members of the Brassicaceae family are used as foods and condiments, including cabbage, rutabaga, turnip, various *Lepidium* species, radish, horseradish, and

white and yellow mustard. Considerable variation in the flavor volatiles occur among these plants due to the different kinds and amounts of hydrolytic products formed (VanEtten et al., 1969; Daxenbichler et al., 1977) during their degradation.

Animals: Many studies have been conducted on the antinutritional effects of rapeseed meal. Such effects are generally attributed to the glucosinolate content and composition of their degradation products (Fenwick et al., 1989). Ingestion of substantial amounts of glucosinolates have been shown to result in reduced performance, enlarged thyroid glands and reduced levels of circulating thyroid hormones in pigs (Thomke, 1984; Thomke et al., 1983, Eggum et al., 1985; Nasi et al., 1985), lambs, mature sheep, beef steers (Bush et al., 1978) and bulls (Iwarsson et al., 1973). High intake of glucosinolates in feedstuff rations has been suspected to cause reproduction problems such as poor conception and goitrogenicity (Laws et al., 1982). The thiocyanate ion has been reported to cause goiter (Astwood, 1943; VanEtten and Tookey, 1983; Heaney and Fenwick, 1987) in pigs (Rundgren, 1983; Fiems and Buysse, 1985), poultry (Fenwick and Curtis, 1980; Clandinin and Robblee, 1981) and dairy cattle (Fiems and Buysse, 1985).

Glucosinolates breakdown products are associated with liver abnormalities and liver hemorrhage in livestock (Bourdon et al., 1980; Barry et al., 1981; Pfirter et al., 1982; Fritz et al., 1983; Bougon and Guyen, 1985). The antinutritional effects of glucosinolates are also associated with skeletal abnormalities (Timms, 1983), low palatability (Lee et al., 1984) and egg taint (Butler and Fenwick, 1984).

Humans: Glucosinolates are important as precursors of the substances giving desirable pungency to mustard and radish and the characteristic flavor of *Brassica* vegetables (Fenwick et al., 1989). Their breakdown products can pass indirectly into the human diet in foods derived from animals which feed on cruciferous material (Fenwick et al., 1989). Although little is known about the metabolic fate of glucosinolates in the digestive tract, it has been suggested that endemic goiter in Finland may be related to goitrogens in milk (Elfving, 1980).

Factors Affecting Glucosinolates Levels

Although the relative amount of glucosinolates in a particular species is under genetic control, the actual levels are subject to a variety of influences (Fenwick et al., 1983). For example, factors such as drought and close plant spacing which impose stress on the developing seeds have been shown to increase glucosinolates levels (Fenwick et al., 1983). The effects of sowing time on glucosinolate content of harvested rapeseed have also been reported by Sang et al. (1986) to influence glucosinolate level of the seed. Harvesting techniques were reported to be responsible for the observed instability of glucosinolates in low-glucosinolate rape cultivars, with harvested seed sometimes containing twice the glucosinolate content of the seed planted (Parnell, 1983).

Field studies have shown that canola/rapeseed exhibit significant cultivar and site variability in chemical composition, particularly for glucosinolate level (Mailer and Wratten, 1985). However, in a study by Mailer and Pratley (1990), the two primary factors which contributed most to environmental variation were soil sulfur and water status. Increased sulfur availability has been shown to cause significant increases in

glucosinolate concentration of the seed (Josefsson and Appleqvist, 1968; Josefsson, 1970; Shnug, 1987 and Mailer, 1989). Similarly, under controlled environmental conditions, water stress has been shown to reduce seed yield and oil level but significantly increase the glucosinolate concentration (Mailer and Cornish, 1987).

SULFUR NUTRITION

Sulfur (S) is characterized as an essential macronutrient (Arnon and Stout, 1939). for which the optimal growth requirement varies between 0.2 and 0.5% of the dry weight of plants (Marschner, 1986). Loneragan (1968) defines a crop's requirement for a specific nutrient as "the minimum content of that nutrient associated with the maximum yield" or the minimum rate of intake of the nutrient associated with the maximum growth rate.

Both uptake and requirement for S differ greatly among species and cultivars, as well as stage of crop development (Gerloff, 1973; Thompson et al. 1970). Crops that commonly contain the largest amounts of sulfur include most of the *Brassicaceae* family. In the temperate regions, agricultural crops are reported to contain 0.1 to 1.5% S (Eaton, 1966), whereas in tropical areas, crops generally contain between 0.1 to 0.5% (Whitehead, 1964).

Plant roots absorb most of their S from the soil in the form of sulfate ion (SO_4^{2-}) (Bardsley, 1960). Supplies of Soil SO_4^{2-} are supplemented at varying rates during the growing seasons by buildup from atmosphere, fertilizers, pesticides, and from soil organic matter degradation (Duke and Reisenauer, 1986).

Plant Sulfur Metabolism

Sulfur (S) is one of 6 major macroelements including nitrogen, potassium, phosphorus, magnesium, and calcium (Marschner, 1986). Because of its status as a major plant nutrient, knowledge of plant sulfur metabolism is essential for understanding the roles of plant sulfur compounds.

Plants receive most of their S as SO_4^{2-} (Epstein, 1976). Since SO_4^{2-} is inert, its activation is a necessary first step in its utilization (De Meio, 1975). The activation process involves 2 reactions forming adenosine 5'-phosphosulfate (APS) which is transformed into adenosine 3'-phosphate 5'-phosphosulfate (PAPS) (Lipmann, 1958; Wilson and Bandurski, 1956).

In higher plants, APS, activated S, is reduced and the reduced sulfur is incorporated into cysteine. The S of cysteine is transferred into methionine and other compounds. Cysteine and methionine are incorporated into amino acids.

Role of Sulfur in Plants

Sulfur is a constituent of the amino acids cysteine and methionine and, hence, of proteins. Both of these amino acids are precursors of other sulfur-containing compounds such as coenzymes and secondary plant products. Sulfur is a structural constituent of these compounds or acts as a functional group (e.g. R-SH) directly involved in several metabolic reactions.

Disulfide bonds between cysteine residues in proteins play a very important role in connecting different parts of a polypeptide and/or several polypeptides (Torshinsky, 1981). Furthermore, free cysteinyl sulfhydryl groups, SH or thiol groups, may

contribute to the biological activity of plant protein by functioning in a number of roles including binding of substrates and coenzymes to enzymes and metal cofactors to enzymes and other proteins. Anderson (1978) estimated that as many as 40% of all enzymes require a free cysteinyl SH group for catalytic activity.

Sulfur may also be found in sulfolipids and therefore constitute a structural component of all biological membranes. Sulfolipids are particularly abundant in the thylakoid membranes of chloroplasts. Sulfolipids are also involved in the regulation of ion transport across other membranes (Marschner, 1986).

Volatile compounds such as isothiocyanates and sulfoxides with sulfur as a structural constituent are mainly responsible for the characteristic odor of such plant species as onion (*Allium cepa* L.), garlic (*Allium spp.* L.) and mustard (*Brassica juncea* L.). Of these compounds glucosinolates (previously called "mustard oils") accumulate in intact cells predominantly as non-volatile glucosides containing sulfur (see above).

The objective of the present research was to study the effects of sulfur fertilization rates on the morphological traits, seed glucosinolate concentration, oil and protein content, and fatty acid profile of spring canola grown under Michigan field conditions.

MATERIALS AND METHODS

Three Spring canola (*B. napus*) cultivars, Bounty, Delta and Westar, were used in this study. These varieties were selected for their low glucosinolate content and similar maturity period.

Planting, Sulfur Fertilization and Experimental Design

This study was conducted at the Michigan State University Agronomy Farm in East Lansing, MI. Seed of each cultivar was planted at rate of 5.6 kg/ha in a completely randomized block design with four replications. Each replication consisted of a five-row plot 6 m long and 92 cm wide. Urea (46% nitrogen) was applied at the rate of 140 kg N/ha. In the first year, the spring cultivars were planted on May 4, 1991 using a small plot planter and harvested on August 15, 1991. In the second year, the cultivars were planted on May 5, 1992 and harvested on August 31, 1992.

In both years, when plants were at the rosette stage, three sulfur treatments were applied by hand at rates of 0 kg/ha, 45 kg /ha and 95 kg/ha of potassium sulfate (K_2SO_4), respectively. Potassium (K_2O) at 45 kg/ha was applied to the control plot to compensate for the potassium added to sulfur fertilized plots.

Material Sampling

Twenty leaf samples were collected from each replication. The leaves were dried, ground and analyzed for total sulfur content. Sulfur analysis of leaf samples were

performed at the Ohio State University Research Extension Analytical Laboratory in Wooster, OH. The procedure used in the analysis is described by (Hern, 1984).

Twenty whole plant samples were randomly taken from each replication at physiological maturity for use to determining yield components. Variables determined included plant height, branches/pod, pod length, seeds/pod, and weight of seeds/plant. Plant height is a measure in centimeters (cm) of the primary stem length. All 20 pods used to determine the pod length and seeds per pod were randomly selected on the primary stem adjacent to the last secondary branch.

At harvest, the moisture content of the seed was determined using a Dickey-John Multi-Grain portable moisture tester (Seedburo Equipment Company, Chicago, IL). Seed yield was determined by adjusting the seed moisture to 8%.

Ground seed samples from all experiments were analyzed for total seed sulfur content by the Ohio State University Research Extension Analytical Laboratory in Wooster, OH.

Determination of Lipid Content by Gas Chromatography (GC)

Lipid content and fatty acid profile were determined using the Sodium Methoxide Method. About 20 seeds were placed in 4 ml glass vials and crushed, using a glass rod. Lipids were extracted by adding 3 ml of heptane containing an internal standard, the fatty acid (C17:0). The vials were left overnight on a shaker to enhance the extraction. A 900-μl aliquot was pipeted to 10 ml volumetric flask to which 1 ml of 0.4 N sodium methoxide was added.

The flasks were allowed to stand for 30 min, brought to a 10 ml volume with deionized water and mixed by gently inverting them 10 times. Finally, the samples were allowed to stand for 10 min to allow separation of phases. The organic phase of all samples was then pipeted into autosampler vials for gas chromatography analysis. The injection volume was 0.5 μl. Results were reported in percent (%) fatty acid methyl-esters (FAME).

Determination of Lipid Content by Nuclear Magnetic Resonance

Oil content of seed samples was also determined by Near Magnetic Resonance (NMR) analyzer. 1.2 g of clean, whole, and unbroken seeds were dried for 2 hours at 130 °C in aluminum cups. After drying, the seeds were immediately transferred to plastic NMR vials to bring them to equilibrium with NMR room temperature. The NMR analyzer was calibrated using samples containing 0, 50, 100% oil. Oil content of seed samples was determined individually. Two reading of each samples were taken.

Protein Content (Nitrogen Content)

The protein content of the seed was determined using the modified Kjeldahl method as outlined by Warner and Jones (1970). 0.15g ($\pm$ 0.02) of ground sample was weighed into a 75 ml digestion tube. Kjeldahl catalyst tablets (a mixture of 5 g K_2SO_4 and 150 mg $CuSO_4$) were put in each digestion tube to which 7 ml of concentrated sulfuric acid (H_2SO_4) were added. The tubes were mixed on a Vortex mechanical shaker for 5 seconds. They were then set on a digestion block and the temperature was gradually increased to 190°C. The samples were digested for 90 min or until digestion was complete, after which digestion, the tubes were allowed to cool for 20-25 minutes.

20 ml of distilled-deionized water were added to each tube. They were gently mixed and allowed to cool for 30 min.

The digestion tubes were then brought to a 75 ml volume with distilled-deionized water, capped with a rubber stopper and mixed thoroughly. A 20-ml sample of was filtered through a Whatman No. 1 filter paper and total nitrogen of an aliquot of filtrate measured by a Lachat Flow Injection Analyzer using the colorimetric procedure.

The % total nitrogen was multiplied by a factor of 6.25 to obtain the crude protein content of the seeds.

Glucosinolate Content Determination

The determination of seed glucosinolate content method was done using the method adopted by the Canadian Grain Commission Grain Research Laboratory (Daun and McGregor, 1981). This procedure involves the extraction and isolation of desulfo-glucosinolates on DEAE Sephadex micro-columns. It is a lengthy process involving several intermediate chemical steps that are performed in 2 to 3 days.

Preparation of Samples and Extraction of Glucosinolates

Five grams of clean seed were ground as fine as possible using a Braun Coffee Grinder and put in small plastic bags. Exactly 200 mg of ground seed were weighed directly into 4 ml glass vial.

Vials with the ground seed were put on a heating block set to 95°C for 15 min. One ml of boiling, deionized H_2O was added to each sample. The samples were heated for exactly 1 min and withdrawn from the heating block since a longer time may have degraded the samples and limited the extraction. One ml of internal standard benzyl-

glucosinolate was added to each sample. Vials were then mixed by a stirrer (vortex type) for 10 seconds and then put on the heating block for 1 min. After this, the samples were put in the freezer for 15 min and 100 μl of lead and barium acetate solution added to each sample. Then samples were mixed on vortex for 10 seconds. Finally, the samples were centrifuged for 20 min at 3500 rpm and held in the refrigerator until needed.

Micro Column Preparation

Fifty mg of DEAE Sephadex A-25 ion exchange resin were put into micro columns to which 10 ml of deionized water was added. Bubbles were removed by gentle swirling and then allowed to settle overnight.

Preparation of Columns and Sample Loading

The ion exchange micro columns were uncapped to allow deionized H_2O to pass through the resin column. The columns were first washed by 5 ml of NaOH (0.5N) followed by three washes using 5 ml of deionized H_2O. At each wash, the washing solution was allowed to completely drain. After these preliminary washes, 5 ml of pyridine acetate (0.5 M) were added to the micro column, allowed to drain, and followed by 2 final washes using 5 ml deionized H_2O.

Following the column washing steps, 1 ml of glucosinolate extract was added to the ion exchange micro column, allowed to drain and then washed by a 3 ml of pyridine acetate. 0.5 ml of purified sulfatase (Aryl sulfatase, type H1 from *Helix pomatia*) was added to micro columns to desulfate the glucosinolates. Sulfatase was also added to a control micro column loaded with a mixture of 1 ml benzyl glucosinolate and 1 ml allyl glucosinolate (BA sample). This sample served as a control for the sulfatase activity.

The micro columns were capped and covered with Saran wrap and kept overnight in room temperature.

Isolation and Purification of Desulfoglucosinolates

The pure desulfoglucosinolates were eluted from columns with 2 ml of dH_2O in clean 4 ml glass vials. One ml of eluted solution was pipeted into a centrifuge membrane filter and centrifuged for 5 min at 1500 rpm. After centrifugation, 500 μl of purified desulfoglucosinolates were pipeted into autosampler HPLC vials.

Derivatization of Desulfoglucosinolates

Samples in HPLC vials were frozen and freeze-dried. Residue in each vial was dissolved in 150 μl of sillylation grade pyridine. The solution was transferred to a 1 ml Reacti-Vial. One hundred μl of N-methyl-N-trimethylsilyl-trifluoroactamide (MSTFA) and 10 μl of trimethylchloro-silane (TMCS) were added to the samples after which the vials were capped and heated at 120°C for 20 min. One μl of sample was injected into a gas chromatograph (Perkin Elmer 8500 Gas Chromatograph) for analysis. The column was a Supelco SPB-20 11 meter with 0.32 mm internal diameter and 25 μm film diameter.

Individual Glucosinolates Identified

Individual glucosinolates identified were allylglucosinolate (ALL), 3-butenylglucosinolate (3BUT), 4-pentenylglucosinolate (4PEN), 2-hydroxy3-butenylglucosinolate (3BOH), 2-hydroxy4pentenylglucosinolate (4POH), benzylglucosinolate , 4-methylthiobutylglucosinolate + 5-methylthiopentylglucosinolate (MSGL), indol-3-ylmethylglucosinolate (3IME) and N-methoxyindol-3-

ylmethylglucosinolate (3IM).

Second Year Study

Field experiments and agronomic practices, laboratory tests, and seed analyses for the second year study were performed as described above.

Statistical Analysis

Analysis of variance, least significant difference, Duncan's multiple range test, and simple correlation analysis were used to analyze the data using the statistical package MSTAT (Michigan State University, East Lansing, Michigan).

RESULTS

Climatic Conditions

In 1991, the average maximum temperature in May was 24°C and increased in June, July and August to 28, 28, 26, respectively. In 1992, the average maximum temperature in May was 21°C but did not reach 27°C. Average temperatures for June, July and August were 24°C, 24°C, and 24, respectively (Tables 1 and 2). In 1991, average monthly maximum and minimum temperatures in May and June were higher than those during the past 30-year period. In 1992, the average monthly maximum and minimum temperatures for June, July and August were all below the 30 year averages for these months.

In 1991, the average minimum temperature in May was 12°C but increased in June, July to 14° and 16°C. The average minimum temperature in August was 13°C. In 1992, the minimum temperatures for May, June, July, and August did not reach 15°C. The average minimum temperatures for these months were, 6, 10, 13, 12°C, respectively (Tables 1 and 2).

In 1992, precipitation (in mm) was 9% higher than in 1991 (Tables 1 and 2). These weather data may help to explain some of the variability in number of days after planting, yield and yield components, seed oil, protein, and glucosinolate concentration.

Table 1. Average monthly maximum and minimum temperatures (T.) (°C) and monthly precipitation in 1991 and summary for the East Lansing area during a 30 year period (1951-1980)[1].

Month	1991 Tmax	1991 Tmin	30-year Tmax	30-year Tmin	Precipitation 1991	Precipitation 30-year
	°C					
May	24	12	20	7	43	69
June	28	14	25	13	76	89
July	28	16	28	15	92	76
August	26	13	26	14	54	79

[1] Data produced by the Michigan Department of Agriculture climatology program.

Table 2. Average monthly maximum and minimum temperature (Temp.) (°C) and monthly precipitation in 1992 and summary for the East Lansing area during a 30 year period (1951-1980)[2].

Month	1992 Tmax	1992 Tmin	30-year Tmax	30-year Tmin	Precipitation 1992	Precipitation 30-year
	°C					
May	21	6	20	7	18	69
June	24	10	25	13	46	89
July	24	13	28	15	185	76
August	24	12	26	14	38	79

[2] Data produced by the Michigan Department of Agriculture climatology program.

YIELD AND YIELD COMPONENTS

Plant Height

The two sulfur fertilization treatments did not produce any particular pattern of plant growth. Plant height varied from 102.2 to 111.3 cm in 1991 (Table 3) and 117 to 134.5 in 1992 (Table 4) without any significant effect from sulfur or cultivar ($P > 0.05$).

Branches per Plant

The number of branches per plant was not affected by the sulfur fertilization treatments. However, the cultivar effect was significant in both 1991 ($P < 0.01$) and 1992 ($P < 0.05$) (Tables 5 and 6). Westar had significantly greater number of branches per plant in 1991. Bounty and Delta had comparable number of branches per plant in 1991. However, no significant interaction was found between cultivar and treatment in either 1991 or 1992.

Pod Length

Pod length was not significantly affected by sulfur fertilization treatments, however, cultivar effect was highly significant ($P < 0.01$). In 1991, Bounty had significantly lower pod length than both Delta and Westar (Table 7). Due to extensive bird feeding damage on pods, pod length data in 1992 were not collected.

Table 3. The effects of three sulfur fertilization rates on plant height of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Plant height (cm)		
	BOUNTY	DELTA	WESTAR
0	111.3 ab[§]	111.3 ab	102.2 c
45	105.3 abc	112.8 a	107.8 abc
90	104.6 bc	105.1 bc	105.5 abc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 4. The effects of three sulfur fertilization rates on plant height of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Plant height (cm)		
	BOUNTY	DELTA	WESTAR
0	129.0 ab[§]	125.5 abc	119.5 bc
45	128.5 abc	134.5 a	117.0 c
90	119.5 bc	120.5 bc	126.0 abc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 5. The effects of three sulfur fertilization rates on number of branches per plant of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Branches per plant		
	BOUNTY	DELTA	WESTAR
0	5.0 bc§	4.5 bc	6.5 a
45	4.8 bc	5.1 b	6.9 a
90	5.1 b	4.3 c	6.5 a
Treatment (T)	ns¶	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 6. The effects of three sulfur fertilization rates on number of branches per plant of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Branches per plant		
	BOUNTY	DELTA	WESTAR
0	7.5 a§	5.0 cd	6.5 abc
45	6.0 abcd	5.5 bcd	5.5 bcd
90	5.5 bcd	4.5 d	7.0 ab
Treatment (T)	ns¶	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 7. The effects of three sulfur fertilization rates on pod length of three spring canola cultivars in 1991[1].

Sulfur Rates (Kg/ha)	Pod length (cm)		
	BOUNTY	DELTA	WESTAR
0	5.7 bc[§]	6.1 ab	5.9 abc
45	5.6 c	6.3 a	6.0 abc
90	5.7 bc	6.3 a	5.8 abc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01 probability level.

[1] Due to extensive bird feeding damage on almost all pods, pod length data were not collected in 1992.

Seed per Pod

Sulfur fertilization treatments had a significant effect on the number of seeds per pod ($P < 0.05$) (Table 8); however there was no particular pattern to this effect. The 45-kg treatment of S appeared to increase the number of seeds per pod in Delta and Westar. The cultivar effect on the number of seeds per pod was highly significant ($P < 0.01$). Delta had overall greater number of seeds per pod than Bounty and Westar.

Seed Yield

Sulfur fertilization treatments generally had no significant effect in 1991 (Table 9) on the seed yield of spring canola cultivars, with the exception of Delta. Differences in seed yield obtained in 1991 are due to cultivar effect, with Bounty showing a significantly greater seed yield than Westar and less than Delta.

In 1992, Bounty and Delta had greater overall yields than Westar with both control and sulfur treatments (Table 10). Although sulfur treatments appeared to stimulate larger yields than the control, the overall effect of sulfur treatments was statistically not significant ($P > 0.05$) in either 1991 or 1992.

Table 8. The effects of three sulfur fertilization rates on number of seeds per pod of three spring canola cultivars in 1991[1].

Sulfur Rates (Kg/ha)	Seed per pod		
	BOUNTY	DELTA	WESTAR
0	23.3 bcd[§]	24.3 bc	20.4 d
45	23.4 bcd	29.6 a	24.0 bcd
90	21.4 cd	26.4 ab	20.3 d
Treatment (T)	*[¶]	*	*
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (*, **) significant at the 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

[1] Due to extensive bird feeding damage on almost all pods, seed per pod data were not collected in 1992.

Table 9. The effects of three sulfur fertilization rates on seed yield of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Yield (Kg/ha)		
	BOUNTY	DELTA	WESTAR
0	1060 a[§]	704 bc	468 c
45	1108 a	720 bc	628 bc
90	1105 a	887 ab	611 c
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 10. The effects of three sulfur fertilization rates on seed yield of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Yield (Kg/ha)		
	BOUNTY	DELTA	WESTAR
0	1673 bcd[§]	1537 cd	1489 cd
45	1654 bcd	2425 a	1387 d
90	2012 abc	2205 ab	1658 bcd
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

PLANT SULFUR NUTRITION

Leaf Sulfur Content

Sulfur treatments had a highly significant effect on the plant sulfur nutrition ($P < 0.01$). Spring canola leaves accumulated significant sulfur amounts by the beginning of flowering in response to the sulfur supply (Table 11). Cultivar also had a significant effect on the leaf sulfur content ($P < 0.05$).Sulfur fertilization treatment increased sulfur content of leaves of all cultivars. In Bounty, sulfur concentration was increased by 68% over the control with 45 S kg/ha and by 72% with 90 S kg/ha. In Delta, sulfur concentration was increased by 66% at the 45 S kg/ha and by 72% at the 90 S kg/ha. Leaf sulfur content of Westar was increased by 65 and 67% with 45 and 90 S kg/ha, respectively (Table 11).

Seed Sulfur Content

In 1991, the sulfur content of mature seed was significantly increased by sulfur fertilization ($P < 0.05$). However, the cultivar effect on the seed sulfur content was not significant ($P > 0.05$) (Table 12). In 1992, the opposite occurred. The seed sulfur content was not significantly increased by sulfur treatment ($P > 0.05$), however, the cultivar effect was significant ($P < 0.05$) (Table 13). In 1991, at the 90 S kg/ha treatment, Bounty and Delta had nearly 10% greater sulfur in the seeds compared to controls (Table 12). However, in 1992, the overall seed sulfur concentrations of all

cultivars were lower than those in 1991. Although no significant pattern of seed sulfur concentration was found among cultivars, Delta tended to have significantly higher levels than either Bounty or Westar.

Table 11. The effects of three sulfur fertilization rates on leaf sulfur content of three spring canola cultivars in 1991[1].

Sulfur Rates (Kg/ha)	Leaf sulfur content (%)		
	BOUNTY	DELTA	WESTAR
0	0.48 c[§]	0.52 c	0.45 c
45	1.52 ab	1.53 ab	1.31 b
90	1.75 a	1.84 a	1.40 b
Treatment (T)	**[¶]	**	**
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) and (**) significant at 0.05 and 0.01, respectively.

[1] Leaf sulfur content data for 1992 are not available.

Table 12. The effects of three sulfur fertilization rates on seed sulfur content of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Seed sulfur content (%)		
	BOUNTY	DELTA	WESTAR
0	0.62 bcd§	0.60 cd	0.55 d
45	0.63 bc	0.60 cd	0.65 abc
90	0.69 ab	0.71 a	0.60 cd
Treatment (T)	*¶	*	*
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 13. The effects of three sulfur fertilization rates on seed sulfur content of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Seed sulfur content (%)		
	BOUNTY	DELTA	WESTAR
0	0.46 b§	0.54 a	0.48 ab
45	0.50 ab	0.55 a	0.50 ab
90	0.49 ab	0.54 a	0.53 ab
Treatment (T)	ns¶	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01.

SEED COMPOSITION

Protein Content

In 1991, seed protein content was significantly affected by cultivar ($P < 0.05$) but not by sulfur fertilization treatments ($P > 0.05$). Delta had significantly greater protein content than Westar and Bounty (Table 14). The sulfur rate of 45 Kg S/ha resulted in a 7% increase in protein content of Delta over the control.

In 1992, sulfur fertilization treatments had a significant effect on the seed protein content ($P < 0.01$). Like several other variables, cultivars had a highly significant effect on the seed protein content ($P < 0.01$). Overall seed protein concentration of Delta were higher than those of Westar and Bounty. This result is consistent with data found in 1991. Increased sulfur availability produced higher protein concentrations in Bounty and Delta (Table 15). However, in Westar, protein concentrations were consistent and did not show any treatment effect.

Oil Content (by NMR)

Sulfur treatments did not influence the oil concentration of the seeds. However, oil concentrations were highly influenced by cultivar ($P < 0.01$). Oil concentration in Bounty were significantly greater than that of Delta and Westar (Table 16). These data are consistent with the protein content results (Tables 14 and 15).

In 1992, neither sulfur fertilization treatment nor cultivar influenced the overall oil

concentrations in the mature seeds. However, these concentrations were substantially higher than those found in 1991. No particular pattern of sulfur treatment nor cultivar effect on seed oil concentrations occurred (Table 17).

Oil Content (by GC)

Oil concentrations, determined by GC analysis of seeds at 8.5%, were closely related data of oil concentrations determined by NMR (on a 2% seed moisture basis) (Table 18 and 19).

Table 14. The effects of three sulfur fertilization rates on seed protein content of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Seed protein content (%)		
	BOUNTY	DELTA	WESTAR
0	25.6 bc[§]	27.3 ab	26.0 abc
45	25.6 bc	29.2 a	26.5 abc
90	23.8 c	26.9 abc	27.9 ab
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 15. The effects of three sulfur fertilization rates on seed protein content of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Seed protein content (%)		
	BOUNTY	DELTA	WESTAR
0	25.3 c[§]	27.2 ab	27.0 b
45	26.4 b	26.6 b	27.1 b
90	26.6 b	28.0 a	27.1 b
Treatment (T)	*[¶]	*	*
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level, (*) and (**) significant at the 0.05 and 0.01 levels, respectively.

Table 16. The effects of three sulfur fertilization rates on the oil content of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Oil content (%)		
	BOUNTY	DELTA	WESTAR
0	39.3 abc[§]	39.7 ab	39.8 a
45	37.5 bcd	36.9 d	37.5 cd
90	37.9 abcd	37.5 cd	38.0 abcd
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 17. The effects of three sulfur fertilization rates on the oil content of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Oil content (%)		
	BOUNTY	DELTA	WESTAR
0	43.7 a[§]	42.9 abc	43.4 ab
45	41.9 bc	42.4 abc	42.5 abc
90	43.0 abc	41.6 c	44.0 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 18. The effects of three sulfur fertilization rates on the oil content, determined by GC, of three spring canola cultivars in 1991.

Sulfur Rates (Kg/ha)	Oil content (%)		
	BOUNTY	DELTA	WESTAR
0	35.9 ab[§]	36.3 ab	36.4 a
45	34.3 bc	33.8 c	34.3 bc
90	34.7 abc	34.3 bc	34.8 abc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 19. The effects of three sulfur fertilization rates on the oil content, determined by GC, of three spring canola cultivars in 1992.

Sulfur Rates (Kg/ha)	Oil content (%)		
	BOUNTY	DELTA	WESTAR
0	40.0 a[§]	39.3 abc	39.7 ab
45	38.4 bc	38.8 abc	38.9 abc
90	39.3 abc	38.1 c	40.3 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

FATTY ACID PROFILE

GC analysis of canola seeds indicated the presence of at least the following 12 fatty acids:

* Palmitic	16:0
* Palmitoleic	16:1
* Stearic	18:0
* Oleic	18:1
* Linoleic	18:2
* Linolenic	18:3
* Eicosanoic	20:0
* Eicosenoic	20:1
* Eicosadinoic	20:2
* Docosanoic	22:0
* Erucic	22:1
* Docosadinoic	22:2

Since some of these fatty acids were detected at very small concentrations, only those fatty acids with concentrations of more than 1% will be discussed, except for erucic acid (22:1).

Palmitic Acid

Palmitic acid concentrations were not influenced by sulfur fertilization or by cultivar ($P<0.05$). In 1991, palmitic acid concentrations ranged from 3.93 to 4.22% (Table 20). In 1992, palmitic acid concentrations were significantly influenced by cultivar ($P<0.01$) but not by sulfur treatment ($P>0.05$) (Table 21). The interaction between sulfur treatment and cultivar was also significant ($P<0.05$); sulfur fertilization

significantly reduced palmitic acid concentrations in Westar from 4.13 to 3.93% with 45 kg S/ha rate and 3.87% with 90 kg S/ha. Similarly, sulfur availability reduced palmitic acid concentration from 4.14% at 45 Kg S/ha rate to 3.87% at 90 kg S/ha. The reduction in palmitic acid concentrations did not result from a direct effect of sulfur fertilization but rather from the interaction between sulfur treatment and cultivar.

Stearic Acid

In 1991, stearic acid concentration ranged from 2.16 to 2.65%. Sulfur fertilization treatments did not significantly influence the stearic acid concentrations ($P > 0.05$) (Table 22). Similarly, no significant difference occurred among cultivars.

In 1992, stearic acid concentrations were, in general, lower than those obtained in 1991, varying from 1.52 to 1.88% and constituting about 30% reduction in this saturated fatty acid. Unlike sulfur fertilization treatments, differences in stearic acid concentrations among cultivars were highly significant ($P < 0.01$), with Delta having a significantly smaller stearic acid concentration than Bounty and Westar (Table 23).

Oleic Acid

Oleic acid concentration ranged from 62.2 to 67.3% in 1991 and from 58.8 to 64.7% in 1992 (Table 24 and 25). In 1991, sulfur fertilization treatment had a significant but inconsistent influence on oleic acid concentration. Unlike Delta, in which no influence occurred, sulfur fertilization significantly increased the oleic acid content of Westar and decreased its concentration in Bounty (Table 24).

In 1992, sulfur fertilization treatments had no significant effect on oleic acid concentrations. However, these concentrations were significantly influenced by cultivar,

with Westar having a significantly higher concentration than Bounty or Delta (Table 25).

Linoleic Acid

Linoleic acid concentrations ranged between 17.0 and 20.2% in 1991 and between 17.6 and 20.9% in 1992 (Table 26 and 27). In 1991, sulfur fertilization treatment did not significantly influence the linoleic concentration of seeds. Slight differences between Bounty, Delta, and Westar were not significant ($P > 0.05$) (Table 26).

In 1992, sulfur fertilization treatments were not significantly different, although significant differences in linoleic acid concentrations did occur among cultivars ($P < 0.01$) (Table 27).

Linolenic Acid

Linolenic acid concentrations ranged from 5.4 and 8.3% in 1991 and from 8.8 to 11.6% in 1992. In 1992, linolenic acid concentrations increased by more than 30% for all cultivars. In 1991, sulfur fertilization treatments had a significant influence on the linolenic acid concentration ($P < 0.01$). Increased sulfur availability tended to reduce linolenic acid levels in Westar. The differences in linolenic acid concentrations among cultivars were not significant ($P > 0.05$) (Table 28).

In 1992, sulfur fertilization treatments did not significantly effect linolenic acid concentrations. However, differences in linolenic acid concentrations among cultivars were highly significant. Linolenic acid concentrations in Westar seeds were significantly lower than those in Bounty and Delta (Table 29).

Erucic Acid

In 1991, erucic acid concentrations ranged between 0.025 and 0.05. In 1992, concentrations varied between 0.03 and 0.64 (Table 30 and 31). Concentrations were not influenced by the sulfur fertilization treatments in either 1991 or 1992. Differences in erucic acid concentrations among cultivars were not significant ($P > 0.05$) (Table 30 and 31).

Ratio of Linolenic to Linoleic Acid

The ratio of linolenic to linoleic fatty acid was significantly influenced by the sulfur fertilization treatments ($P < 0.05$) in 1991 but not in 1992 ($P > 0.05$). However, unlike 1991, differences in the linolenic to linoleic ratio among the cultivars were highly significant (Tables 32 and 33).

In 1991, the overall ratios of linolenic to linoleic fatty acids ranged between 31.6 and 41.1%, whereas in 1992, ratios were closer to the desired 1:2 ratio, ranging between 49.9 and 56.9% (Table 33).

Table 20. Palmitic acid (16:0) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Palmitic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	4.07 a[§]	4.18 a	3.93 a
45	3.95 a	4.22 a	3.99 a
90	3.96 a	4.19 a	3.98 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 21. Palmitic acid (16:0) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Palmitic acid (%)		
	BOUNTY	DELTA	WESTAR
0	4.02 bcd[§]	4.07 abc	4.13 ab
45	4.14 bc	4.21 a	3.94 cd
90	3.87 d	4.18 ab	3.87 d
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	*	*	*

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level; (*) and (**) significant at 0.05 and 0.01 probability levels, respectively.

Table 22. Stearic acid (18:0) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Stearic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	2.41 ab[§]	2.18 b	2.30 b
45	2.22 b	2.21 b	2.65 a
90	2.25 b	2.16 a	2.24 b
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 23. Stearic acid (16:0) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Stearic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	1.77 ab[§]	1.60 bc	1.75 abc
45	1.67 abc	1.52 c	1.80 ab
90	1.72 abc	1.56 bc	1.88 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01.

Table 24. Oleic acid (18:1) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Oleic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	65.0 bc[§]	63.9 bcd	65.2 b
45	62.2 d	63.6 bcd	67.3 a
90	62.9 cd	63.2 bcd	64.8 bc
Treatment (T)	**[¶]	**	**
Cultivar (V)	ns	ns	ns
T x V	*	*	*

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) and (**) significant at 0.05 and 0.01, respectively.

Table 25. Oleic acid (18:1) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Oleic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	59.5 c[§]	61.3 bc	61.1 bc
45	58.8 c	59.2 c	64.0 ab
90	59.3 c	59.3 c	64.7 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ not significant at the 0.05 probability level. (**) significant at 0.01 level.

Table 26. Linoleic acid (18:2) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Linoleic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	19.1 a[§]	18.9 a	18.9 a
45	20.2 a	19.2 a	17.0 b
90	19.7 a	19.4 b	19.2 a
Treatment (T)	*[¶]	*	*
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 27. Linoleic acid (18:2) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Linoleic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	20.8 a[§]	19.5 a	19.4 ab
45	20.9 a	20.8 a	18.1 bc
90	20.6 a	20.4 a	17.6 c
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01.

Table 28. Linolenic acid (18:3) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Linolenic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	6.2 cd[§]	7.7 abc	6.5 bcd
45	8.3 a	7.6 ab	5.4 d
90	8.0 ab	8.0 ab	6.7 bcd
Treatment (T)	**[¶]	**	**
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01.

Table 29. Linolenic acid (18:3) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Linolenic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	10.8 a[§]	10.5 ab	9.0 b
45	11.5 a	11.4 a	9.0 b
90	11.4 a	11.6 a	8.8 b
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 30. Erucic acid (22:1) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Erucic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	0.040 a[§]	0.030 a	0.025 a
45	0.045 a	0.035 a	0.035 a
90	0.045 a	0.050 a	0.040 a
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 31. Erucic acid (22:1) concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Erucic Acid (%)		
	BOUNTY	DELTA	WESTAR
0	0.045 b[§]	0.030 b	0.640 a
45	0.050 b	0.035 b	0.035 b
90	0.050 b	0.035 b	0.030 b
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 32. Ratio of linolenic to linoleic acid concentrations of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Linolenic:Linoleic ratio (%)		
	BOUNTY	DELTA	WESTAR
0	32.8 cd[§]	40.8 ab	34.4 bcd
45	41.4 a	39.6 abc	31.6 d
90	40.6 ab	41.1 ab	35.2 abcd
Treatment (T)	*[¶]	*	*
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 33. Ratio of linolenic to linoleic acid concentrations of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Linolenic:Linoleic ratio (%)		
	BOUNTY	DELTA	WESTAR
0	51.9 abc[§]	53.4 abc	46.5 d
45	55.2 ab	54.7 abc	50.0 bcd
90	55.6 a	56.9 a	49.9 cd
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at the 0.01.

SEED GLUCOSINOLATE CONTENT

Total Glucosinolate

Sulfur fertilization treatments did not significantly affect the glucosinolate content of seeds in either 1991 or 1992 (Table 34 and 35). In 1991, total glucosinolate concentrations in the seed were 36.0, 40.9, and 37.5 μmoles/g of defatted meal with Bounty, 41.7, 43.2, and 43.2 μmoles with Delta, and 32.0, 33.9, and 35.8 μmoles all at 0, 45, 90 kg S/ha, respectively (Table 34). In 1992, total glucosinolate concentrations were 26.4, 45.1, and 28.0 μmoles/g of defatted meal with Bounty, 29.2, 28.5, and 33.5 μmoles with Delta, and 26.1, 33.0, and 30.5 μmoles all at 0, 45, 90 kg S/ha, respectively (Table 35)

In 1991, differences in seed glucosinolate concentration among cultivars were significant ($P<0.05$) but not in 1992 ($P>0.05$). Furthermore, in 1991 Delta had significantly greater glucosinolate concentrations than Bounty and Westar, however, Westar had the lowest glucosinolate concentration.

Table 34. Total glucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Glucosinolate concentration (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	36.0 abc[§]	41.7 abc	32.0 c
45	40.9 abc	44.7 a	33.9 bc
90	37.5 abc	43.2 ab	35.8 abcd
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at the 0.05.

Table 35. Total glucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Glucosinolate concentration (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	26.4 b[§]	29.2 b	26.1 b
45	45.1 a	28.5 b	33.0 b
90	28.0 b	33.5 b	30.5.b
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Individual glucosinolates

*** Allylglucosinolate**

The concentrations of allylglucosinolate (ALL) in 1991 and 1992 were less than 1μmole/g defatted meal (Tables 36 and 37). In both 1991 and 1992, neither sulfur fertilization treatments nor cultivar had a significant influence on ALL concentration. However, in 1992, a highly significant sulfur treatment-cultivar interaction was obtained with Delta ($P < 0.01$) (Table 35).

*** 3-Butenylglucosinolate**

The concentration of 3-butenylglucosinolate (3BUT) varied between 4.1 and 6.5 μmoles/g of defatted meal in 1991, and between 4.3 and 8.6 μmoles/ g in 1992 (Tables 38 and 39). In 1991, sulfur treatments had no effect on 3BUT concentration. Differences in concentration among cultivars were significant in 1991 but not in 1992.

*** 4-Pentenylglucosinolate**

The concentration of 4-pentenylglucosinolate (4PEN) varied between 0.5 and 2.18 μmoles/g of defatted meal in 1991, and between 0.82 and 3.78 μmoles/ g in 1992 (Tables 40 and 41). Sulfur treatments had no effect on 4PEN concentration in either 1991 or 1992. Differences in concentration among cultivars were significant in 1991 but not in 1992.

3-Hydroxybutenylglucosinolate

The concentration of 3-hydroxybutenylglucosinolate (3BOH) varied between 8 and 15 μmoles/g of defatted meal in 1991 and between 11 and 22 μmoles/g in 1992 (Tables 42 and 43). In 1991, 3BOH concentration in the mature seed was almost 30% the total amount of glucosinolate detected in the seed. In 1992, 3BOH constituted about 50% of the total glucosinolate concentration. Unlike in 1992, differences in 3BOH concentration among cultivars were significant in 1991 ($P<0.05$). Sulfur treatments had no consistent influence on the concentration of 3BOH (Tables 42 and 43).

*** 4-Hydroxypentenylglucosinolate**

The concentration of 4-hydroxypentenylglucosinolate (4POH) varied between 0.14 and 0.69 μmoles/g of defatted meal in 1991 (Table 44) and between 0.28 and 1.97 in 1992 (Table 45).

Sulfur fertilization treatments significantly increased the concentration of 4POH in Westar but not in Delta or Bounty which had significantly higher 4POH concentrations than in 1992. A highly significant interaction between sulfur treatments and cultivar was also detected.

3-Methoxyindolylmethylglucosinolate

The concentration of 3-Methoxyindolylmethylglucosinolate (3IM) varied between 5.0 and 11.2 μmoles/g of defatted meal in 1991 and 6.2 and 9.2 in 1992 (Tables 46 and 47). Neither sulfur fertilization treatments nor cultivar had any significant effect on 3IM concentration in either year.

Other individual glucosinolates

Indoly-3-ylmethylglucosinolate, 4-methylthiobutylglucosinolate, 5-methylthiopentenylglucosinolate, were detected in concentrations of less than 0.1 μmoles/g of defatted meal (Data are not reported).

Table 36. Allylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	Allylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	0.90 a§	0.12 b	0.23 ab
45	0.18 ab	0.27 ab	0.26 ab
90	0.11 b	0.20 ab	0.30 ab
Treatment (T)	ns¶	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 37. Allylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	Allylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	0.07 abc§	0.03 bc	0.10 ab
45	0.09 ab	0.01 c	0.10 ab
90	0.08 abc	0.12 a	0.06 abc
Treatment (T)	ns¶	ns	ns
Cultivar (V)	ns	ns	ns
T x V	**	**	**

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 38. 3-Butenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	3-butenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	4.47 ab§	6.55 a	4.10 c
45	5.49 ab	6.54 ab	4.27 bc
90	4.95 ab	6.53 a	5.43 ab
Treatment (T)	ns¶	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 39. 3-Butenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	3-butenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	4.78 bc§	5.37 bc	6.75 ab
45	8.65 a	4.88 bc	4.38 c
90	5.66 bc	4.72 c	5.72 bc
Treatment (T)	ns¶	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 40. 4-Pentenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	4-pentenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	1.41 ab[§]	1.92 a	0.56 c
45	1.79 ab	1.65 ab	0.50 c
90	1.53 abc	2.18 a	1.08 bc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.05.

Table 41. 4-Pentenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	4-pentenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	1.83 bc[§]	2.34 b	0.82 c
45	3.78 a	1.79 bc	2.24 b
90	2.08 bc	1.97 bc	1.97 bc
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

Table 42. 3-Hydroxybutenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	3-hydroxybutenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	8.0 c[§]	12.8 abc	11.3 abc
45	11.2 abc	15.0 a	11.9 abc
90	9.5 bc	14.4 ab	13.8 ab
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	*	*	*
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 43. 3-Hydroxybutenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	3-hydroxybutenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	11.9 b[§]	12.1 b	11.1 b
45	22.9 a	12.6 b	14.5 b
90	12.5 b	16.8 b	14.6 b
Treatment (T)	*[¶]	*	*
Cultivar (V)	ns	ns	ns
T x V	*	*	*

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 44. 4-Hydroxypentenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	4-hydroxypentenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	0.29 bcd§	0.56 abc	0.18 cd
45	0.46 abcd	0.69 a	0.20 cd
90	0.36 abcd	0.63 ab	0.14 d
Treatment (T)	ns¶	ns	ns
Cultivar (V)	**	**	**
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.05.

Table 45. 4-Hydroxypentenylglucosinolate concentration of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	4-hydroxypentenylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	1.04 b§	1.62 ab	0.28 c
45	1.97 a	1.20 b	2.23 a
90	0.94 bc	1.65 ab	1.12 b
Treatment (T)	**¶	**	**
Cultivar (V)	ns	ns	ns
T x V	**	**	**

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (**) significant at 0.01.

Table 46. 3-Methoxyindolylmethylglucosinolate content of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1991.

Sulfur Rates (Kg/ha)	3-methoxyindoylmethylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	9.48 a[§]	9.41 a	5.22 a
45	10.37 a	11.28 a	6.68 a
90	10.36 a	9.31 a	5.01 a
Treatment (T)	*[¶]	*	*
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level. (*) significant at 0.05.

Table 47. 3-Methoxyindolylmethylglucosinolate content of seed of three spring canola cultivars grown under three sulfur fertilization rates in 1992.

Sulfur Rates (Kg/ha)	3-methoxyindoylmethylglucosinolate (μmoles/g defatted meal)		
	BOUNTY	DELTA	WESTAR
0	6.37 b[§]	7.20 ab	6.38 b
45	7.35 ab	7.62 ab	9.21 a
90	6.28 b	8.25 ab	6.82 b
Treatment (T)	ns[¶]	ns	ns
Cultivar (V)	ns	ns	ns
T x V	ns	ns	ns

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

¶ (ns) not significant at the 0.05 probability level.

DISCUSSIONS

Plots were harvested 103 days after planting in 1991 and 117 days in 1992. In 1992, cooler summer conditions and unusually high precipitation delayed pod and seed maturity.

Sulfur fertilization treatments did not influence plant height, branches per plant, pod length, seeds per pod, or seed yield. The results of this study did not support the suggestion by Beaton and Soper (1986) that sulfur fertilization increases growth and improves crop appearance. Furthermore, this study does not support previous reports that sulfur increases canola yields in sulfur-deficient soils in Saskatchewan, Canada (Ukrainetz, 1982). Other authors reported similar yield increases by sulfur fertilization in sulfur deficient soils with maize in Zimbabwe (Vogt, 1966) and with rice in Indonesia (Blair et al., 1978).

Sulfur is an essential element for plant growth and its deficiency may result in reduced yield and crop quality (Rendig, 1986). Since no significant differences in yield, agronomic traits, and crop quality were found between the 2 sulfur fertilization rates and control treatment, the results may be explained by adequate sulfur levels of Michigan soils (5.6 kg/ha) by addition from atmospheric sulfur. Sulfur levels in 40 to 60 cm deep soil horizons were found to be greater than 5.6 kg/ha, resulting from mineralization of organic compounds and percolating sulfates from rain on surface horizons (Vitosh,

Personal communication). Hoeft and Fox (1986) reported that in coarse-textured soils with marginal sulfur availability, atmospheric sulfur contributions supplied adequate levels of sulfur. In order to successfully study the influence of sulfur on yield and yield components under field conditions, monitoring atmospheric sulfur should be considered.

Accumulation of sulfur in the leaves during flowering clearly suggests that sulfur-treated plants actively responded to higher soil sulfur availability without any influence on growth and development of the crop. Leaf sulfur content of the control treatment was about 0.5% which is about normal for canola leaves. Under Michigan field conditions, optimum leaf sulfur concentrations for soybean and corn at flowering were between 0.2 and 0.4% without any sulfur fertilizers application (Vitosh, personal communication). This indicates that sulfur levels of non-sulfur treated plots were adequate, and no favorable response to sulfur fertilization was found. Leaf sulfur content of sulfur-treated plots were more than double that of controls, with 45 kg S/ha and more than triple with 90 kg S/ha (Figure 3). At 0 kg S/ha, Bounty, Delta, and Westar had very similar leaf sulfur content. At 45 and 90 kg S/ha, Bounty and Delta had clearly greater concentrations of total sulfur in the leaves than Westar (Figure 3). This indicates varietal differences in the uptake and accumulation of sulfur in the leaves, as soil sulfur availability increases. Sulfur accumulation may have been primarily in non-organic forms that are unavailable to leaf and plant metabolic processes, i.e. in vacuoles (Kaiser et al., 1989). Janzen and Bettany (1984) reported that sulfur in excess of plant requirements tends to accumulate in the leaves in forms unavailable for redistribution.

Although plants from treated plots produced seed with slightly greater sulfur

content, this increase was not consistent with the of pattern sulfur accumulation in leaves in both 1991 and 1992 (Figure 3). Differences in seed sulfur content between 1991 and 1992 could be attributed to differences in seasonal weather. These may suggest a mechanism for sulfur regulation in the leaves by storage in non-active forms and genetically controlled accumulation of seed sulfur in the form of aminoacids and proteins and other sulfur compounds.

Consistent with previous discussion seed protein content was not influenced by sulfur fertilization treatments, however, there were significant differences due to cultivar. In 1991 and 1992, Delta had significantly higher protein concentration; Bounty had the lowest protein concentration, but the highest oil levesl.

In 1991, oil concentration was influenced by cultivar as well as by the weather. During the cool summer of 1992, overall oil concentrations were higher than in 1991, a much warmer year. In 1991, the average maximum temperature for July and August were 26.6°C and the rainfall was 145 mm compared to 23.8°C and 224 mm for the same period in 1992. These differences in weather conditions during pod development, seed filling and maturity could have influenced final oil concentration of seeds of 1991 and 1992 growing seasons. This is consistent with reports by Mailer and Cornish (1987) and Mailer and Pratley (1990) in rapeseed and Turnip rape in Australia.

Sulfur fertilization treatment had no particular influence on fatty acid profile Fertilization treatments had no significant effect on either palmitic acid or stearic acid concentration of the seeds in 1991 and in 1992 which were similar. In 1992, significant difference in palmitic and stearic acid concentrations were found among cultivars.

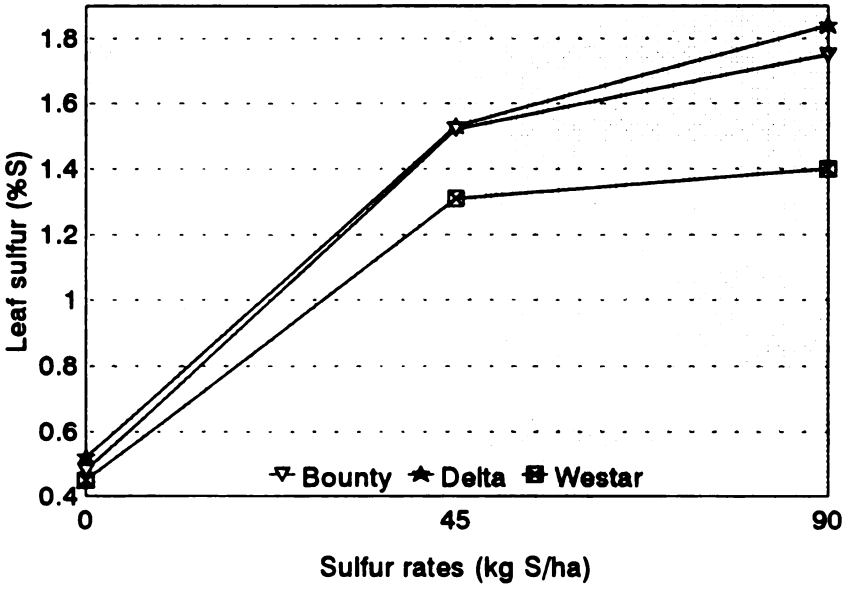

Figure 3. The effects of sulfur fertilization treatments on the total sulfur content of leaves during flowering of three spring canola cultivars in 1991.

Overall oleic acid concentrations in 1991 were greater in 1991 than in 1992. Sulfur treatments had a significant influence on oleic acid concentration in 1991 but not in 1992. This variability may be due to seasonal variation in temperature and rainfall amounts. In contrast to oleic acid concentration, linoleic acid concentrations were lower in 1991 than in 1992, however, linolenic acid concentration were lower in 1991 than in 1992. As for oleic and linoleic acid concentrations, sulfur fertilization had a significant effect in 1991 and differences among cultivar were significant in 1992. This suggests that oleic, linoleic, and linolenic fatty acid concentrations in the seed were somewhat sensitive to sulfur fertilization along with changes in seasonal weather conditions.

The ratio of linolenic to linoleic acid which should be ideally a 1:2 ratio (50%) was affected in 1991 but not 1992. In 1991, this ratio ranged between 31 and 41%, indicating that linolenic acid levels were less than 10%; in 1992, the ratio ranged between 46 and 56%, which is closer to the ideal range as suggested by (Ackman, 1990).

Erucic acid concentrations were less than 0.1% in both 1991 and 1992 except for Westar under control conditions (no sulfur) which had 0.64%. Erucic acid levels were not affected by either sulfur treatments and cultivar. They were significantly lower than than the canola standard for erucic acid of 2%. Therefore, results of this study do not suggest any relationship between sulfur nutrition of plants and final erucic acid content. This may be due to genetic stability of the double-low cultivars used in this study.

Fertilization treatments appeared to affect the glucosinolate content of seed in an inconsistent manner (Figures 4 and 5). In 1991, glucosinolate content of Westar seeds appeared to slightly increase, as the sulfur content of leaves increased, however, this

increase is not statistically significant (Figure 4). Similar results occurred with Bounty and Delta, although glucosinolate content of seed at 90 kg S/ha for both cultivars was smaller than those concentrations observed at 45 kg S/ha (Figures 4 and 5).

Several authors reported that the most important factor regulating glucosinolate content in vegetative tissues and seeds is the sulfur nutritional status of plants (Nutall et al., 1987; Schnug, 1987, and Ramans, 1989). However, glucosinolate concentrations of seed were not significantly increased by sulfur treatments ($P > 0.05$) in 1991 and 1992. As discussed above, sulfur fertilization treatments increased sulfur content in leaf tissue (lamina and petiole) but did not increase glucosinolate concentrations in seed. These results do not support the hypothesis of close linear relationship between total sulfur concentrations of leaves and glucosinolate content in double-low cultivars (Schnug, 1990).

Low glucosinolate concentrations of seed were consistent with the notion of genetically-fixed low glucosinolate in double-low cultivars. Josefsson and Appelqvist (1968) observed that Bronowski, a polish spring rapeseed cultivar, had genetically low controlled low glucosinolate content which was attributed to a metabolic block in the biochemical pathway (Josefsson, 1970). Later, all double-low cultivars derived from Bronowski. Accumulation of sulfur in the leaves and low glucosinolate content in the

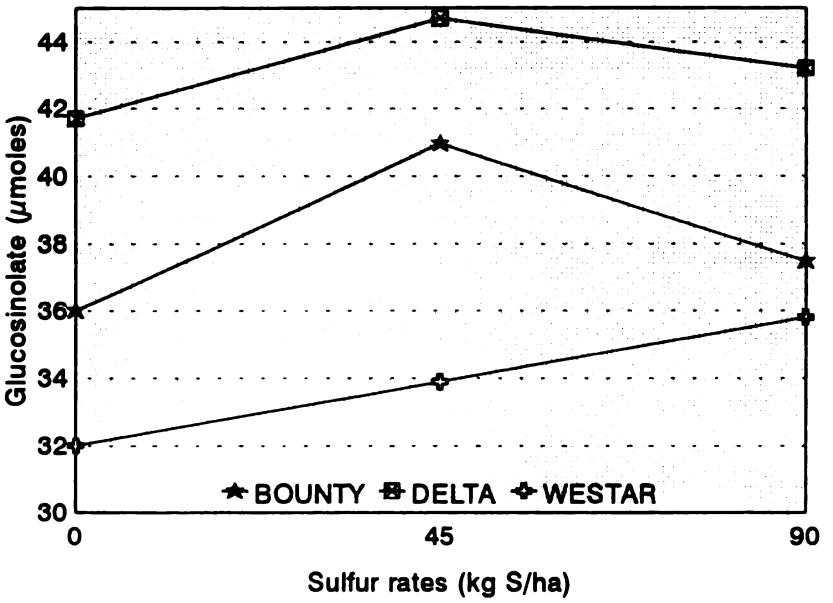

Figure 4. Effects of sulfur fertilization on total glucosinolate content of Bounty, Delta and Westar seed in 1991.

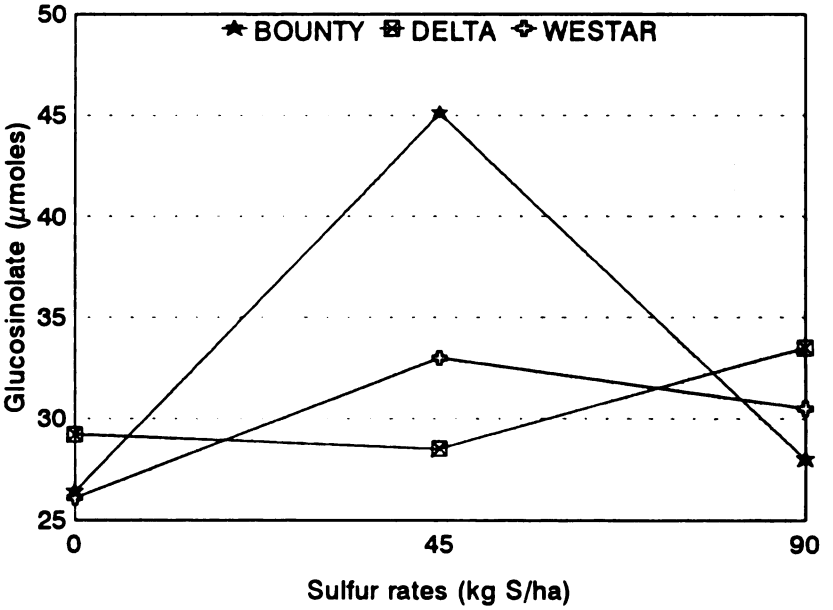

Figure 5. Effects of sulfur fertilization on total glucosinolate content of Bounty, Delta, and Westar seed in 1992.

seed, are in agreement with reports by Lein (1972) that glucosinolates are synthesized in the pods, transported into the seeds, and that *de novo* synthesis of glucosinolate was possible in the seeds.

Under mid-Michigan field conditions, results of 2-year experiments indicate that spring canola cultivars receive adequate sulfur supplies. Application fertilizers had no tangible effect on yield, yield components, protein content, oil concentration, and fatty acid profile. While sulfur fertilization treatments increased total sulfur content of leaves, Accumulation of sulfur in the leaves did not significantly increase seed sulfur or glucosinolate content.

CONCLUSIONS

* Spring canola plants responded to application of potassium sulfate fertilizers by increasing their uptake of sulfur and accumulating it primarily in the leaves. Differences in final seed sulfur content were not significant.
* Sulfur fertilization did not significantly affect spring canola yield and yield components, although it tended to increase seed yield depending on cultivar.
* Sulfur fertilization increased protein content in Delta but not in Bounty or Westar and had little effect on oil content of seeds.
* No significant differences in glucosinolate content among cultivars were obtained, although sulfur fertilization tended to increase the glucosinolate content of seeds.

CHAPTER II

THE EFFECTS OF FIVE SULFATE CONCENTRATIONS UNDER GREENHOUSE CONDITIONS ON CANOLA (*B. napus* L.) MORPHOLOGICAL TRAITS, SEED GLUCOSINOLATE, PROTEIN, OIL CONTENT AND FATTY ACID PROFILE.

ABSTRACT

Levels of glucosinolates have become important criteria for quality of canola (*B. napus* L.) seeds. Although canola cultivars are genetically fixed for low glucosinolate content in the seed, increased sulfur availability has been suggested to increase the concentration of this compound. Effects of sulfate concentrations in modified Hoagland's nutrient solution on seed glucosinolate content of spring canola plants were studied. Spring canola morphological traits, seed yield, oil and protein content and fatty acid profile were also determined. The variety Delta was grown in 5 nutrient solutions containing 0.001, 0.01, 0.1, 1.0, 5.0 mM SO_4^{2-} in a randomized complete block design with 5 replications under controlled greenhouse conditions. Medium-granular Rockwool culture medium was used and pots were watered by hand regularly.

The effects of sulfur treatments were highly significant ($P<0.01$). Total sulfur contents in the leaves were 0.47, 0.7, 0.71, 1.23, and 1.27% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM, suggesting a favorable response of canola to increasing sulfate concentrations. A similar pattern was obtained for total S in the stems. Concentrations

of nitrogen in leaves and stems were increased with increased sulfate in solution. Sulfate treatments had a significant effect on potassium, phosphorous, and micronutrients but differences among the means were not physiologically significant.

Plant height, number of primary and secondary branches, pods per plant, and seed yield were highly correlated with sulfate concentration in solution. Seed yield increased with increasing sulfate concentration, however, 5 mM sulfate concentration had no effect on morphological traits, suggesting an optimum concentration of 1 mM sulfate concentration.

Sulfate treatments had a significant effect on oil and protein content ($P<0.01$). Increasing sulfate concentrations tended to decrease oil content and increase crude protein content in the seed. Glucosinolate concentrations were 31.8, 31.0, 38.3, 39.8, and 42.7 μmoles/g of defatted meal at 0.001, 0.01, 0.1, 1.0 and 5.0 mM sulfate in solution. These concentrations exceeded the canola standard of 30 μmoles glucosinolate/g of defatted meal by between 20 and 30% .

INTRODUCTION

Under field conditions, sulfur is taken up mostly in the sulfate form (SO_4^{2-}). This sulfate uptake is supplemented by SO_2 uptake from the air and by sulfur impurities present in some fertilizers. Under greenhouse conditions, atmospheric sulfur is present at very low levels, and therefore constitute an ideal environment to study the effects of different sulfates concentrations in a modified Hoagland's nutrient solution on canola growth and development under controlled conditions as well as its impact on seed oil, protein, and glucosinolate contents. Sulfate concentrations used in this research were 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-} in the nutrient solution. Evans (1975) reported that in most arable lands optimum sulfur levels are 0.5 mM and therefore, any sulfate concentration between 0.1 and 1.0 mM concentration could be considered as representative of concentrations normally observed under field conditions.

The purpose of the research reported in this chapter was to study the effects of five sulfate concentration on morphological traits, seed glucosinolate concentration, oil and protein content, fatty acid profile, and mineral composition of spring canola Delta grown in modified Hoagland's nutrient solution under controlled greenhouse conditions.

MATERIALS AND METHODS

A greenhouse experiment was designed to study the influence of different concentrations of sulfur (sulfate ion) in a nutrient solution culture on the glucosinolate content of canola (*B. napus*) seeds and its influence on lipid, fatty acid profile, and protein content of seeds.

Planting, Experimental Design, and Treatments

This controlled environment experiment was conducted at the Plant Science Greenhouses, Michigan State University, from September 91 to March 92. Sulfate concentration (SO_4^{2-}) was controlled by culturing the plants in a hydroponic system using rock wool and modifying the concentration of SO_4^{2-} within the nutrient solution.

Seed of the spring canola cultivar Delta (Allelix Seed Company, Ontario, Canada) was planted on September 20, 1991 in rock wool propagation blocks (Grodan SBS 36/77). Deionized water was used for watering during the first 10 days of germination.

Three 10-day-old, seedlings of similar size were transplanted into 3.8 l plastic pots filled with granular (medium grade) horticultural rock wool (Mollema Company, Grand Rapids, MI). The experimental design was a randomized complete block with five replications. Approximately, 1 l of a modified Hoagland solution (Epstein, 1972) with 0.001 mM SO_4^{2-} was applied during the first 30 days, until the plants reached the rosette stage of 4 true leaves. This starting solution was provided to ensure good initial growth.

Five (5) different nutrient solutions (treatments) differing only in concentration of sulfur (SO_4^{2-}) were prepared (Table 1). SO_4^{2-} treatment formulations were prepared in such a way that the other anions accompanying the nutrient solution remained in optimal balance. Micronutrient and iron concentrations in the solution were not modified (Epstein, 1972). A micronutrient stock solution was prepared and added to all five solutions. It consisted of KCl, H_3BO_3, $MnCl_2.4H_2O$, $ZnCl_2$, $CuCl_2.2H_2O$, H_2MoO_4 ($85\%MoO_3$), and Fe Sequestrene. The 5 levels of sulfur concentration in the nutrient solutions were:

* Very low 0.001 mM SO_4^{2-}
* Moderately low 0.01 mM SO_4^{2-}
* Near optimal 0.1 mM SO_4^{2-}
* Moderately high 1.0 mM SO_4^{2-}
* Very high 5.0 mM SO_4^{2-}

The treatments were initiated when plants reached the rosette stage of development with 4 true leaves (about 30 days after planting). One of the 3 seedlings in each pot was then removed and pots were watered daily with modified Hoagland's nutrient solution contained in five 155 l plastic barrels.

After stem elongation, and beginning flowering, one of the 2 remaining plants was sampled for analysis of total sulfur content in the leaves (lamina and petiole) and stems. The remaining plants were grown to maturity and seed production.

Table 1. Mineral composition of modified Hoagland's nutrient solution.

Salt	Stock solution concentration in mM				
	0.001	0.01	0.1	1	5
KNO_3	3	3	3	3	3
$Ca(NO_3)_2$	4	4	4	4	4
NaH_2PO_4	1	1	1	1	1
$MgSO_4$	0.001	0.01	0.1	1	1
$MgCl_2$	0.999	0.99	0.9	0	0
KCl	2	2	2	2	0
$NH_4(NO_3)$	2	2	2	2	1
$(NH_4)_2SO_4$	0	0	0	0	1
K_2SO_4	0	0	0	0	1
Na_2SO_4	0	0	0	0	2

Greenhouse Conditions

Since temperature and light may interfere with normal growth, and consequently on uptake of mineral nutrients, day/night temperatures were maintained close to 25/20°C. The photoperiod was set at 14 hours by automatic timer control. Light intensity, determined by a Li-Cor photometer above the plant canopy on a cloudy day one hour after sunrise and at about 1 o'clock on a sunny day, averaged 295.7 and 560-760 μmol.cm.s, respectively.

Leaf and Stem Analysis

The plants were sampled at the beginning of flowering. The leaves and stems were dried, ground, and analyzed for total sulfur content. Analysis of sulfur content was performed by the Ohio State University Research Extension Analytical Laboratory in Wooster, OH. Procedures used for this analysis are described above.

Leaf and stem elemental composition analysis was performed at the Michigan State University Soil Testing Laboratory. The analysis of mineral elements included N, P, K, B, Ca, Mg, Cu, Fe, Mn, Zn, Al, and Mo. 0.5 ± 0.02 g of plant tissue (leaf and stem) were weighed into numbered covered crucibles. One standard tissue sample and blank crucibles were included. Crucibles were dried to ash in muffle furnace for 5 hours at 500°C (932°F). After cooling, 25 ml of digestion solution (3 N HNO_3 in 1000 ppm LiCL) were added to each crucible and allowed to set for 1 hour. The solution of each crucible was filtered using Whatman No. 1 filter paper and analyzed in a Direct Current Plasma Emission Spectrometer (ALR Model, Beckman Instruments, Inc., Fullerton, CA). Results are reported in percent of dry weight of tissue for macronutrients and in ppm for

micronutrients.

Parameters Measured

The irrigation of plants was stopped 95 days after planting, based on visual assessment of pod/seed physiological maturity and plants were left to naturally dry in the greenhouse. Data collected include plant height, branches/plant, secondary branches per plant, pods/plant, pod length, pod width, seeds/pod, seed/plant, and weight of seeds/plant.

Seed Lipid, Protein, and Glucosinolate Content

Seed lipid, fatty acid profile, protein, and glucosinolate content were determined as described above.

Replicate Experiment

The greenhouse experiment, variables measured, and seed analyses for the replicate study were performed as described above.

Statistical Analysis

Analysis of variance, least significant difference, Duncan's multiple range test, and simple correlation coefficient were used to analyze the data using the statistical package MSTAT (Michigan State University, East Lansing, Michigan).

RESULTS AND DISCUSSIONS

MINERAL COMPOSITION OF CANOLA

Sulfur and Nitrogen

Sulfate concentration treatments had a highly significant influence on the total sulfur (S) content of leaves during flowering. As sulfate concentration in the nutrient solution increased, total S in the leaves during flowering also increased. At 0.001 mM sulfate concentration, total S content of leaves was 0.47% and gradually increased with increased sulfate concentration to 1.7% at 5.0 mM sulfate level (Figure 1). At 0.01 and 0.1 mM sulfate, total S content of leaves was about 0.7%. Sulfate treatments increased total S in leaves of plants grown in 1.0 mM sulfate concentration by almost 43% and by 58% in the 5.0 mM sulfate nutrient solution.

Sulfate treatments had a significant effect on total S concentration in stems ($P<0.01$). Total S content of stems increased with increasing sulfate concentration of the nutrient solution. Sulfur content of stems was 0.17, 0.37, 0.37, 0.40, 0.64% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM, respectively (Figure 1). These results agreed with reports by Janzen and Bettany (1984) that excess sulfur application relative to nitrogen availability resulted in accumulation of sulfur primarily in leaves and to some extent in the stems. Sulfate treatments also had a significant effect on total S content of seeds

which ranged between 0.53 and 0.68% (Figure 1).

Canola plants reacted to incremental increases of sulfates in the nutrient solution by increasing the uptake of sulfates and transport via the xylem and accumulation in the leaves where most of the reduction and assimilation occur. Since sulfur is essential for chlorophyll formation, leaves of plants grown in 0.001 mM sulfate had pale green coloration whereas plants grown in higher sulfate concentrations had larger and "greener" leaves. This observation is in agreement with the report by Gilbert (1951) that under severe conditions, leaves may undergo some loss of green color. Furthermore, although no characteristic deficiency symptoms were observed (e.g, chlorosis) on plants treated with 0.001 mM sulfate, their overall appearance showed that canola plants adjusted to the sulfur deficiency by adapting morphological changes (discussion below) which ultimately result in yield losses. Grant and Bailey (1993) reported that moderate sulfur deficiency may not cause deficiency symptoms but could still reduce yield.

Low total S concentration of stems during flowering was expected because sulfates are transported from roots mainly by xylem vessels and accumulated in the leaves. That total stem S concentrations were much lower than in the leaves can be explained by a slow remobilization of reduced sulfur products to other plant parts via the phloem. However, total stem S concentrations followed a similar pattern which suggested a close correlation between sulfate uptake by roots and transport via the xylem and sulfur-containing photosynthate accumulation and retranslocation via the phloem.

Total sulfur content of seeds was mearly constant and inconsistent with overall plant response to sulfates in the nutrient solution. There may be several reasons for low

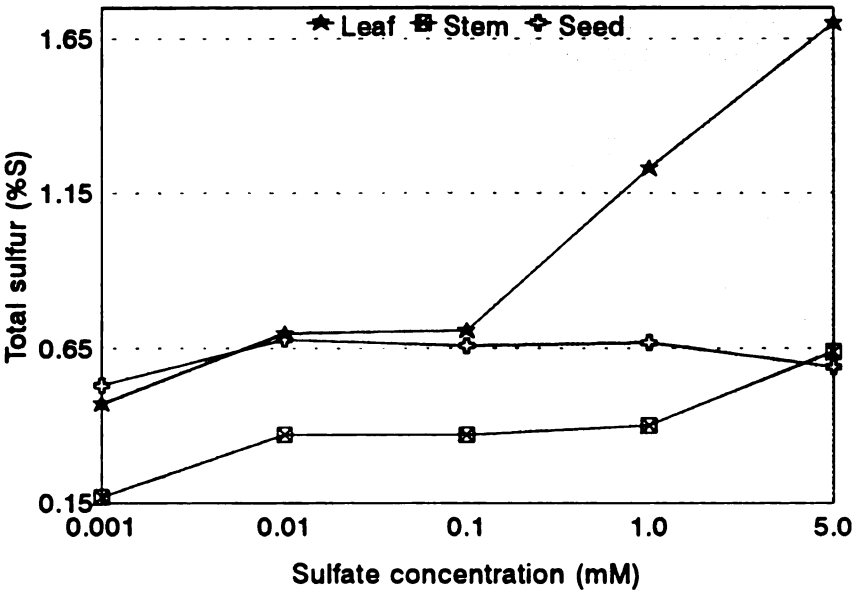

Figure 1. Total sulfur content of leaves, stems, and seeds of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

sulfur concentration in the seed, including the complexity of the physiological processes that take place at the whole plant level and the integrative nature of physiological response to variable concentrations of certain mineral nutrients in solution (Widders, personal communication).

During reproductive growth, the carbohydrate supply to the roots, and thus root activity and nutrient uptake decrease rapidly (Marschner, 1986). During this period, remobilization of nutrients from the leaves depends on many factors, including the number of developing pods. These reproductive structures constitute sinks that actively compete for phloem remobilized assimilates and nutrients by a regulated physiological process. The extent of remobilization depend on various factors, including specific requirements of the seed, mineral nutrient level in the vegetative parts, the ratio between vegetative mass and number and size of seeds and fruits, and the nutrient uptake by the roots during the reproductive stages (Marschner, 1986). Total sulfur content of the seed may not have a similar response in the seed than in leaves or stems because in the xylem tissue sulfur is transported in the form of sulfates to leaves, whereas sulfur is transported to seeds primarily in organic forms using different pathways which are highly regulated.

Sulfate concentration in the growth medium had a significant effect on the nitrogen content of leaves during flowering ($P < 0.01$). Nitrogen concentration of the leaves were 3.47, 4.08 4.2, 4.35, and 4.44 % at sulfate concentrations of 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-} concentration, respectively. As sulfate concentration increases in the nutrient solution, nitrogen content of leaves also increases (Figure 2). Although nutrient solutions had equal concentrations of total nitrogen (nitrates and

ammonium ions), low sulfate treatments particularly affected uptake and reduction of nitrogen by plants. Sulfate treatments also had also a significant effect on the concentration of S in the stems ($P<0.01$). Stem nitrogen concentrations were 1.89, 2.12, 2.10, 2.02, and 2.15 % at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentrations, respectively (Figure 3).

This study suggests that adequate availability of sulfates in the nutrient solution is essential for nitrogen nutrition and therefore for growth and development of canola plants. Inhibition of nitrate and ammonium ion uptake by environmental factors such as light intensity, temperature of the nutrient solution, and volume of nutrient solution used was unlikely. Therefore, only low sulfate concentrations in solution could explain the abnormally lower nitrogen concentrations in leaves, stems, and seeds since equal nitrogen was present in all five nutrient solutions. It is not clear from this experiment how low sulfate concentrations (0.001 and 0.01 mM) influenced the plant nitrogen uptake efficiency.

Both sulfur and nitrogen levels in the leaves, stems, and seeds increased with increasing sulfates in solution, indicating a close relationship between the uptake, assimilation, and utilization (i.e. protein synthesis) of these 2 essential elements by canola plants grown in nutrient solutions. Consequences of this relationship on canola morphological traits are discussed below.

Nitrogen-to-sulfur ratios have been related to the sulfur status of many species (Dijkshoorn et al., 1960). In this study, the nitrogen-to-sulfur ratio decreased with increasing sulfate concentration in growth solutions (Table 2). This is in agreement with

Spencer et al. (1984) who suggested that N/S ratio in subterranean clover (*Trifolium subterraneum*) (Spencer, 1978) and Spencer et al. (1984) in rapeseed is a suitable index for the evaluation of sulfur status in these plants.

Table 2. **Nitrogen-to sulfur ratios in the leaves, seeds, and stems of canola grown in modified Hoagland's solution containing 5 concentrations of sulfur under greenhouse conditions.**

Sulfur Conc. (mM)	N/S Leaves	N/S Stems	N/S Seeds
0.001	7.86 a	11.40 a	7.19 b
0.01	5.78 b	5.81 b	7.60 ab
0.1	4.55 c	5.03 bc	7.65 ab
1.0	3.54 d	5.02 bc	7.62 ab
5.0	2.61 e	3.37 c	8.40 a
Treatment	**[¶]	**	ns

[§] Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

[¶] (**) significant at the 0.01 probability level.

Phosphorous

Sulfate treatments had a significant effect on the concentration of phosphorus in the leaves at flowering ($P<0.01$). Phosphorous content of the leaves was 0.26, 0.20, 0.22, 0.24, and 0.31% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration in solution (Figure 2). Phosphorous concentrations of the stems during flowering were 0.20, 0.16, 0.17, 0.15, and 0.22 at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration, respectively (Figure 3). Although the concentration of phosphorous in all five nutrient solutions was 1.0 mM, an optimal concentration for normal canola growth and development, phosphorous levels in plant tissue during flowering varied significantly. Because canola nutrition studies in nutrient solutions are not available, it is not clear how sulfate concentrations could have affected the levels of phosphorus in the vegetative tissues. However, it possible that the sulfate treatments influenced phosphorous levels in tissues by altering plant physiological processes which resulted in morphological changes of the plants.

Potassium

Sulfate treatments had a significant influence on potassium concentrations of leaves and stems ($P<0.01$). Potassium (K^+) concentrations in leaves during flowering were 2.95, 2.42, 2.27, 2.18, and 3.81% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-} concentrations, respectively (Figure 2). In the stems, the K^+ concentrations were 2.68, 3.61, 3.2, 3.22, and 4.66% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-} concentrations, respectively (Figure 3).

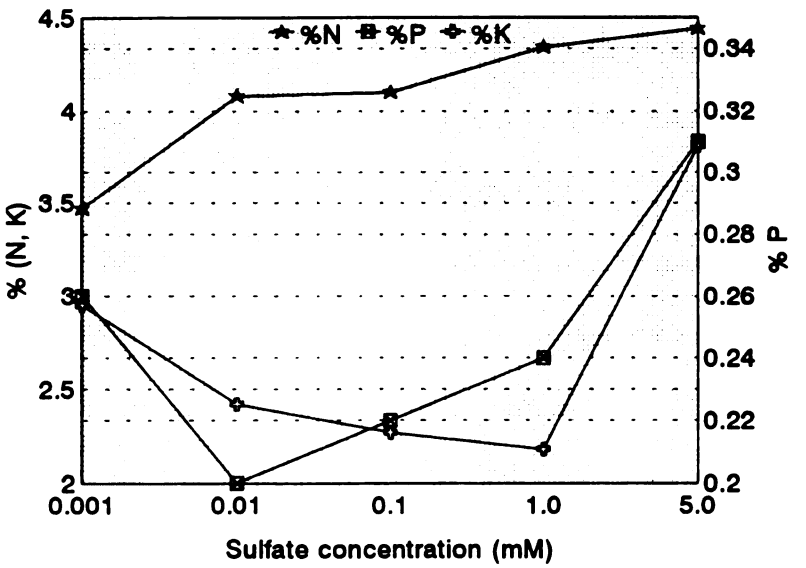

Figure 2. Concentration of N, P, and K in leaves of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

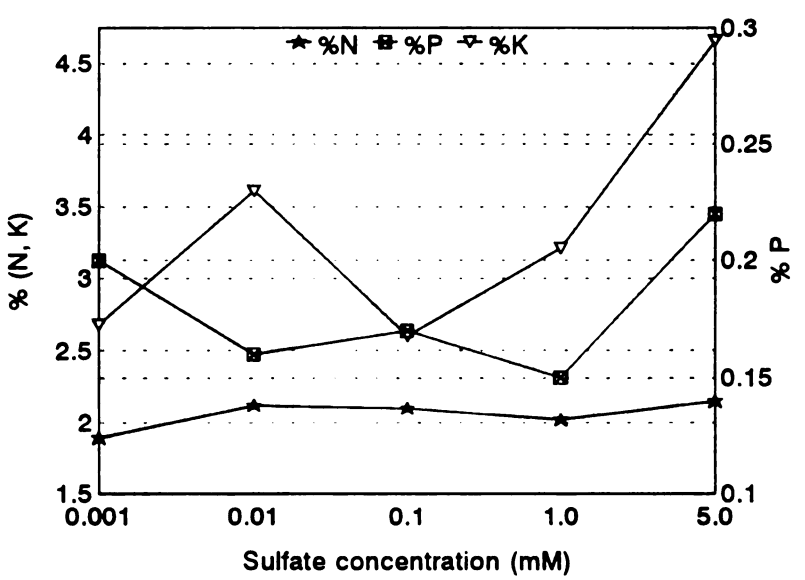

Figure 3. Concentration of N, P, and K in stems of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

The potassium requirement for optimal plant growth is 2-5% of the dry weight of the vegetative parts. The concentrations of K^+ found in this experiment show that although sulfur treatments had a significant effect on the relative concentration of K^+ in plant tissue, low sulfate treatments did not have a profound effect by causing deficiencies. Potassium concentrations are normally greater in leaves than in stems. However, in this study, potassium concentrations were greater in the stems than in the leaves, which may indicate the start of K^+ retranslocation during flowering via the phloem where it plays an essential role in counterbalancing mobile ions (Clarkson and Hanson, 1980).

Magnesium, Calcium and Boron

Sulfate treatments had a significant effect on the concentration of magnesium (Mg^{2+}) in the leaves during flowering ($P<0.01$). Magnesium concentrations in the leaves were 0.64, 0.42, 0.49, 0.36, and 0.51% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration in the nutrient solution (Table 3). However, sulfate treatments did not effect the concentration of magnesium in the stems. Mg^{2+} levels in the stems were were 0.37, 0.36, 0.33, 0.33, and 0.42% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentrations in the nutrient solution (Table 4).

Magnesium is a major essential element that is a component of the chlorophyll molecule. It serves as a cofactor for most phosphorylation enzymes (Jones et al., 1991) and as a bridging element for the aggregation of ribosome subunits (Cammarano et al., 1972), a process that is necessary for protein synthesis. Although the effects of sulfate treatments were statistically significant, Mg^{2+} concentrations in leaves for all sulfate

treatments were about 0.5%, which is optimal for plant growth (Marschner, 1986). In the stems, Mg^{2+} concentrations varied between 0.33 and 0.42%, yet these concentrations are above the deficiency levels of 0.25% and less (Jones et al, 1991).

Sulfate treatments had a significant effect on calcium (Ca^{2+}) levels in the leaves during flowering ($P<0.01$). Calcium concentrations in the leaves were 1.31, 1.26, 1.20, 1.22, and 1.85 at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration in the nutrient solution (Table 3). Sulfate treatments also had a significant effect on the calcium concentrations in the stems during flowering ($P<0.01$). Ca^{2+} concentrations in the stems were 0.38, 0.60. 0.40, 0.65, and 0.89% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentrations in the nutrient solution (Table 4).

Calcium plays a fundamental role in membrane stability and cell integrity. Its concentration in vegetative parts ranges between 0.1 and 5% of plant dry weight depending on many factors, including plant species (Marschner, 1986).
Although sulfate concentration treatments were statistically significant ($P<0.01$), they did not cause the concentration of calcium in the leaves and stems to reach deficient levels.

Sulfate treatments had a significant effect on the concentration of boron (B) in both leaf and stem tissues ($P<0.01$). Borate (BO_3^{3-}) concentration in canola leaves during flowering were 49.0, 42.6, 40.5, 40.6, and 61.9 ppm at 0.001, 0.01, 0.1, 1.0, and 5.0 mM of sulfate solutions. Borate concentrations of stems were 24.4, 25.6, 24.2, 32.1, and 29.8 ppm at 0.001, 0.01, 0.1, 1.0, and 5.0 mM of sulfate solutions (Tables and 3 and 4). Although sulfate treatments had a statistically significant effect on the

concentration of borate ions in the leaves and stems, slight differences in concentration due to these treatments had no physiological implications since borate concentrations in leaves and stems were all above critical deficiency levels.

Micronutrients

Sulfate treatments had no effect on the concentrations of aluminum, manganese, copper, iron, molybdenum, zinc, sodium, and chlorine in leaves and stems of the spring canola cultivar Delta (Data are not reported). Concentrations of these micronutrients were all above the critical deficiency and toxicity levels, but were not affected by the large variation in sulfate concentration in the nutrient solution.

Table 3. **Magnesium, calcium, and boron content of spring canola leaves grown in modified Hoagland's solution containing 5 concentrations of sulfur under greenhouse conditions.**

Sulfur Rates (mM)	Mg %	Ca %	B ppm
0.001	0.64 a[§]	1.31 b	49.0 b
0.01	0.42 bc	1.26 b	42.6 c
0.1	0.49 b	1.20 b	40.5 c
1.0	0.36 c	1.22 b	40.6 c
5.0	0.51 b	1.85 a	61.9 a
Treatment	**[¶]	**	**

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (**) significant at the 0.01 probability level.

Table 4. Magnesium, calcium, and boron content of spring canola stems grown in modified Hoagland's solution containing 5 concentrations of sulfur under greenhouse conditions.

Sulfur Rates (mM)	Mg %	Ca %	B ppm
0.001	0.37 ab[§]	0.38 c	24.4 c
0.01	0.36 ab	0.60 b	25.6 bc
0.1	0.33 b	0.40 c	24.2 c
1.0	0.33 b	0.65 b	32.1 a
5.0	0.42 a	0.89 a	29.8 ab
Treatment	ns[¶]	**	**

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (**) significant at the 0.01 probability level. (ns) not significant at the 0.05 probability level.

Oil and Protein Content

Sulfate treatments significantly affected the oil and protein content of the seed ($P<0.01$). At the 0.001 mM sulfate level, oil content of seeds was 40.3%. But as the concentration of sulfate increased, oil content tended to decrease to about 34% at 0.1, 1.0, and 5.0 mM SO_4^{2-} levels (Table 5). Thus, oil content was negatively correlated with sulfate treatment ($r= -0.49$, $P<0.05$). Wetter et al. (1970) and Holmes and Ainsley (1977) reported that whenever sulfur supply had a significant effect on the yield, oil content tended to decrease with increased sulfur supply. In this study, sulfate treatments had a significant effect on seed yield per plant and oil content of seeds.

Protein content was 24% at 0.001 mM sulfate concentration, but increased to about 31% at 0.1, 1.0, and 5.0 mM SO_4^{2-} levels (Table 5). Unlike oil content, protein content was positively correlated with the sulfate treatments ($r= 0.54$, $P<0.01$). These results are in agreement with those of Appleqvsit (1968) who suggested that extreme sulfur deficiency strongly reduced seed protein content.

Oil and protein synthesis were, as expected, antagonistic processes that showed a high inverse correlation coefficient of -0.74 which was significant at the 0.001 probability level. Similar results have been reported by Vogel et al. (1967) with various rapeseed cultivars in Switzerland. Holmes (1980) suggested that increased nitrogen supply reduces the oil concentration by increasing the synthesis of nitrogen-containing protein precursors. Consequently protein synthesis competes strongly for photosynthates and less photosynthates for fatty acid synthesis reduces oil formation. This could explain the reason for the highest oil and lowest protein concentration at 0.001 mM sulfate in

solution. Also increased nitrogen content in vegetative tissues coincided with increasing protein content and decreasing oil concentration in the seed.

Table 5. Oil and protein content of seeds of Delta canola grown in modified Hoagland's solution with 5 sulfur concentrations under greenhouse conditions.

Sulfur Conc. (mM)	Oil (%)	Protein (%)
0.001	40.3 a§	24.0 b
0.01	33.0 b	32.4 a
0.1	34.1 b	30.9 a
1.0	34.2 b	31.6 a
5.0	34.8 b	30.9 a
Treatment	**¶	**
Correlation[1]	-0.49 *	0.54 **

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (*, **) significant at the 0.05 and 0.01 probability levels, respectively.

[1] Simple correlation coefficient between sulfate concentrations in the nutrient solution and seed oil and protein content.

FATTY ACID PROFILE

GC analysis of harvested Delta canola seeds from 1991 and 1992 field experiments showed the presence of at least 12 fatty acids shown below[1]:

Fatty Acid	Chemistry	Delta
Palmitic	16:0	4.4
Palmitoleic	16:1	0.2
Stearic	18:0	2.0
Oleic	18:1	59.6
Linoleic	18:2	21.4
Linolenic	18:3	9.4
Eicosanoic	20:0	0.7
Eicosenoic	20:1	1.4
Eicosadinoic	20:2	0.09
Docosanoic	22:0	0.39
Erucic	22:1	0.04
Docosadinoic	22:2	0.01

[1]Data presented are mean fatty acid concentration (in %) of spring canola cultivar Delta grown on Kalamazoo loam under well-watered conditions in 1991 and 1992.

Since some of these fatty acids were present at very low concentrations, only those with concentrations greater than 1% will be discussed, except for erucic acid (22:1) which is a critical level in canola oil quality.

Palmitic acid

Sulfate treatments did not significantly affect palmitic acid concentration ($P>0.05$). Palmitic acid concentrations were 4.7, 4.8, 5.0, 4.7, 4.9% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively (Figure 4). Although, these concentrations are slightly higher than those of Delta grown under field conditions, they are still considered normal and therefore extreme concentration of sulfates (0.001 and 5.0 mM SO_4^{2-}) did not affect the final concentration. Palmitic acid concentrations in the harvested seed were not significantly correlated with the sulfate treatments ($r= 0.20$, $P>0.05$) (Table 6).

Stearic acid

Sulfate treatments did not significantly affect stearic acid concentration ($P>0.05$) which were 2.5, 2.3, 2.1, 2.0, and 2.3% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively (Figure 4). It is possible that because spring double-low canola cultivars have naturally low levels of stearic acid content in the seed, that large variations in sulfate concentration in the nutrient solution would have no effect on its stearic acid level. Also, stearic acid concentrations in the seed were not significantly correlated with sulfate treatments ($r= -0.17$, $P>0.05$) (Table 6).

Oleic acid

Sulfate treatments had a highly significant effect on the concentrations of oleic acid in the harvested seed ($P<0.01$). Oleic acid concentrations were 67.4, 65.0, 59.5, 61.3, and 63.3% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively (Figure 4). Oleic acid concentrations were negatively correlated with sulfate

treatments ($r = -0.43$, $P < 0.05$) (Table 6). Oleic acid concentration was highest at 0.001 mM sulfate, decreased to 59.5% at 0.1 mM sulfate in solution, and slightly increased to 61.3 and 63.3% at 1.0 and 5.0 mM sulfate, respectively (Figure 4). Since lipid synthesis was negatively correlated with protein synthesis and oleic acid constitutes the largest fatty acid component, it is possible that oleic acid concentration was notably affected by the interaction between oil and protein synthesis. Also, given the impact of sulfate treatments on other morphological and physiological aspects, it may be expected that oleic acid would be substantially affected since it constitutes the largest fraction of fatty acid in the seed.

Linoleic acid

Sulfate treatments had a significant effect on linoleic acid levels of the harvested seed ($P < 0.05$) which were 16.9, 17.6, 21.3, 19.4, and 18.7% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration in solution, respectively (Figure 4). These concentrations were not significantly correlated with sulfate treatments ($r = 0.37$, $P > 0.05$), but were highly correlated with oleic acid concentration ($r = -0.97$, $P < 0.01$) (Table 6).

Linolenic acid

Sulfate treatments had a significant effect on the concentration of linolenic acid in the seed ($P<0.01$) which were 6.2, 7.0, 8.8, 9.4, and 7.3% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively (Figure 4). Concentration of linolenic was positively correlated with sulfate concentration in solution ($r=0.42$, $P<0.05$) and linoleic acid concentration ($r= 0.76$, $P<0.01$) but negatively correlated with oleic acid level of the seed ($r= -0.84$, $P<0.01$) (Table 6).

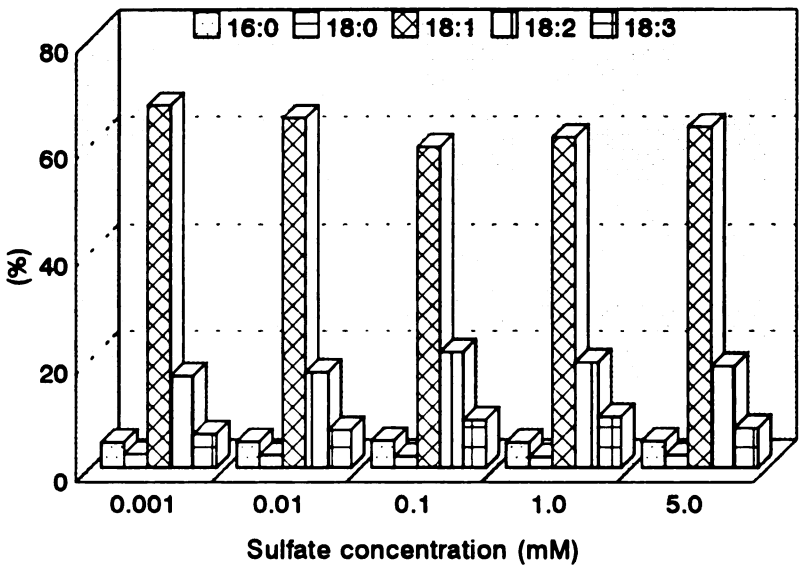

Figure 4. Partial fatty acid profile of seeds of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

Table 6. Simple correlation coefficients between sulfate treatments in the nutrient solution and concentration of several fatty acids in the seed.

Fatty Acid	18:1	18:2	18:3	SO_4^{2-} [1]
16:0	-0.47 *	0.46 *	0.05 ns	0.20 ns
18:0	0.31 ns	-0.40 *	-0.50 **	-0.17 ns
18:1	----	-0.97 **	-0.84 **	-0.43 *
18:2	----	----	0.76 **	0.37 ns
18:3	----	----	----	0.42 *

[1] (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

Erucic acid

Sulfate treatments had no significant effect on erucic acid concentration ($P > 0.05$). Erucic acid concentrations were 0.02, 0.04, 0.03, 0.04, and 0.03% at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in solution, respectively. As sulfate concentration increased in the nutrient solution seed erucic acid concentrations varied little and final concentrations were between 0.02 and 0.04%. These concentrations are much smaller than the canola standard of 2%. This study show that, unlike earlier rapeseed cultivars (Appleqvist, 1968), sulfur had no effect on erucic acid levels in mature seeds.

MORPHOLOGICAL TRAITS

The five different sulfur concentrations in the nutrient solution had a significant effect ($P < 0.05$) on the height of canola plants (Table 7). Mean height of plants grown in 0.001 mM SO_4^{2-} concentration was 174 cm, but decreased with increasing sulfate concentration in the nutrient solution to 131 and 155 cm at 1.0 mM and 5.0 mM sulfate solution, respectively (Table 7). Since nutrient solutions are identical except for the sulfate ion concentration, inadequate sulfate availability in the 0.001 and 0.01 mM solutions could explain the unusual canola plant height with thinner stem and lower number of leaves (results not reported). A lower availability of organic sulfur compounds may have affected the vegetative growth (leaves and branches) of plants.

Unlike plant height, the number of primary and secondary branches per plant significantly increased with increasing sulfate concentration in the nutrient solution, which is consistent with the impact on plant height ($P < 0.01$) (Table 7). Mean number of branches increased from 16 at 0.001 mM to 49 at 1.0 mM SO_4^{2-} concentration (Table 7). Since neither macro and micronutrients, water, nor photosynthetic energy were limiting, reduced sulfur-containing compounds increase in leaves with increasing sulfate concentration in the nutrient solution. This resulted in larger plants, numerous differentiated leaves, larger stems, and increased number of branches.

Table 7. Plant height and total number of branches of Delta canola grown in modified Hoagland's solution with 5 sulfur concentrations under greenhouse conditions.

Sulfur Conc. (mM)	Plant height (cm)	Total branches
0.001	174.2 a[§]	16.6 b
0.01	143.4 bc	39.8 a
0.1	153.4 abc	42.6 a
1.0	131.8 c	49.4 a
5.0	155.0 ab	46.6 a
Treatment	*[¶]	**

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively.

Concentration of sulfates in nutrient solution had a highly significant effect on the number of pods per plant ($P < 0.01$) (Table 8) which were 347 and 400 at 0.001 and 0.01 mM SO_4^{2-} concentration, respectively. At 0.1, 1.0 and 5.0 mM SO_4^{2-} levels, the number of pods/plant increased, but the difference among the means was not significant at these concentrations (Table 8). This is consistent with previously discussed morphological traits. That is, increased sulfates in the nutrient solution resulted in plants with numerous large leaves and primary and secondary branches which, under non-limiting conditions, produced a large number of flowers (Data not shown) and consequently pods per plant.

Pod length was significantly affected by sulfate treatments in the nutrient solution ($P < 0.05$). Mean length of pods were 6.3, 6.4, 7.1, 6.0, 5.9 cm at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in solution, respectively (Table 8). Although sulfate treatment was significant, differences among the means at 0.1, 1.0 and 5.0 mM were not significantly different except at 0.1 mM sulfate concentration which had the highest pod length among all means.

Sulfate concentration in the nutrient solution did not significantly affect the number of seeds per pod ($P > 0.05$), which ranged from 24 to almost 33 seeds per pod (Table 9). The effect of sulfate concentration on the number of seeds per pod showed no particular trend.

Seed yield per plant was significantly ($P < 0.01$) increased by sulfate levels in the nutrient solution. At the 0.001 mM SO_4^{2-} level, the mean seed yield per plant was 14.6g which increased to 15.6 and 20.0 at the 0.01 and 0.1 mM solution

Table 8. Pods per plant and pod length of Delta canola grown in modified Hoagland's solution with 5 sulfur concentrations under greenhouse conditions.

Sulfur Conc. (mM)	Pods/plant	Pod length (cm)
0.001	347.2 b[§]	6.3 ab
0.01	400.0 b	6.4 ab
0.1	531.0 a	7.1 a
1.0	550.6 a	6.0 b
5.0	564.0 a	5.9 b
Treatment	**[¶]	*

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively.

concentration, respectively (Table 9). In a sand culture experiment, Josefsson and Appleqvist (1965) reported that canola developed very weakly and produced no seed when little or no sulfate was supplied. At the 1.0 and 5.0 mM sulfate concentration, yield remained constant at 21.4 g of seed per plant (Table 9). Plants grown at near-optimum concentration of 1.0 mM SO_4^{2-} in solution had about 32% higher seed yield than at 0.001 mM and 27% higher than at 0.01 mM. Canola plants grown in limiting concentration of 0.001 mM sulfate in the nutrient solution, developed fewer pods per plant, and consequently lower seed yield. As sulfate concentration increases, plants produced more pods and, therefore, seed yield increased.

Because of the close relationship between sulfur and nitrogen nutrition (discussed above), seed yield increased as the N/S ratio decreased (Figure 5). This is particularly meaningful because it shows the correlation between the sulfur and nitrogen content in the leaves during flowering and maximum seed yield (Figure 5).

The presence of adequate sulfate available to canola root systems is essential to optimum growth, development, and economic yield of canola plants. Plant height, total number of branches per plant, pods per plant, and seed yield were highly correlated with sulfate concentration in the nutrient solution (Table 10). Plant height was negatively correlated to sulfate concentration ($r = -0.45$ with $p < 0.05$). Total number of branches, pods per plant, and seed yield per plant were positively correlated to sulfate concentration. As sulfate concentration increased in the nutrient solution, plants were more vigorous and produced more and larger leaves, thicker stems, greater number of branches, and pods, and consequently, greater seed yields.

Table 9. Seeds per pod and seed yield of Delta canola grown in modified Hoagland's solution with 5 sulfur concentrations under greenhouse conditions.

Sulfur Conc. (mM)	Seed per pod	Seed yield g/plant
0.001	28.4 ab[§]	14.6 c
0.01	27.0 b	15.6 bc
0.1	32.8 a	20.0 ab
1.0	24.7 b	21.4 a
5.0	25.2 b	21.4 a
Treatment	ns[¶]	**

§ Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

¶ (ns) and (**) not significant and significant at the 0.01 probability level, respectively.

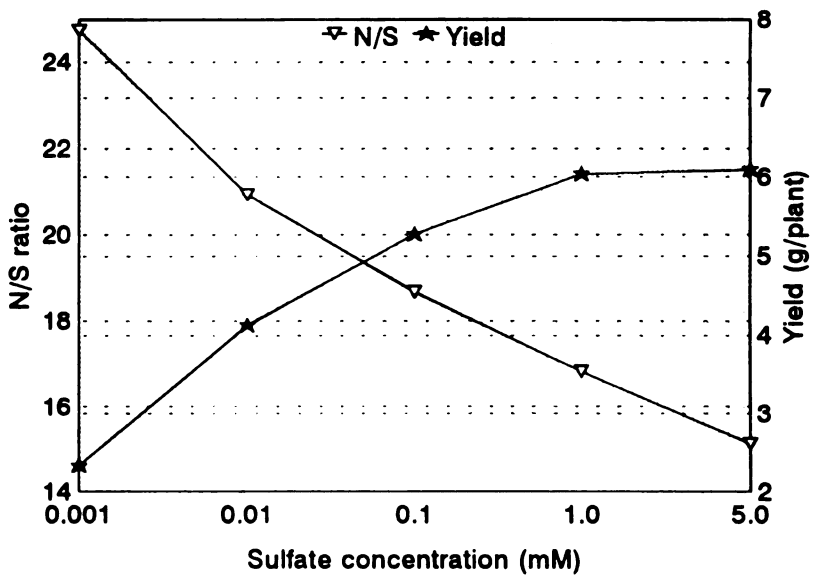

Figure 5. Variation in seed yield with N/S ratio in leaves of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

Table 10. Simple correlation coefficients between sulfate concentrations in the nutrient solution and morphological traits of canola.

Traits	Sulfate Conc.	P[1]
Plant height	-0.45	*
Primary branches	0.45	*
Secondary branches	0.66	**
Pods per plant	0.73	**
Seeds per pod	-0.36	ns
Pod length	0.17	ns
Seed Yield	0.75	**

[1] (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

Total and Individual Glucosinolate Content

Sulfate treatments had a significant effect on the concentration of glucosinolates in the harvested seed ($P<0.05$). Glucosinolate concentrations were 31.8, 31.0, 38.3, 39.8, and 42.7 μmoles/g of defatted meal at 0.001, 0.01, 0.1, 1.0 and 5.0 mM sulfate in solution (Figure 6). Means of glucosinolates in the seed at 0.001 and 0.01 mM were not significantly different. However, at 0.1 mM and 1.0 mM sulfate in solution, glucosinolate content in the seed increased to 38.3 and 39.8 μmoles/g of defatted meal, constituting a 19 to 21% increase. At 5.0 mM sulfate concentration, glucosinolate concentration in the seed was 42.7 μmoles which constitutes a 27% increase over that of the lower 2 sulfate concentrations. This increase in glucosinolate level as a result of increased sulfate concentration in solution is particularly important because it exceeded that of the canola standard of 30 μmoles/g of defatted meal by 20 to 30%.

Large increases in glucosinolate content with increased sulfur application had been previously reported in rapeseed (Josefsson and Appleqvist, 1968), however, that glucosinolate content of seed was increased, suggests that double-low rapeseed cultivars could have excessive glucosinolate levels as a result of high sulfur fertility.

Individual glucosinolates detected were allylglucosinolate (ALL), 3-butenylglucosinolate (3BUT), 4-pentenylglucosinolate (4PEN), 2-hydroxy3-butenylglucosinolate (3BOH), 2-hydroxy4-pentenylglucosinolate (4POH), 4-methylthiobutylglucosinolate + 5-methylthiopentylglucosinolate (MSGL), indol-3-ylmethylglucosinolate (3IME) and N-methoxyindol-3-ylmethylglucosinolate (3IM).

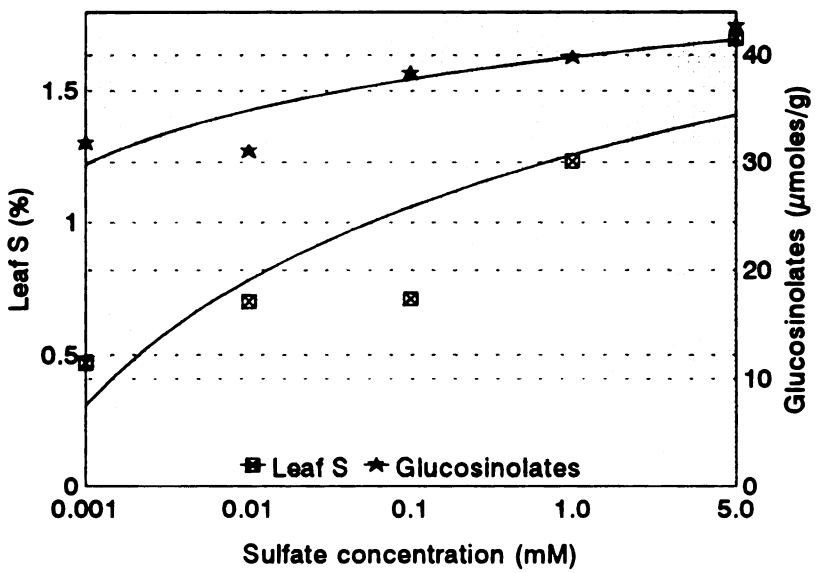

Figure 6. Variation in leaf sulfur with glucosinolate content in seeds of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

(ALL), (MSGL), and (3IME) were detected at concentrations of less than 1 μmoles/g of defatted meal which were insignificant concentrations and therefore are not shown on Figure 7.

3-Butenylglucosinolate, 4-pentenylglucosinolate, 2-Hyrdroxy3-butenylglucosinolate, 2-hydroxy4-pentenylglucosinolate, and N-methoxyindol-3-ylmethylglucosinolate were detected at concentrations greater than 1 μmoles/g defatted meal (Figure 7).

Sulfate treatments had a significant effect only on the concentration of 3BOH and 4POH ($P < 0.01$). 2-Hydroxy3-Butenylglucosinolate concentrations were 7.4, 6.2, 11.5, 12.0, 13.6 μmoles g of defatted meal respectively at 0.001, 0.01, 0.1, 1.0, 5.0 mM sulfate in solution (Figure 7). Differences in sulfate levels in the nutrient solution resulted in almost a 54% increase in concentration between the lowest and highest value obtained.

4-pentenylglucosinolate concentrations were 0.82, 0.98, 2.24, 1.81, 1.78 at 0.001, 0.01, 0.1, 1.0, 5.0 mM sulfate, respectively (Figure 7). Differences in sulfate levels in the nutrient solution resulted in a 55% increase in concentration between the lowest and highest concentration obtained.

2-Hydroxy4-pentenylglucosinolate in the seed were 0.29, 0.33, 0.99, 1.33, 1.21 μmoles at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively (Figure 7). Differences in sulfate levels in the nutrient solution resulted in a 78% increase in concentration between the lowest and highest value.

Concentrations of N-methoxyindol-3-ylmethylglucosinolate were 9.26, 8.76, 8.42,

9.40, nd 9.61 μmoles at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate in the nutrient solution, respectively. Although, sulfate treatments had little effect on this individual glucosinolate, it constituted between 20 and 30% of the total (Figure 7).

These results show that increases in total glucosinolate content of the seed could largely be attributed to increases in the various individual glucosinolates, and particularly 3BOH which constituted 23, 20, 30, 30, 32% of the total glucosinolate at 0.001, 0.01, 0.1, 1.0, and 5.0 mM sulfate concentration, respectively.

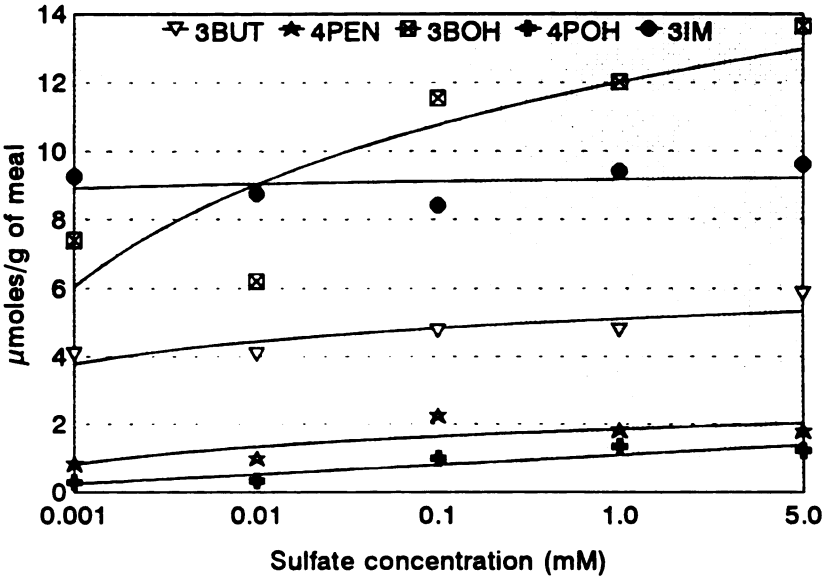

Figure 7. Profile of some selected glucosinolates in seed of Delta canola grown in modified Hoagland's nutrient solution containing 0.001, 0.01, 0.1, 1.0, and 5.0 mM SO_4^{2-}.

CONCLUSIONS

* Sulfur content of leaves, stems, and seeds increased with increasing sulfate in Hoagland's modified nutrient solutions.
* Morphological traits of Delta canola were significantly affected by different sulfate levels. Plant height, number of primary and secondary branches, pods per plant, and seed yield were highly correlated with sulfate concentrations in the nutrient solution.
* Increased sulfate concentrations in the nutrient solution tended to decrease the oil content and increase the protein content in the seed.
* Increasing sulfate concentrations in the nutrient solution increased seed glucosinolate concentrations and caused it to exceed the canola standard of 30 μmoles/g of defatted meal.

CHAPTER III

ABSTRACT

THE EFFECTS OF WATER STRESS AND SOIL TYPE ON THE MORPHOLOGICAL TRAITS, SEED GLUCOSINOLATE CONCENTRATION, OIL AND PROTEIN CONTENT, AND FATTY ACID PROFILE OF CANOLA (*B. napus* L.) SEEDS.

Glucosinolates are sulfur-containing compounds in the secondary plant metabolism found typically in Brassica species. Levels of these compounds have become important criteria for quality of canola (*B. napus* L.) seeds. Although most canola cultivars are genetically fixed for low glucosinolate content in the seed, water stress has been suggested to increase the concentration of this compound in the harvested seed.

This experiment was conducted to study the effects of water stress and soil type on the glucosinolate content of spring canola seeds under Michigan field conditions. The effects of water stress on yield, yield components, oil and protein content of the seed as well as fatty acid profile have were determined. Water stress experiments were conducted under a rainshelter at the Kellogg Biological Station near Hickory Corners, Michigan. The experimental design was a double splitplot with 2 soil types: Spinks sand (sandy, mixed, mesic Psammentic Hapludalfs) and Kalamazoo loam (fine-loamy, mixed,

mesic typic Hapludalfs). Three spring canola cultivars, Bounty, Delta, Westar and two water regimes were used. Plots were stressed by withholding water during late flowering/beginning pod development.

Water stress significantly reduced plant height, number of pods per plant, seed per pod and yield. On loam, however, means of these variables were higher than those obtained on the sandy soil, clearly suggesting a strong soil type effect. Oil content was significantly higher on loam than on sandy soil, however, water stress was not significant ($P > 0.05$). Consistent with oil content results, protein content of seeds was lower on sandy than on loam soil, with no significant effect of water stress. Water stress significantly increased the glucosinolate content of seeds on the sandy soil, however, on the loam, the increase in glucosinolate was not significant ($P > 0.05$). Overall seed glucosinolate contents were 36% higher in 1991 and 18% higher in 1992 than the normally expected level of less than 30 μmoles/g of defatted meal.

INTRODUCTION

Water stress affects practically every aspect of plant growth, modifying its anatomy, morphology, physiology and biochemistry (Kramer, 1969). Several investigators showed that water stress produces different effects at different stages in the growth cycle. However, there is general agreement that stress during the reproductive stages (flowering, pollination, and fertilization) is the most critical.

LITERATURE REVIEW

Effects of Drought Stress on Yield and Yield Components

Drought is one of the most important factor limiting crop yields (Jones and Corlett, 1992). It is the primary cause of reduced crop growth worldwide (Rosenberg et al., 1983). The effects of drought on yield and yield components depend on the supply of water from the soil, the demands of water by the crop, and the manner in which the crop is able to use the limited water supply. Drought may limit the productivity of crop plants by affecting photosynthetic processes at the canopy, leaf, or chloroplast level, either directly or by feedback inhibition if transport of photosynthate to sink organs is limited. The productivity of many diverse crops can be closely related to light interception (Monteih, 1977), and the critical effect of drought on canopy light

interception, leaf area, and yield have been well-documented (Legg et al., 1979). Expansive growth of leaves was found to be the most sensitive process to water stress (Hsiao and Jing, 1987; Hsiao et al., 1985). A number of relationships have been published describing the effect of water deficit on leaf growth and transpiration (Meyer and Green, 1980; Acevedo et al., 1971; Ritchie et al., 1972).

Yield decreases resulting from drought stress depend both on the phenological timing of the stress and on the degree of yield component compensation (Korte et al., 1983b). Drought stress applied during flowering, pod formation, or seed-filling stages has been reported to reduce soybean (*Glycine max* L.) seed yield due to decreases in the number of pods per plant, seeds per plant, or in individual seed weight, respectively (Korte et al., 1983b; Kadhem et al., 1985b). Muchow (1989) reported large reductions in grain yields of maize (*Zea mays* L.) in later vegetative stages, shortly or after anthesis (Begg and Turne, 1976). Yield losses due to water deficits during anthesis were attributed to poor synchronization in emergence of male and female flower components (Herrero and Johnson, 1981; Hall et al., 1981; Freier et al., 1984) and to embryo abortion (Westgate and Boyer, 1985).

Final seed yield of canola (*B. napus*) is determined by a number of contributing factors, including number of pods, seeds per pod, and individual seed weight. Therefore, it is expected that water deficits during flowering, pod development, and seed filling adversely affect yield components and result in losses of seed yield.

Effects of Drought Stress on Plant Metabolism

Besides drought stress, significant effects on the overall crop vegetative and reproductive stages, its consequences on seed protein, lipids, and mineral nutrients have been well-documented for a variety of crops as discussed below.

Hsiao (1973) reported that water deficits have a profound effect on plant metabolism; photosynthesis is inhibited and photosynthate levels and nitrogen metabolism are altered (Lawlor and Fock, 1977).

Yamada and Fukutoku (1983) reported that as leaf water potential decreases, insoluble sugar content decreased rapidly with a simultaneous suppression of soybean plant growth; Also free amino acid content increased while protein content declined. Batchelor et al. (1984) found that drought stress during reproductive growth decreased the concentration of calcium (Ca) in the stems, leaves, and pods of soybean.

Foroud et al. (1993) reported that soybean seed proteins were greatly reduced by water deficits. The synthesis of proteins is affected at various levels: absorption and reduction of soil nitrogen, photosynthetic production of amino acid precursors, synthesis of nucleic acids, and synthesis of proteins from amino acids. Barnett and Naylor (1966) reported that considerable protein hydrolysis is observed in wilted plants along with increasing levels of free amino acids. As water deficits become severe, seed protein content sometimes tends to be comparable to that of well-watered seed, since water deficits during the anthesis result in low seed yield (Terman et al., 1969); therefore, the proteins concentrate and tend to result in high protein seeds (Jarrell and Beverly, 1981). This is consistent with the findings of Henry et al. (1986) in wheat (*Triticum aestivum*)

in Western Canada.

Effects of Drought Stress on Seed Quality

Seed quality (germinability and vigor) is essential to establishing adequate plant stands for crop production. Seed quality can be adversely affected by a variety of environmental factors while seeds are still maturing on the plant, during harvesting or processing, or during postharvest storage conditions (Heydecker, 1977; and Maguire, 1977). Tekrony et al. (1980, 1983, 1984) reported a decline in seed germination and vigor when unfavorable environmental conditions (e.g. moisture stress) occur after physiological maturity (PM).

However, results of drought stress effects during seed development on seed quality are often inconsistent (Tekrony, personal communication). Rassini and Lin (1981) reported decreased soybean seed vigor when drought stress was imposed between pod development and early seed filling. Smiciklas et al. (1989) reported that drought stress imposed during soybean seed formation decreased germination of harvested seed which was positively correlated with the decrease in calcium level in the seed. Ketring (1991) also reported losses in germinability of peanut (*Arachis hypogea* L.) seed grown under water deficient conditions.

Dornbos et al. (1989) reported that drought stress reduced germination, seedling axis dry weight, and increased single-seed conductivity. However, they indicated that stress reduced yield and seed number more significantly than germination and vigor. In another experiment, Dornbos and Mullen (1991) reported that severe drought stress during seed fill caused soybean plants to exceed their capacity to buffer seed number,

shifting seed weight distributions towards a larger proportion of small seed, resulting in lower germination and vigor.

Effects of drought stress on seed glucosinolate content

A review of the effects of drought stress on glucosinolate content of canola seeds was presented in the first chapter.

In this study the research objective was to determine the influence of water stress and soil type on the morphological traits, seed glucosinolate concentration, oil and protein content, and fatty acid profile of spring canola under Michigan field conditions.

MATERIALS AND METHODS

Three Spring canola (*B. napus*) commercial cultivars Bounty, Delta, and Westar were used in this experiment.

Planting, Plot Management and Experimental Design

This experiment was conducted at the Kellog Biological Station (KBS) in Hickory Corners, MI. Seed of each cultivar was planted at a rate of 5.6 kg/ha in a randomized complete block with two replications. The experiment was established in a Rainshelter on two adjacent soils types: Kalamazoo loam (fine-loamy, mixed, mesic typic Hapludalfs) and Spinks sand (sandy, mixed, mesic Psammentic Hapludalfs). Each replication consisted of a five-row plot 6 m long and 92 cm wide. Urea (46% nitrogen) was applied at the rate of 140 kg N/ha.

In the first year, the plots were planted on May 3, 1991 using a small plot planter and harvested on July 29, 1991. In the second year, the plots were planted on May 1, 1992 and harvested on August 21, 1992.

Rainshelter Characteristics

A complete review of KBS Rainshelter technical characteristics are described in Martin et al. (1988). The Rainshelter facility covers 0.13 ha and consists of two buidlings which are moved by a single drive system. The buidings, located at opposite ends of a set of tracks, move toward each other during rainfall, and meet at the center

to enclose the entire plot area. A programmable controller (PC) governs opening and closing of facility based on rainfall and darkness. When rainfall occurs, it activates the PC's "raining" mode and the shelter closes. The PC is also equipped with a dusk-to-dawn photocell input so that the shelter will not open during times of darkness. The shelter is also fitted with overhead irrigation sprinklers which are set to allow irrigation of desired plots.

Irrigation Treatments

Following planting, plots on both soils were watered weekly at a rate of 25.4 mm per week for 6 weeks, until the beginning of flowering. This amount of moisture was estimated based on evapotranspiration data for the month of May. This starting irrigation treatment was provided to maintain a normal plant growth on both soils.

After 8 weeks, drought stress treatments started. Water was withheld on loam, while for well-watered loam plots, the irrigation schedule consisted of applying 38.1 mm of water every 3 days. On sandy soil, the well-watered plots were also given 38.1 mm of water every 3 of water days, while stressed plots were given only 12 mm of water every 3 days for 4 weeks. Irrigation water was withheld on all the plots for a week before harvest.

Material Sampling

At physiological maturity, 20 whole plant samples were randomly taken from each replication for determination of yield components including: plant height, branches/pod, pods per plant, pod length, seeds/pod, and 100-seed weight. Plant height is a measure, in centimeters (cm), of the primary stem length. Branches per plant are the number of

secondary branches on the primary stem. All 20 pods used to determine the pod length and seeds per pod were randomly selected on the primary stem adjacent to the last secondary branch. At harvest, the moisture content of the seed was determined using a Dickey-John Multi-Grain portable moisture tester. Seed yield was determined by adjusting the seed moisture to 8%.

Seed Lipid, Protein, and Glucosinolate Content

Seed lipid, fatty acid profile, protein, and glucosinolate content were determined as described above.

Second Year Study

Field experiments and agronomic practices, laboratory tests, and seed analyses for the second year study were performed as described above.

Statistical Analysis

Analysis of variance, least significant difference, Duncan's multiple range test, simple correlation coefficient were used to analyze the data using the statistical package MSTAT (Michigan State University, East Lansing, Michigan).

RESULTS

Climatic Conditions

In 1991, the average maximum temperature in May was 25°C and increased steadily in June, July, and August to 29°C, 29°C, 28°C, respectively. In 1992, the average maximum temperature in May was 23°C and did not reach 27°C. Average temperatures for June July and August were 26, 26, and 25°C, respectively (Table 1).

In 1991, the average minimum temperature in May was 13°C, but increased gradually in June and July to 15°C and 17°C, respectively. The average minimum temperature in August was 15°C. In 1992, the minimum temperatures for May, June, July, and August did not reach 15°C. The average minimum temperatures for these months were 7, 7, 14, and 13, respectively (Table 1).

Water stress treatment were started 56 days after planting and maximum weekly temperatures until harvest were in the mid to upper 20s in 1991 and in the low 20s (°C) in 1992. This notable difference in average weekly maximum temperatures is particularly important, since higher temperatures enhance stress response (Figures 1 and 2).

Table 1. Average monthly maximum and minimum temperature (°C) during the spring canola growing season in 1991 and 1992[1].

	May		June		July		August	
	91	92	91	92	91	92	91	92
Maximum Temperature	25	23	29	26	29	26	28	25
Minimum Temperature	13	7	16	7	17	14	16	13

[1] Data produced by the Michigan Department of Agriculture climatology program.

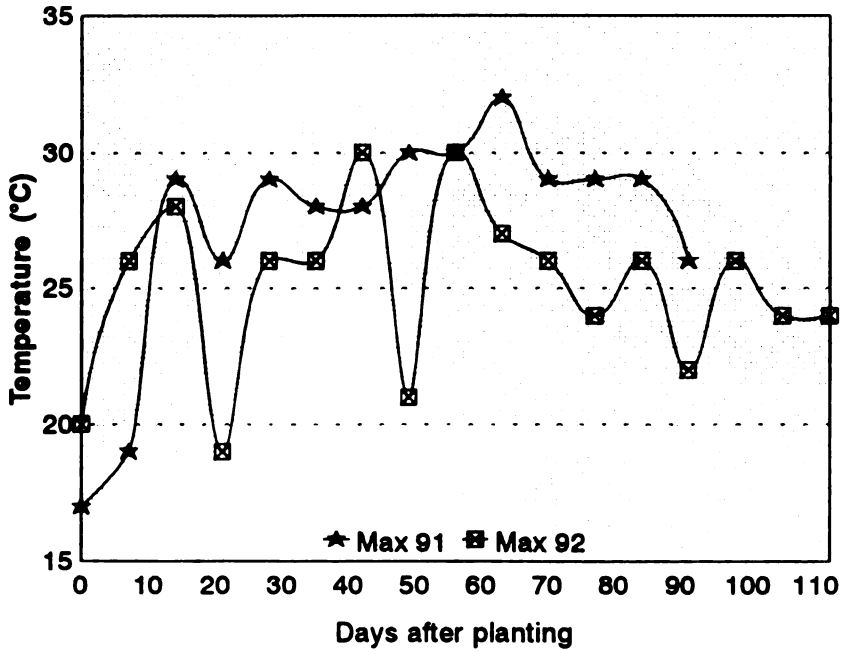

Figure 1. Weekly maximum temperatures (°C) in 1991 and 1992.

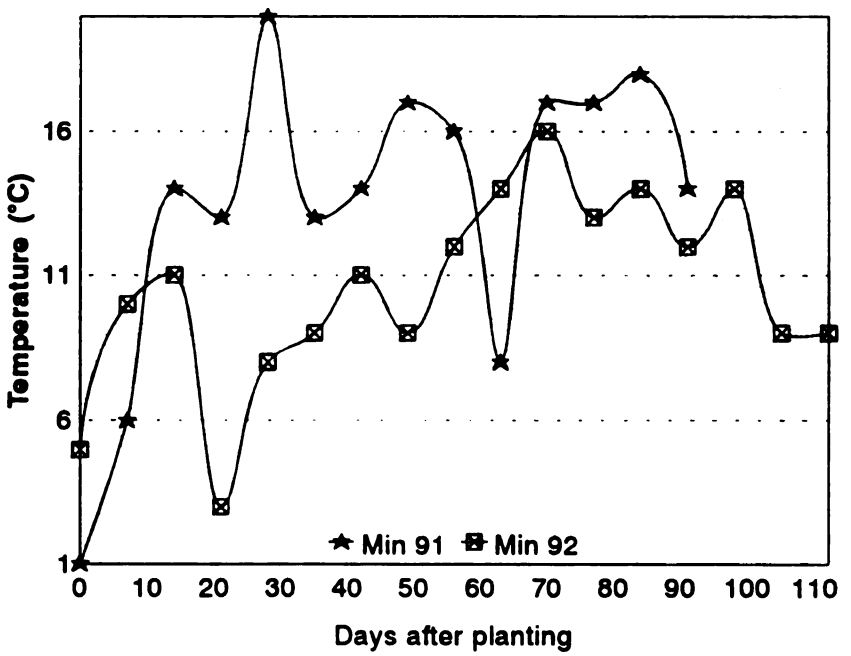

Figure 2. Weekly minimum temperatures (°C) in 1991 and 1992.

YIELD AND YIELD COMPONENTS

Significance of the treatment effects, soil type, water stress, and cultivar determined by the Analysis of Variance procedure for 1991 and 1992 data are presented in Tables 2 and 3. Soil type had a significant effect at the 0.01 probability level on all yield components, plant height, branches per plant, pods per plant, pod length, and seeds per pod. The soil type had no effect on the actual yield, perhaps because actual yield data were not adjusted for the extensive bird feeding damage. However, soil type had a highly significant effect ($P < 0.01$) on the estimated yield.

In 1991, water stress treatment had a significant effect on plant height, branches per plant, pods per plant, and seed per pod, but affected neither pod length nor seed yield (Table 2). In 1992, water stress had a significant effect on all yield components ($P < 0.01$) and seed yield ($P < 0.05$). Soil x water stress X cultivar interaction effects were not significant in either 1991 or 1992 (Tables 2 and 3).

Table 2. **Significance levels of soil types (S), treatment (T), variety (V), and interaction effects on yield and yield components in 1991.**

Variable	Source of Variation				
	S	T	V	ST	STV
Plant height	**§	**	ns	*	ns
Branches plant^{-1}	ns	**	*	ns	ns
Pods plant^{-1}	**	**	**	**	ns
Pod length	**	ns	ns	ns	ns
Seed pod^{-1}	**	**	ns	ns	*
Yield	**	ns	**	ns	ns

§ (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively; (ns) not significant at 0.05 probability level.

Table 3. **Significance levels of soil types (S), treatment (T), variety (V), and interaction effects on yield and yield components in 1992.**

Variable	Source of Variation				
	S	T	V	ST	STV
Plant height	**§	**	ns	ns	*
Branches plant^{-1}	**	**	ns	ns	ns
Pods plant^{-1}	**	**	*	**	ns
Pod length	**	**	**	**	ns
Seed pod^{-1}	**	**	**	ns	ns
Yield actual	ns	*	ns	**	*
Yield estimated	**	**	ns	**	ns

§ (*) and (**) significant at the 0.05 and 0.01 probability levels, respectively; (ns) not significant at 0.05 probability level.

Plant Height

Soil type and water stress significantly affected plant height in both 1991 and 1992. In 1991, mean plant height wase 63.5 on sand and 111.6 cm on loam, both under well-watered conditions (Table 4), 57.3 on sand, and 101.3 cm on loam under water stress conditions (Table 4). In 1992, mean plant height was 87.8 cm on sand, and 134.2 cm on loam under well-watered conditions (Table 5), 76.0 cm on sand, and 101.3 cm on loam under water stress conditions (Table 5). Plants were overall smaller on the sandy soil than on loam under both water treatments, suggesting a significant soil type effect on the plant height. Water stress significantly reduced height of plants grown on both loam and sand.

Branches per Plant

The number of branches per plant was affected differently by soil type and water stress (Tables 6 and 7). In 1991, under well-watered conditions, the number of branches per plant was 4.9 on sand, 4.6 on loam under stressed conditions, 3.2 on sand, 4.2 on loam (Table 6). In 1992, under well-watered conditions, the number of branches per plant were 2.9 on sand, 5.1 on loam, under stressed conditions 2.2 on sand, 3.7 on loam.

In both 1991 and 1992, the number of branches per plant was significantly reduced by water stress on the Spinks sand. The reduction in branches was 34% in 1991 and 24% in 1992. On the loam plots, the water stress treatment reduced the number of branches by 8% in 1991 and by 24% in 1992. However, the number of branches per plant for both water treatments on the loam soil was consistently comparable or higher to that on sand.

Pods per Plant

The number of pods per plant was significantly affected by soil type and water stress. In 1991, under well-watered conditions, the number of pods per plant was 33 on sand and 109 on loam; however, under water stress treatments, the number of pods per plant was 23 on sand and 62 on loam (Table 8). In 1992, under well-watered conditions, the number of pods per plant was 66 on sand and 187 on loam. Under water stress, the number of pods per plant was 38 on sand and 87 on loam (Table 9). In 1991, water reduced the number of pods per plant by 43% on the loam soil and by 30% on sandy soil. In 1992, water stress reduced the number of pods per plant by 53% on the loam soil and by 42% on the sandy soil. The difference in pods per plant between the two soils under well-watered treatment was 69 % in 1991 and 64% in 1992. Under water stress, the reduction in pods per plant between sand and loam soil was 62% in 1991 and 56% in 1992.

Table 4. Effect of drought stress and soil type on plant height of spring canola in 1991.

	Plant height (cm)	
Water Regime	Soil type	
	Sand	Loam
Watered	63.5 c[§]	111.8 a
Stressed	57.3 c	101.3 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 5. Effect of drought stress and soil type on plant height of spring canola in 1992.

	Plant height (cm)	
Water Regime	Soil type	
	Sand	Loam
Watered	87.8 c[§]	134.2 a
Stressed	76.0 d	101.3 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 6. Effect of drought stress and soil type on the number of branches per plant of spring canola in 1991.

	Branches per plant (cm)	
Water Regime	Soil type	
	Sand	Loam
Watered	4.9 a[§]	4.6 a
Stressed	3.2 b	4.2 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 7. Effect of drought stress and soil type on the number of branches per plant of spring canola in 1992.

	Branches per plant	
Water Regime	Soil type	
	Sand	Loam
Watered	2.9 c[§]	5.1 a
Stressed	2.2 d	3.7 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 8. Effect of drought stress and soil type on the number of pods per plant of spring canola in 1991.

	Pods per plant	
Water Regime	Soil type	
	Sand	Loam
Watered	33.8 c[§]	109.1 a
Stressed	23.2 c	62.3 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 9. Effect of drought stress and soil type on the number of pods per plant of spring canola in 1992.

	Pods per plant	
Water Regime	Soil type	
	Sand	Loam
Watered	66.8 bc[§]	187.2 a
Stressed	38.6 c	87.9 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Pod Length

In 1991, under well-watered conditions, mean pod length was 5.1 on sand and 5.9 cm on loam, under non-stress conditions compared to 4.9 on sand and 5.5 on loam under water stress treatment (Table 10). In 1992, mean pod length was 5.9 on sand and 6.6 on loam under well watered conditions and 5.7 on sand and 5.9 on loam under water stress treatment (Table 11). Pod length was affected by soil type more than water regime (Tables 10 and 11). Under well-watered conditions, pod length was significantly smaller on sand than on loam. On Spinks sand, water stress reduced pod length by only 3% in both 1991 and 1992. On the Kalamazoo loam, water stress reduced pod length by 6% in 1991 and by 10% in 1992.

Seed per Pod

In 1991, mean number of seeds per pod was 14 on sand 21 on loam under well-watered conditions and 9 and 12 under water stress treatment (Table 12). In 1992, mean number of seeds per pod was 27 on sand and 31 on loam under well-watered conditions and 23 on sand and 28 on loam under water stress treatment (Table 13). On the sandy soil, water stress reduced mean number of seeds per pod by nearly 38% in 1991 and by 15% in 1992. On the loamy soil, water stress significantly reduced the number of seeds per pod by 40% in 1991 and by 10% in 1992. Furthermore, in both 1991 and 1992, mean number of seeds per pod was consistently lower on the sandy soil for both water treatments than on loam.

Table 10. Effect of drought stress and soil type on pod length of spring canola in 1991.

	Pod length (cm)	
Water Regime	Soil type	
	Sand	Loam
Watered	5.1 bc[§]	5.9 a
Stressed	4.9 c	5.5 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 11. Effect of drought stress and soil type on pod length of spring canola in 1992.

	Pod length (cm)	
Water Regime	Soil type	
	Sand	Loam
Watered	5.9 b[§]	6.6 a
Stressed	5.7 b	5.9 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 12. Effect of drought stress and soil type on the number of seeds per pod of spring canola in 1991.

	Seed per pod	
Water Regime	Soil type	
	Sand	Loam
Watered	14.7 b[§]	21.8 a
Stressed	9.2 c	12.7 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 13. Effect of drought stress and soil type on the number of seeds per pod of spring canola in 1992.

	Seed per pod	
Water Regime	Soil type	
	Sand	Loam
Watered	27.8 c[§]	31.6 a
Stressed	23.6 c	28.4 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Seed Yield

In 1991, mean seed yield was 145 kg/ha on sand and 797 kg/ha under well-watered conditions compared to 137 on sand and 697 kg/ha under stress treatment (Table 14). In 1992, seed yields were overall greater than those obtained in 1991. On the sandy soil yields were 619 and 235 kg/ha under well-watered and stressed conditions, respectively (Table 15). However, extensive bird feeding on loam plots resulted in unexpected yield reductions. On loam plots, mean seed yield was 412 and 456 kg/ha under well-watered and stressed conditions, respectively (Table 15). Estimation of yield loss to birds on each of the plots produced revised data on Table 15. On loam, mean seed reviised yield was 1647 and 775 kg/ha under well-watered and stressed conditions respectively (Table 15). On sand, estimated mean seed yield was 849 and 261 kg/ha under well watered and stressed conditions, respectively (Table 15).

The estimated data indicate that water stress significantly reduced seed yield both on sand and loam soil. Water stress reduced seed yield by almost 70% on the sandy soil (using actual data, the reduction was 62%). On the Kalamazoo loam, water stress reduced yield by almost 50%, whereas the actual data indicate that water stress did not significantly affect the yield.

Table 14. Effect of drought stress and soil type on the yield of spring canola in 1991.

Water Regime	Yield (kg/ha) — Soil type — Sand	Loam
Watered	145.8 b[§]	797.6 a
Stressed	137.6 b	697.6 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 15. Effect of drought stress and soil type on the actual and estimated yield of spring canola in 1992[1].

Water Regime	Actual yield (kg/ha) — Soil type — Sand	Loam	Estimated yield (kg/ha) Sand	Loam
Watered	14.7 b[§]	21.8 a	849.3 b	1647.0 a
Stressed	9.2 c	12.7 b	261.3 c	775.8 b

1 Estimation of bird damage to potential yield.

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

SULFUR CONTENT

Leaf Sulfur Content

Soil type significantly affected the sulfur content of canola leaves. On loam soil, the sulfur content of leaves was 0.81% compared to 0.33% on the Spinks sand (Table 16). Water stress did not significantly reduce the sulfur content of leaves in either soil type.

Seed Sulfur Content

Soil type and water stress did not affect sulfur content of the mature seed (Tables 17 and 18). In 1991, seed sulfur concentrations were 0.60 and 0.62% on sand (Table 17) and 0.62 and 0.64% on loam under well-watered and stressed conditions, respectively (Table 17). In 1992, seed sulfur concentrations were 0.64 and 0.65% on sand (Table 17) and 0.68 and 0.68% on loam, both under well-watered and stressed conditions, respectively (Table 18).

Table 16. Sulfur content of leaves during flowering of spring canola grown on sand and loam soils under two water regime in 1991[1].

	Sulfur content (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.33 b[§]	0.81 a
Stressed	0.29 b	0.81 a

[1] Actual data for 1992 growing season were not collected but could be assumed similar to those of 1991.

[§] Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 17. Sulfur content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Sulfur content (%)	
Water Regime	Soil type — Sand	Loam
Watered	0.60 a[§]	0.62 a
Stressed	0.62 a	0.64 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 18. Sulfur content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Sulfur content (%)	
Water Regime	Soil type — Sand	Loam
Watered	0.64 a[§]	0.68 a
Stressed	0.65 a	0.68 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Oil Content (NMR)

Soil type significantly affected the seed oil content as determined by NMR analyzer (Tables 19 and 20). In 1991, means of seed oil content were 34.3 and 33.6% on sand 38.8 and 38.4% on loam under well watered and stressed conditions, respectively (Table 19). In 1992, means of seed oil content were 44.1 and 40.0% on sand 41.3 and 41.3% on loam, both under well watered and stressed conditions, respectively (Table 20).

Oil Content (GC)

In 1991 on the sandy soil water stress did not significantly affect seed oil concentrations which were 31.4 and 30.8% respectively under well-watered and stressed conditions (Table 21). On the loam, seed oil concentrations were 35.5 and 35.9% under well-watered and stressed conditions, respectively (Table 21). Soil type had a significant effect on the concentration of oil in the seed ($P < 0.05$).

In 1992, seed oil concentrations obtained on sand were 36.6 and 40.3% under well-watered and stressed conditions, respectively (Table 22). On the loam, seed oil concentrations were 37.5 and 38.0% under well-watered and stressed conditions, respectively (Table 22).

Table 19. Seed oil content, determined by NMR, of harvested spring canola grown on sand and loam soils under two water regimes in 1991.

	Oil content (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	34.3 b[§]	38.8 a
Stressed	33.6 b	38.4 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 20. Seed oil content, determined by NMR, of harvested spring canola grown on sand and loam soils under two water regimes in 1992.

	Oil content (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	44.1 a[§]	41.3 b
Stressed	40.0 c	41.3 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 21. Seed oil content, determined by GC, of harvested spring canola grown on sand and loam soils under two water regimes in 1991.

	Oil content (%)	
Water Regime	Soil type — Sand	Loam
Watered	31.4 b[§]	35.5 a
Stressed	30.8 b	35.9 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 22. Seed oil content, determined by GC, of harvested spring canola grown on sand and loam soils under two water regimes in 1992.

	Oil content (%)	
Water Regime	Soil type — Sand	Loam
Watered	36.6 c[§]	37.5 b
Stressed	40.3 a	38.0 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

FATTY ACID PROFILE

GC analysis of canola seeds indicated the presence of at least 12 fatty acids. The following is a list of some fatty acids found in canola seeds and their relative concentration in three spring canola cultivars[1]:

Fatty Acid	Chemistry	Bounty	Delta	Westar
Palmitic	16:0	4.4	4.4	4.5
Palmitoleic	16:1	0.3	0.2	0.2
Stearic	18:0	2.1	2.0	2.2
Oleic	18:1	59.7	59.6	60.9
Linoleic	18:2	21.3	21.4	21.2
Linolenic	18:3	9.3	9.4	7.9
Eicosanoic	20:0	0.7	0.7	0.8
Eicosenoic	20:1	1.4	1.4	1.4
Eicosadinoic	20:2	0.08	0.09	0.08
Docosanoic	22:0	0.44	0.39	0.39
Erucic	22:1	0.04	0.04	0.03
Docosadinoic	22:2	0.01	0.01	0.02

[1] Data presented are means of 1991 and 1992 data of Bounty, Delta, and Westar spring canola cultivars grown on Kalamazoo loam under well watered regime.

Since some of these fatty acids were detected at very small concentrations, only those with concentrations greater than 1% will be discussed except for erucic acid (22:1) which is a very important criterion for canola oil quality.

Palmitic Acid

In 1991, soil type and water stress treatment did not significantly affect the concentration of palmitic acid. Under well-watered conditions, the concentrations of palmitic acid in the seed were 4.6 and 4.3% on sand and loam, respectively (Table 23). Under stressed conditions, palmitic acid concentrations were 4.6 and 4.5% on sand and

loam respectively (Table 23). In 1992, the concentrations of palmitic acid were 4.2 and 4.4% on sand and 4.3 and 4.3% on loam both under well-watered and stressed conditions, respectively (Table 24).

Stearic Acid

In 1991, the concentration of stearic acid in the seed were 2.5 and 2.52 on sand and 2.55 and 2.52 on loam under well-watered and stressed conditions, respectively (Table 25). In 1992, the concentration of stearic acid in the seed were 1.83 and 2.02 on sand and 1.76 and 1.82% on loam under well-watered and stressed conditions, respectively (Table 26). In 1991, neither water stress nor soil type had any significant effect on seed stearic acid concentration. In 1992, mean stearic acid concentrations were overall smaller than those obtained in 1991, however, water stress significantly increased the stearic acid concentration on the sandy but not on the loam soil.

Oleic Acid

In 1991, water stress did not significantly affect the concentration of oleic acid on either sand or loam soils, however, soil type significantly affected the concentration of oleic acid. Oleic acid concentrations were 58.5 and 58.0% on sand and 61.6 and 61.5 on loam under well-watered and stressed conditions, respectively (Table 27). In 1992, neither soil type nor water stress had significantly affected oleic acid concentration. Oleic acid concentrations were 58.6 and 59.1 on sand and 59.0 and 59.9% on loam under well-watered and stressed conditions, respectively (Table 28). Like in 1991, oleic acid concentrations produced on loam were slightly higher than on sand, however, the difference was not significant in 1992.

Linoleic Acid

In 1991, soil type had a significant effect on linoleic acid concentration. Under well-watered conditions, linoleic acid concentrations were 23.4% on sand and 20.4% on loam under well-watered conditions and 23.8% on sand and 20.9 under stressed conditions (Table 29). Water stress tended to slightly increase the concentration of linoleic acid but this increase was not significant.

In 1992, linoleic acid concentration was significantly increased by water stress on the sand but was not on loam. Linolenic acid concentrations were 20.4 and 21.8% on sand and 21.7 and 21.4% under well-watered and stressed conditions, respectively (Table 30). Unlike in 1991, soil type did not influence linoleic acid concentration.

Linolenic Acid

In both 1991 and 1992, the concentration of linolenic acid was affected by neither soil type nor water stress. Under well-watered conditions, linolenic acid concentrations were 7.7 and 7.9% on sand and loam, respectively compared to 7.9 and 7.4% under stressed conditions (Table 31).

In 1992, linolenic acid concentrations were slightly higher than those obtained in 1991. Under well-watered conditions, linolenic acid concentrations were 10.4 and 10.4% on sand and loam, respectively and 9.5 and 9.1% under stressed conditions (Table 32).

Erucic Acid

Erucic acid concentrations were not significantly affected by either soil type nor water stress. In 1991, erucic acid concentrations were 0.028 and 0.026% on sand and loam, compared to 0.033 and 0.036% under well-watered and stressed conditions,

respectively (Table 33). In 1992, erucic acid concentrations were 1.257 and 0.168% on sand and 0.048 and 0.033% on loam under well watered and stressed conditions, respectively (Table 34). Erucic acid concentrations obtained in both 1991 and 1992 were much lower than the 2% standard for canola quality.

Table 23. Palmitic acid (16:0) concentration of harvested spring canola seeds grown on sand and loam soils under two water regimes in 1991.

	Palmitic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	4.6 a[§]	4.3 b
Stressed	4.6 a	4.5 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 24. Palmitic Acid (16:0) concentration of harvested spring canola grown on sand and loam soils under two water regimes in 1992.

	Palmitic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	4.2 b[§]	4.3 a
Stressed	4.4 a	4.3 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 25. Stearic acid (18:0) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Stearic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	2.50 a[§]	2.55 a
Stressed	2.52 a	2.52 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 26. Stearic acid (18:0) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Stearic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	1.83 b[§]	1.76 b
Stressed	2.02 a	1.82 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 27. Oleic acid (18:1) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Oleic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	58.5 b[§]	61.6 a
Stressed	58.0 b	61.5 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 28. Oleic acid (18:1) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Oleic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	58.6 a[§]	59.0 a
Stressed	59.1 a	59.9 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 29. Linoleic acid (18:2) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Linoleic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	23.4 a[§]	20.4 b
Stressed	23.8 a	20.9 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 30. Linoleic acid (18:2) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Linoleic acid (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	20.4 b[§]	21.7 a
Stressed	21.8 b	21.4 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 31. Linolenic acid (18:3) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Linolenic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	7.7 a[§]	7.9 a
Stressed	7.9 a	7.4 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 32. Linolenic acid (18:3) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Linolenic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	10.4 a[§]	10.4 ab
Stressed	9.5 ab	9.1 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 33. Erucic acid (22:1) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Erucic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	0.028 a[§]	0.033 a
Stressed	0.026 a	0.036 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 34. Erucic acid (22:1) concentration of harvested spring canola seed grown on sand and loam soils under two water regimes and in 1992.

	Erucic acid (%)	
Water Regime	Soil type — Sand	Loam
Watered	1.257 a[§]	0.048 a
Stressed	0.168 b	0.033 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Total Saturated vs Unsaturated Fatty Acids

In 1991, neither soil type nor water treatment significantly influenced the concentration of saturated fatty acid in the seed (Table 35). On the sandy soil, concentration of saturated fatty acid in seeds were 8.41 and 8.47 under well-watered and stressed conditions, respectively (Table 35) compared to 8.16 and 8.29% on loam (Table 35). In 1992, on sandy soil, concentration of saturated fatty acids in seed were 7.11 and 7.65% under well-watered and stressed conditions, respectively (Table 36) compared to 7.28 and 7.32% on loam (Table 36). Unlike in 1991, water stress tended to increase the total concentration of saturated fatty acids and was significant on sand but not on loam. In 1992, concentrations of saturated fatty acids were smaller than in 1991 with differences in concentrations of more than 1% between the 2 years.

In 1991, neither soil type nor water treatment significantly influenced concentration of unsaturated fatty acids in the seed (Table 37). On the sandy soil, concentration of saturated fatty acids in seed were 91.58 and 91.52% under well-watered and stressed conditions, respectively (Table 37) compared to 91.83 and 91.71% on loam (Table 37). In 1992, concentration of unsaturated fatty acids on sandy soil were 92.88 and 92.34% under well-watered and stressed conditions, respectively (Table 38) compared to 92.72 and 92.68% on loam (Table 38). Unlike in 1991, water stress tended to significantly decrease the total concentration of unsaturated fatty acids on sand but not on loam. In 1992, concentrations of unsaturated fatty acids were overall smaller than in 1991, with differences in concentrations of more than 1% between the 2 years.

Ratio of Linolenic to Linoleic Acid

In 1991, linolenic to linoleic ratio was significantly affected by soil type. Ratios of linolenic to linoleic fatty acid concentration were 33.06 and 33.16% on sand and 38.78 and 35.65% on loam under well-watered and stressed conditions, respectively (Table 39). In 1992, this ratio was significantly affected by water stress but not by soil type. Ratios of linolenic to linoleic fatty acid concentration were 50.92 and 43.93% on sand and 46.18 and 42.85% on loam under well-watered and stressed conditions, respectively (Table 40).

Table 35. Total saturated fatty acid content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Total saturated (%)	
Water Regime	Soil type — Sand	Loam
Watered	8.41 a[§]	8.16 a
Stressed	8.47 a	8.29 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 36. Total saturated fatty acid content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Total saturated (%)	
Water Regime	Soil type — Sand	Loam
Watered	7.11 b[§]	7.28 b
Stressed	7.65 a	7.32 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 37. Total unsaturated fatty acid content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Total unsaturated (%)	
Water Regime	Soil type — Sand	Loam
Watered	91.58 a[§]	91.83 a
Stressed	91.52 a	91.71 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 38. Total unsaturated fatty acid content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Total unsaturated (%)	
Water Regime	Soil type — Sand	Loam
Watered	92.88 a[§]	92.72 a
Stressed	92.34 b	92.68 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 39. Ratio of linolenic to linoleic acid of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Ratio 18:3/18:2 (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	33.06 c[§]	38.78 a
Stressed	33.16 c	35.65 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 40. Ratio of linolenic to linoleic acid of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Ratio 18:3/18:2 (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	50.92 a[§]	46.18 ab
Stressed	43.93 b	42.85 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Crude Protein

In 1991, neither soil type nor water stress had any significant effect on crude protein of seed. Mean protein content of seed was 28.1 and 28.2% on sand and 26.1 and 26.9% on loam under well-watered and stressed conditions, respectively (Table 41). In 1992, water stress had a significant effect on the protein content of seed only on sandy soil and not on loam. In 1992, protein content of seed were 23.2 and 24.5% on sand and 25.9 and 25.9 on loam both under well-watered and stressed conditions, respectively (Table 42). In 1992, protein contents of seed were overall lower than in 1991.

SEED GLUCOSINOLATE CONTENT

Total Glucosinolate Content

In 1991, neither soil type nor water treatment had any significant effect on the glucosinolate content of seeds. Glucosinolate contents of seed produced on both sand and loam under control stress treatments were significantly higher than the canola standard of 30 μmoles/g of defatted meal. In 1991, glucosinolate contents of seed were 47.0 and 49.6 μmoles/g of defatted meal on sand and 45.7 and 43.0 μmoles on loam under well-watered and stressed conditions, respectively (Table 43). In 1992, seed glucosinolate contents were overall lower than those of 1991. Water stress significantly increased glucosinolate content of seed on sandy soil but not on loam. Glucosinolate contents of seed were 28 and 37.0 μmoles/g of defatted meal on sand and 34.8 and 35.7 μmoles on loam under well-watered and under stressed conditions, respectively (Table 44).

Table 41. Crude protein content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Protein content (%)	
Water Regime	Soil type — Sand	Loam
Watered	28.1 ab[§]	26.1 b
Stressed	28.2 a	26.9 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 42. Crude protein content of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Protein content (%)	
Water Regime	Soil type — Sand	Loam
Watered	23.2 c[§]	25.9 a
Stressed	24.5 b	25.9 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 43. Glucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Glucosinolate concentration (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	47.0 a[§]	45.7 a
Stressed	49.6 a	43.0 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 44. Glucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Glucosinolate concentration (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	28.0 b[§]	34.8 ab
Stressed	37.0 a	35.7 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Individual Glucosinolates

The total glucosinolate concentration is made up of eight chemically different glucosinolate groups. They exist at various concentrations and their consequent degradation greatly depends on their chemical nature and the concentration of each.

Allylglucosinolate

The concentrations of allylglucosinolate (ALL) in 1991 and 1992 were less than 1μmole/g defatted meal (Tables 45 and 46). These concentrations are very small and had little influence on the final seed glucosinolate content.

3-Butenylglucosinolate

In 1991, 3-Butenylglucosinolate (3BUT) concentrations in the seed were 7.5 and 8.0 μmoles/g of defatted meal on sand and 7.3 and 6.5 μmoles on loam under well-watered and stressed conditions, respectively (Table 47). In 1992, 3BUT were notably lower than those of 1991. They were 3.3 on sand and 3.7 μmoles/g of defatted meal on loam under well watered conditions. These were 56% and 46% lower than those obtained in 1991. Under stress conditions, 3BUT concentrations were 5.8 on sand and 5.8 μmoles/g of defatted meal on loam (Table 48).

4-Pentenylglucosinolate

4-Pentenylglucosinolate was also detected in small concentrations varying between 1.8 to 2.3 μmoles/g of defatted meal in 1991 and 0.7 to 1.8 in 1992 (Tables 49 and 50). These concentrations had little on final seed glucosinolate content.

3-Hydroxybutenylglucosinolate

In 1991, concentrations of 3-Hydroxybutenylglucosinolate (3BOH) were 23.2 and

24.8 μmoles/g of defatted meal on sand and 17.6 and 16.2 μmoles on loam under well-watered and stressed conditions, respectively (Table 51). In 1992, these concentrations were 7.3 and 12.6 μmoles on sand and 10.6 and 11.9 on loam under well-watered and stressed conditions, respectively (Table 52).

In 1991, soil type had a significant effect on 3BOH with concentrations on sandy soil significantly greater than those on loam. In 1992, water stress increased its concentration on the sandy soil but not on loam.

4-Hydroxypentenylglucosinolate

Soil type and water stress did not significantly influence 4-Hydroxypentenylglucosinolate in either 1991 or 1992. Its concentration varied between 0.48 to 0.78 μmole/g defatted meal in 1991 and between 0.3 to 0.99 in 1992 (Tables 53 and 54).

3-Indolylmethylglucosinolate

This glucosinolate was found in very small concentrations (Tables 55 and 56).

3-Methoxyindolylmethylglucosinolate

In 1991, soil type significantly affected the concentration of 3-Methoxyindolylmethylglucosinolate (3IME). Concentrations of 3IME were 1.8 and 2.1 μmoles/g of defatted meal on sand and 7.1 and 7.1 μmoles on loam under well-watered and stressed conditions, respectively (Table 57). In 1992, 3IME was 5.7 and 6.1 μmoles on sand and 6.5 and 6.2 on loam under well-watered and stressed conditions, respectively (Table 58). In both years, water stress tended to increase 3IME but this increase was not significant.

Table 45. Allylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	Allylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.45 a[§]	0.09 c
Stressed	0.29 b	0.19 bc

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 46. Allylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	Allylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.06 ab[§]	0.03 b
Stressed	0.08 a	0.05 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 47. 3-Butenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	3-Butenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	7.5 ab[§]	7.3 ab
Stressed	8.0 a	6.5 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 48. 3-Butenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	3-Butenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	3.3 c[§]	3.7 bc
Stressed	5.8 a	5.2 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 49. 4-Pentenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	4-Pentenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	2.3 a§	2.1 a
Stressed	2.2 a	1.8 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 50. 4-Pentenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	4-Pentenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.7 b§	1.8 a
Stressed	1.3 ab	51.3 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 51. 3-hydroxybutenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	3-hydroxybutenylglucosinolate (%)	
Water Regime	Soil type — Sand	Loam
Watered	23.2 a[§]	17.6 b
Stressed	24.8 a	16.2 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 52. 3-hydroxybutenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	4-Pentenylglucosinolate (%)	
Water Regime	Soil type — Sand	Loam
Watered	7.3 b[§]	10.6 ab
Stressed	12.6 a	11.9 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 53. 4-hydroxypentenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	4-hydroxypentenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.78 a[§]	0.57 ab
Stressed	0.76 a	0.48 b

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 54. 4-hydroxypentenylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	4-hydroxypentenylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.30 b[§]	0.99 a
Stressed	0.54 ab	0.69 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 55. 3-Indolylmethylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	3-Indolylmethylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.11 b[§]	0.21 ab
Stressed	0.49 a	0.21 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 56. 3-Indolylmethylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	3-Indolylmethylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	0.00 b[§]	0.20 a
Stressed	0.15 ab	0.17 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 57. 3-Methoxyindolmethylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1991.

	3-Methoxyindolmethylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	1.8 b[§]	7.1 a
Stressed	2.1 b	7.1 a

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

Table 58. 3-Methoxyindolmethylglucosinolate concentration of harvested spring canola seed grown on sand and loam soils under two water regimes in 1992.

	3-Methoxyindolmethylglucosinolate (%)	
Water Regime	Soil type	
	Sand	Loam
Watered	5.7 b[§]	6.5 a
Stressed	6.1 ab	6.2 ab

§ Means followed by the same letter within a column and row are not significantly different at the 0.05 probability level.

DISCUSSIONS

Yield and Yield Components

Significant difference between average maximum and minimum daily temperatures produced considerable variability between 1991 and 1992. In 1991 plants were harvested 91 days after planting, whereas in 1992, a cooler year, maturity was delayed and plants were harvested 112 days after planting, a difference of about 3 weeks from the previous year.

The water stress treatment was designed to induce plant moisture stress at the late stem development stage prior to beginning flowering. The well-watered treatment was a control treatment for which water is adequately provided from planting until near harvest. Plots under both water treatments are adjacent and therefore equally exposed to identical weather conditions under a Rainshelter. Yield and yield components were all significantly affected by the moisture stress treatment both on the sand and loam soil. Since the water stress treatment was begun during stem elongation and beginning of flowering, plant height, branches per plant, pods per plant, seeds per pod, pod length, and yield were affected.

In 1991, with warmer conditions, plant height on the sandy soil was 40% lower than on loam under the well watered regime, and 43% on loam under the stressed regime, suggesting that plants on the sandy soil might have been subjected to early

moisture stress during the beginning of stem elongation before the actual irrigation treatments started. In 1992, the water stress treatment produced similar results, however, plant height on the sandy soil was 27% higher than in 1991 under well-watered conditions and 25% higher than in 1991 under stressed conditions. These results suggest that water stress treatments were successful in significantly reducing plant height on the two different soil types, especially under warmer conditions during the moisture stress period. This is in agreement with the findings of Sionit and Kramer (1977), Ashley and Ethridge (1978), Korte et al. (1983a), Dornbos and Mullen (1991).

Moisture stress reduced the number of branches per plant by 34% and by 24% on the sandy soil in 1991 and 1992, respectively. However, on the loam, the number of branches per plant was reduced by 8% in 1991 and 27% in 1992 under water stress. As expected, the number of branches per plant were higher on the loam than on the sandy soil in both growing seasons.

In 1991, the number of pods per plant was substantially reduced by moisture stress. As suggested by Korte et al. (1983b) and Kadhem et al. (1985b) for soybean, moisture stress during the reproductive growth of spring canola significantly reduced the number of pods per plant, an important canola yield component (see Table 64). Moisture stress reduced the number of pods per plant by 30% in 1991 and by 40% in 1992 on the sandy soil. The number of pods per plant in 1991 were 70% higher on loam than on sand soil under well-watered regime compared to 65% under stress conditions. These results suggest that in 1991 the final number of pods per plant on the sand may have been reduced not only by moisture stress during the reproductive growth but also by a

combination of warmer temperatures during vegetative growth and limited moisture availability on the sandy soil. This observation was previously reported by Denmead and Shaw (1960), Denmead and Shaw (1962), and Muchow (1989).

The number of seeds per pod was significantly reduced both in 1991 and in 1992. The reduction in the number of seeds per pod may be caused by significant water shortages during anthesis as suggested by Herrero and Johnson (1981) and Hall et al. (1981), and Freier et al. (1984) for soybean and by Eastin et al. (1983), Garrity et al. (1982), and Meyers et al. (1984) for sorghum (*Sorghum bicolor* (L.) Moench) for which anthesis was found to be the most sensitive stage that determines the number of seeds per panicle. Under stressed conditions, the number of seeds per pod on loam was 28% higher than on sand in 1991 and only 16% higher in 1992, suggesting that higher temperatures in 1991 associated with moisture stress may be attributed to the substantial reduction in the number of pods and seeds per pod.

Yield was affected by both soil type and moisture stress. It is well known that moisture stress reduces the yield of soybean (Korte et al., 1983b) and sorghum (Meyers et al., 1984). Spring canola yields in these studies were significantly reduced by a combination of moisture stress and soil type. On the sandy soil, the yield averaged 140 kg/ha which is an extremely low for canola. On the loam soil, moisture stress reduced seed yield by only 13% (not statistically significant). As noted above, during 1991, higher temperatures and possible early water deficits during vegetative growth may have enhanced the influence of moisture stress on both soils. This may explain the lack of a significant yield reduction on both soils during 1991. Cooler weather in 1992 resulted

in overall more vigorous, taller plants, with greater number pods per plant and pod length, more seeds per pod, and higher seed yield. However, higher seed yields of 1992 were seriously reduced by bird feeding. Thus bird feeding losses were visually estimated at time of harvest as percentage of yield loss per plot. However, the crop on the sandy soil did not attract as many birds, and feeding loss was limited.

Yield losses due to moisture stress was almost 50% on the loam and 70% on the sandy soil. This result is expected, given the difference in moisture availability, as well as the various physical and chemical properties of both soils. Yield components and ultimately seed yield were affected by both moisture stress and soil type. On the sandy soil, the impact of moisture stress on the various yield components is particularly accentuated during warmer growing season.

In 1991 and 1992, all yield components except number of branches per plant in 1991 were significantly and positively correlated with yield (Table 59). In 1992, the number of seed per pod was highly correlated to the estimated yield ($r = + 0.85$, $P > 0.001$) whereas in 1991, yield was highly correlated with plant height ($r = + 0.76$, $P > 0.001$) (Table 59).

The moisture treatments in 1991 and 1992 clearly stressed the canola plants, as shown by the effect they had on yield and yield components.

Table 59. Simple correlation coefficients between yield and yield components of spring canola in 1991 and 1992[1].

	Yield	
	1991	1992
	r	
Plant height	0.76 **[1]	0.78 **
Branches plant^{-1}	-0.05 ns	0.83 **
Pods per plant	0.44 *	0.85 **
Pod length	0.44 **	0.61 **
Seeds pod$_{-1}$	0.38 ns	0.75 **

[1] (*) and (**) simple correlation coefficient significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

SEED OIL AND PROTEIN CONTENT

Oil content was determined by gas chromatography (GC) and nuclear magnetic resonnance (NMR) at seed moisture contents of 8.5% and 2%, respectively. In 1991, water stress did not affect the oil content on either soils. On the loam, however, seed oil level was higher than that on the sandy soil. In 1992, under well watered conditions, seed oil content on the sandy soil was 6% higher than that on the loam. In 1992, moisture stress reduced oil concentration in the seed on the sandy soil but not on the loamy soil. Similar results were reported by Mailer and Cornish (1987) for canola and Foroud et. al. (1993) with soybeans. Furthermore, in 1992, seed oil concentrations from both treatments and soil types averaged 20% and 6% (on sand and loam, respectively) higher than those in 1991, indicating the extent of variability in oil concentration as a result perhaps of seasonal weather variability. Mailer and Pratley (1990) reported similar variability in oil concentration of canola under different Australian ecosystems. In 1991 a combination of warmer temperatures during vegetative and reproductive growth of canola and moisture stress may have caused lower oil concentrations. This deduction is in agreement with an earlier report by Downey (1983), indicating that cool and moist growing conditions of Northern Europe favored high oil contents of canola.

The effect of moisture stress on the seed protein concentration was reported by several authors. Foroud et al. (1993) reported increases in soybean protein content while

oil content was drastically reduced. In spring canola, as expected, moisture stress increased the protein content but the increase was not significant on the sand and loam in 1991. However, the protein content of the seed on the sandy soil was 7% higher than on the loam under well-watered conditions and 4% higher under stressed conditions. In 1992, moisture stress significantly increased protein content on the sandy soil but not on the loam. However, unlike in 1991, protein content on the loam was 10% higher than on the sandy soil under well watered conditions compared to 5% under stressed condition. A combination of warmer temperatures during 1991 and possibly early water deficits enhanced the stress on the sandy soil and produced significantly less pods and seeds per pod which may also explain the higher protein concentration in the seed. In 1992, cooler temperatures and lower stress on both the sandy and loam soils may have resulted in more pods and seeds per pod, and consequently, an overall decrease of protein content in comparison with 1991. This is further supported by the highly significant correlation between pods per plant, seeds per pod, and oil and protein content in 1991; protein content was negatively correlated with the number of pods ($r = -0.42$, $P < 0.001$) (Table 60) and seeds per pod ($r = -0.46$, $P < 0.001$) (Table 60), indicating a linear relationship between these variables. In 1992, correlation coefficients between number of pods ($r = 0.35$) and seeds per pod ($r = 0.47$) and protein were not significant ($P > 0.05$) (table (60).

Oil and protein content were, as expected, significantly and negatively correlated at the 0.001 probability level with $r = -0.61$ (Table 60), indicating that 37% of the variation in the mean oil content is explained by a linear function of protein content of

the seed. Similar results have been reported for soybean (Latifi, 1980 and Hobbs and Mundel 1983. In 1992, oil and protein content were negatively but not significantly correlated ($r = -0.21$ with $P > 0.05$). Similarly Thompson (1978) found little influence of water availability on the oil and protein content in Australia.

Fatty Acid Profile

The concentration of palmitic acid was very low, making it less susceptible to influence by moisture stress. However, in 1992, moisture stress resulted in a 4% increase in palmitic acid and was positively correlated with water availability ($r = 0.46$,$P < 0.05$) (Table 61). In 1991, soil type was negatively correlated with palmitic acid concentration ($r = -0.49$, $P < 0.05$) (Table 61). This result suggests that interaction between sandy soil and moisture stress significantly increased the palmitic acid concentration.

Palmitoleic acid was present at less than 1% concentration. Neither moisture stress nor soil type affected its concentration during 1991 and 1992 .

Stearic acid was also detected at low concentrations. In 1991, the stearic acid concentration was 28% higher than in 1992. As noted above, warmer temperatures may have accentuated the moisture stress and produced higher concentration of stearic acid. This conclusion may be supported by the significant increase in stearic acid concentration caused by moisture stress in 1992.

Oleic acid is a major fatty acid of canola seeds, constituting about 60% of the total fatty acids in the seed. Moisture stress did not significantly affect oleic acid

concentration in 1991 ($r = -0.06$, $P > 0.05$), however, it was positively correlated to soil type ($r = 0.64$, $P < 0.001$) (Table 61). Oleic acid concentration was significantly higher on the loam than on the sandy soil. However, in 1992, neither water regime nor soil type were correlated to oleic acid concentration. Differences in temperatures during seed formation, development, and maturation between 1991 and 1992 may explain the difference in oleic acid concentration.

Linoleic acid and linolenic acids should normally constitute about 30% of the total fatty acids in canola seed (Ackman, 1990). Linoleic acid (18:2) is an essential fatty acid while linolenic acid (18:3) is regarded as potentially functional in reducing cardiovascular risk and, together, constitute an ideal (1:2) ratio. In 1991, moisture stress was not correlated with levels of linoleic and linolenic acid, however, soil type was negatively correlated to both fatty acids ($r = -0.72$ with $P < 0.01$ and $r = -0.06$ with $P < 0.01$, respectively) (Table 61). In 1991, the sandy soil had a significant influence on the concentration of linoleic acid in particular. The ratio of linolenic to linoleic was also correlated with soil type. In 1992, neither moisture stress nor soil type were correlated with linoleic and linolenic acid, however, their ratio was negatively correlated with the water regime ($r = -0.44$ with $P < 0.05$) (Table 61). This underscores the indirect effect of moisture stress as well the influence of seasonal weather variability on the composition of fatty acids.

Canola has a relatively low concentration of saturated fatty acids. In 1991, the total saturated fatty acid concentration was not correlated with water stress or soil type. In 1992, total saturated fatty acids were correlated with water regime ($r = 0.40$ with

$P<0.01$) (Table 61). Thus, 16% increase in the total concentration of saturated fatty acids may be explained by water availability. In 1991, unsaturated fatty acids composition was generally 1 or 2% lower than in 1992. Total concentration of unsaturated fatty acids was not correlated with soil type or water regime; however, in 1992, unsaturated fatty acids were positively correlated with water regime ($r= 0.42$ with $P<0.05$). The reduction in unsaturated fatty acid and increase in saturated fatty acid suggest a close relationship between moisture availability on one hand and seed development and lipid biosynthesis on the other.

Table 60. **Simple correlation coefficients between oil content (by NMR), oil content (by GC), protein content, number of pods, and number of seeds per pod of spring canola in 1991 and 1992.**

	Oil (GC)	Oil (NMR)	Protein	Pods plant^{-1} pod^{-1}	Seed
			1991		
Oil (GC)	----	0.94**[1]	-0.57**	0.74**	0.49**
Oil (NMR)		-----	0.61**	0.71**	0.56**
Protein			-----	0.42**	-0.46**
Pod plant^{-1}				----	0.77**
Seed pod^{-1}					----
			1992		
Oil (GC)	-----	0.68**	0.03ns	-0.27ns	-0.58**
Oil (NMR)		-----	0.21ns	-0.09ns	0.39ns
Protein			----	0.35ns	0.47**
Pod plant^{-1}				----	0.70**
Seed pod^{-1}					----

[1] (*) and (**) simple correlation coefficient significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

Table 61. Simple correlation coefficients between soil type and moisture stress and palmitic, oleic, linoleic, linolenic, total saturated, total unsaturated fatty acids content of spring canola in 1991 and 1992.

Fatty acid	1991		1992	
	Soil type	Water regime	Soil type	Water regime
Palmitic	-0.49 *	0.30 ns	0.08 ns	0.46 *
Stearic	0.07 ns	0.00 ns	-0.36 ns	0.33 ns
Oleic	0.64 **	-0.06 ns	0.23 ns	0.26 ns
Linoleic	-0.72 **	0.10 ns	0.20 ns	0.26 ns
Linolenic	-0.06 **	-0.08 ns	-0.18 ns	-0.38 ns
Saturated	-0.27 ns	0.11 ns	-0.11 ns	0.40 **
Unsaturated	0.27 ns	-0.11 ns	0.11 ns	-0.40 *
18:3/18:2	0.42 *	-0.15 ns	-0.24 ns	-0.44 *

[1] (*) and (**) simple correlation coefficient significant at 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

SEED GLUCOSINOLATE CONTENT

In 1991, total glucosinolate content of double-low cultivar seed was 35% higher than the canola standard of 30 μmoles/g of defatted meal. The overall increase in glucosinolate content cannot be attributed only to moisture stress but to a probable combination of moisture deficit and warmer temperatures during the vegetative and reproductive growth in 1991.

The glucosinolate concentration of seeds harvested on the sandy soil was higher than that on the loam, demonstrating higher moisture stress of sandy soil because of its low water holding capacity. In 1992, total glucosinolate concentration of the seed was near the normal canola standard of 30 μmoles/g of defatted meal, almost 35% lower level than in 1991. Moisture stress significantly increased glucosinolate concentration of seed grown on sandy soil, but not on the loam ($P > 0.05$). Since location, soil type, agronomic practices, and treatments were similar to those in 1991, lower overall glucosinolate concentrations in 1992 and attributed to cooler temperatures during late spring and summer. Yield and yield component results support this hypothesis.

In 1992, seed were harvested 3 weeks later than in 1991. Furthermore, the plants were more vigorous, with more pods/plant, seed per pod, and higher seed yield than those in 1991. However, variations in glucosinolates concentrations were expected. Earlier, Mailer and Wratten (1985) and Sang et al. (1986) reported variability in

glucosinolate concentrations under different growing conditions. Increased temperatures have also been reported to increase the glucosinolate content in the seed (Mailer, 1988), although the mechanisms for that increase are not clear.

Sulfur nutrition of canola plants has been suggested to influence glucosinolate concentrations. In this study, leaf sulfur content of plants grown on the sandy soil was 0.31% and 0.81% on the loam, a difference of almost 60%, while moisture stress had no significant effect on leaf sulfur status. This substantial difference in leaf sulfur content between sand and loam did not translate into any significant differences in seed sulfur content at harvest. This is an indication that the sulfur nutrition status of canola plants grown on sand and loam did not influence the glucosinolate concentration of the seeds. Available sulfur in the seeds of plants grown on the sandy soil could have been translocated from maturing leaves and pods and accumulated in the seeds by a "concentration effect". On the sandy soil, stressed plants were smaller and had smaller numbers of pods and seeds per pod, which may have taken up available sulfur to levels comparable to those on loam. Therefore, in this study, only moisture stress and higher temperatures during the reproductive stages could have influenced glucosinolate concentrations. This conlcusion is supported by data obtained in 1992 in which seeds had comparable sulfur content to those of 1991, while seed glucosinolate concentrations remained closer to the canola standard of 30 μmoles/g of defatted meal.

Total glucosinolate content represents a total of eight individual glucosinolates having different peaks and retention times. Four of the eight detected individual glucosinolates were present in concentrations of less than 1 μmole/g of defatted meal.

Allylglucosinolate, 4-hydroxypentenylglucosinolate, and methylthioglucosinolate are derived from methionine while 3-Indolmethylglucosinolate is derived from tryptophan. Moisture stress and differences in temperatures during the reproductive stages between 1991 and 1992 did not produce substantial increases in these compounds. However, in 1991, moisture stress and warmer temperatures had caused substantial increases in 3-Hydoxybutenylglucosinolate (3BOH) which had the highest concentration of about 50% of the total glucosinolate concentration. In 1991, moisture stress had no apparent effect on 3BOH level while sandy soil caused a significant increase, perhaps by enhancing the moisture deficits and/or by concentrating this glucosinolate in smaller numbers of seeds. On the loam, 3BOH concentration in 1991 was greater than in 1992, strongly demonstrating how seasonal weather variability, particularly warmer temperatures, could influence synthesis of these secondary metabolites in the seeds.

CONCLUSIONS

* Water stress significantly reduced plant height, number of pods per plant, seeds per pod, and seed yield on both sandy and loamy soil. Means of yield components obtained on loam were greater on that those on sandy soil.

* Water stress did not produce a significant effect pattern on the oil and protein contents of the seed.

* Water stress significantly increased the glucosinolate content of seeds on the sandy more than on the loamy soil.

CHAPTER IV

THE EFFECTS OF WATER STRESS UNDER GREENHOUSE CONDITIONS ON CANOLA (*B. napus* L.) MORPHOLOGICAL TRAITS, PROTEIN CONTENT AND GLUCOSINOLATE PROFILE.

ABSTRACT

Glucosinolates are sulfur-containing compounds in the secondary plant metabolism found typically in Brassica species which have become important criteria for canola (*B. napus*) quality. This experiment was conducted to study the effects of moderate and severe water stress treatments on spring canola morphological traits and seed protein and glucosinolate content.

This study was conducted under controlled greenhouse conditions in the Plant and Soil Sciences Teaching Greenhouses. Delta canola was grown in plastic pots containing 14 kg sterilized sandy loam with 30% Canadian peat and 20% washed sand. The experimental design was a randomized complete block with 3 replications. 7.5g of K_2NO_3 and 4.5g of superphosphate were added to each pot. Treatments were started at the beginning of stem elongation, consisting of a control by restoring weight of pots to field capacity, a moderate treatment providing 50% of the water in the control treatment, and a severe treatment providing only 25% of the saturation volume.

Moderate and severe treatment had a significant effect on all canola morphological traits. Plant height was reduced by 16% under moderate stress and by 47% under severe

stress. The number of branches per plant was reduced by 53% under severe treatment. The pod length was also significantly reduced by water stress treatment. Mean numbers of pods per plant were 90.7, 59.5, 32.8 at control, moderate, and severe stress treatment, respectively. Consequently, seed yields per plant were 4.27, 2.80, and 1.11 g/plant, a reduction of almost 34% and 74% compared to the control treatment.

Water stress treatment increased seed protein content. Mean protein contents were 22.6, 24.6, and 31.1% at control, moderate, and severe water stress treatment, respectively.

Seed glucosinolate contents were increased more significantly by the severe water stress treatment than by the moderate stress treatment. Under severe water stress, seed glucosinolate content was 20% above the canola standard of 30 μmoles/g of defatted meal.

MATERIALS AND METHODS

This greenhouse experiment was conducted to study the effects of moderate and severe water stress treatments during stem elongation on some canola (*B. napus*) morphological traits, protein content of seeds, and glucosinolate profile.

Planting, Experimental Design, and Treatments

This controlled environment experiment was conducted at the Plant Science Greenhouses, Michigan State University, from January to July 1993. Seed of the spring cultivar Delta was planted on January 18, 1993 in plastic pots with diameter and depth both equal to 27 cm and containing sterilized field sandy loam soil with 30% Canadian peat and 20% washed sand (2NS). A second replicate experiment was started on April 21, 1993 in the same facility with similar soil media. Photoperiod was set at 16 hours by automatic control, with day/night temperatures of 26.7 and 21.1°C.

The experimental design was a randomized complete block with 3 replications consisting of one canola plant per pot in the first experiment and 4 replications with 2 plants per pot in the second experiment. Empty pots were weighed and filled with 14 kg of soil. 7.5 g of K_2NO_3 and 4.5 g of superphosphate were added to all pots, which were saturated with 8 l of water and kept draining for 3 days. Weight of pots at saturation was 17 kg. Pots were then allowed to dry out for 15 days.

Treatments were started at the beginning of stem elongation, consisting of a

control by restoring weight of pots to about 17 kg by saturation, a moderate treatment providing 50% of the water at saturation level and a severe treatment providing only 25% of the volume of saturation. Pots were watered weekly.

Parameters Measured

Irrigation of all treatments was stopped at physiological maturity, after which the plants were allowed to naturally dry in the greenhouse. Data collected included plant height, branches/plant, pods/plant, pod length, seeds/pod, and weight of seeds/plant.

Seed Protein and Glucosinolate Content

Seed protein and glucosinolate content were determined as described above.

Replicate Experiment

The greenhouse experiment, measurement of variables, and seed analyses for the replicate second study were performed as described above.

Statistical Analysis

Analysis of variance, least significant difference, Duncan's multiple range test, and simple correlation coefficient were used to analyze the data using the statistical package MSTAT (Michigan State University, East Lansing, Michigan).

RESULTS AND DISCUSSIONS

Water stress treatments were started during stem elongation, coinciding with the bud stage and flowering initiation. Consequently the treatments had a distinctive effect on plant morphology. Plant height, number of branches per plant, pods per plant, pod length, seeds per pod, and seed yield were all negatively correlated with water regimes at probability levels of 0.01 (Table 1). This negative correlation suggested that moderate and severe stress treatments had significant effects on yield and yield components of plants ($P < 0.01$).

Morphological Traits

Moderate and severe water stress treatments had a significant effect on the height of canola plants ($P < 0.01$). Mean heights of plants were 111.6, 93.2, and 59.2 cm at control, moderate, and severe water regimes, respectively (Table 2). Plant height was reduced by 16% under moderate and 47% by the severe treatment. These results are consistent with data discussed in the previous chapter. Moisture stress significantly reduced plant height, particularly when initiated at the crucial stage of stem elongation.

Table 1. Simple correlation coefficients between water stress treatments and morphological traits of Delta canola grown under controlled greenhouse conditions.

Water Regime	Correlation Coefficient	P[1]
Plant height	-0.89	**
Primary branches	-0.77	**
Pods per plant	-0.81	**
Pod length	-0.80	**
Seeds per pod	-0.83	**
Seed Yield	-0.88	**

[1] (**) significant at the 0.01 probability levels.

Moderate and severe treatments had a significant effect on the number of branches per plant ($P<0.01$). Mean numbers of branches per plant were 3.8, 3.0, and 1.8 at the control, moderate, and severe water treatments (Table 2). No significant difference in number of branches per plant occurred between control and moderate water stress. The number of branches per plant was reduced by 53% by the severe stress treatment compared to controls. Although Holmes (1980) suggested number of pods, number of seeds per pod, and seed size as components of seed yield, plant height and number of branches per plant also indirectly influenced components of seed yield by reducing the number of pods, and therefore, seed yield.

Stress treatments also had a significant effect on the pod length ($P<0.01$). Mean lengths of pods were 6.7, 5.7, and 4.5 cm at control, moderate, severe stress treatments, respectively (Table 2). Pod length data were consistent with effects of moderate and severe stress treatments on overall vegetative and reproductive growth (i.e. shorter stems, fewer leaves, and notably less vigorous plants).

Water stress treatments had a singificant effect on the number of pods per plant ($P<0.01$). Mean numbers of pods per plant were 90.7, 59.5, and 32.8 at control, moderate, and severe stress treatment, respectively (Table 3). The number of pods per plant was reduced by 34% with moderate stress and 64% under severe stress, suggesting a serious effect of water stress on an important component of seed yield of canola as indicated by (Holmes, 1980).

Water treatments had a significant effect on the number of seeds per pod ($P<0.01$). Mean numbers of seeds per pod were 27.6, 23.2, and 15.8 at control,

moderate, and severe stress treatments, respectively (Table 3). Difference in the number of seeds per pod between control and moderate stress treatment was not significant, however, the number of seeds per pod was reduced by 43% with severe stress treatment. These results are consistent with plant height, branches per plant, and pods per plant data discussed above.

Seed yield per plant was significantly affected by both moderate and severe water stress treatments ($P<0.01$), with yields of 4.27, 2.80, and 1.11 g/plant at control, moderate, and severe water stress treatments, respectively (Table 3). Seed yield was reduced by 34% under moderate and 74% under severe stress conditions. Yield and yield components results were all in agreement with observations discussed in the previous chapter.

Morphological traits data indicated that moderate and severe stress treatments caused significant differences in plants morphological traits and seed yield.

Table 2. **The effects of moderate and severe water stress on the height and number of branches of Delta canola grown under controlled greenhouse conditions[1].**

Water Regime	Plant height (cm)	Branches plant^{-1} (no.)	Pod length (cm)
Control	111.6 a	3.8 a	6.7 a
Moderate	93.2 b	3.0 a	5.7 b
Severe	59.2 c	1.8 b	4.5 c
Treatment	** ¶	**	**

[1] Means of 7 replications using data of combined replicate experiments.

[§] Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

[¶] (**) significant at 0.01 probability level.

Table 3. **The effects of moderate and severe water stress on the number of pods per plant, seeds per pod and seed yield of Delta canola grown under controlled greenhouse conditions[1].**

Water Regime	Pods plant^{-1}	Seeds pod^{-1}	Seed yield (g)
Control	90.7 a	27.6 a	4.27 a
Moderate	59.5 b	23.2 a	2.80 b
Severe	32.8 c	15.8 b	1.11 c
Treatment	** ¶	**	**

[1] Means of 7 replications using data of combined replicate experiments.

[§] Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

[¶] (**) significant at 0.01 probability level.

Seed Protein and Glucosinolate Content

Moderate and severe water stress treatments had a singificant effect on the protein content of seeds ($P<0.01$). Mean seed protein contents were 22.6, 24.6, and 31.1% at the control, moderate, and severe water stress treatment, respectively (Table 4). The simple correlation coefficient between the three water regimes and protein content of seeds was 0.89 ($P<0.01$) (Table 4). Protein content of seeds was increased by 9% under moderate stress and by 27% under severe stress. This is consistent with data discussed in the previous chapter. It is also consistent with results reported by Henry et al. (1986) for wheat.

Glucosinolate content of seeds was significantly increased by moderate and severe water stress to levels exceeding the canola standard of 30 μmoles/g defatted meal. Means of glucosinolates in the seed were 22.7, 26.5, and 37.5 μmoles/g of defatted meal at control, moderate, and severe water treatments, respectively (Table 4). The simple correlation coefficient between water regimes and glucosinolate content of seeds was 0.90 ($P<0.01$), indicating a high positive correlation between the two variables. Severe water stress increased the glucosinolate content of seed by 39% compared to that of the control. Under severe water stress, glucosinolate content of seeds exceeded the canola standard of 30 μmoles/g of defatted meal by 20%.

Table 4. **The effects of moderate and severe water stress on protein and glucosinolate content of seeds of Delta canola grown under controlled greenhouse conditions[1].**

Water Regime	Protein Content (%)	Glucosinolate μmoles/g
Control	22.6 c	22.7 c
Moderate	24.6 b	26.5 b
Severe	31.1 a	37.5 a
Treatment	**[¶]	**
Correlation[2]	0.89 **	0.90 **

[1] Means of 7 replications using data of combined replicate experiments.

[2] Simple correlation coefficient between water regimes and seed protein and glucosinolate content and significance level.

[§] Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

[¶] (**) significant at 0.01 probability level.

This increase in seed glucosinolate content is consistent with data obtained from field water-stress experiments discussed in the previous chapter.

Glucosinolates Profile

Total glucosinolate content was a summation of eight individual glucosinolates having different peaks and retention times. Concentrations of individual glucosinolates ALL, 4PEN, 4POH, MSGL, and 3IME were less than 1 μmoles/g of defatted meal. However, although these glucosinolates were present in low concentrations, they tended to be higher at the severe water stress treatment (Figure 1).

Concentrations of ALL were 0.03, 0.03, and 0.10 μmoles/g of defatted meal under the control, moderate, and severe water stress treatments, respectively (Figure 1). Although ALL was present at very low levels, severe water stress increased ALL concentration by 88% compared to control.

Concentrations of 4PEN were 0.29, 0.23, and 1.08 μmoles under the control, moderate, and severe water stress treatments, respectively (Figure 1). 4PEN concentration was increased by 73%, compared to the control.

Concentrations of 4POH were 0.05, 0.06, and 0.26 μmoles under the control, moderate, and severe treatments, respectively (Figure 1). Severe stress increased the 4POH level by 57% compared to the control.

Concentrations of MSGL were 0.11, 0.13, and 0.30 μmoles under the control, moderate, and severe treatments, respectively (Figure 1). Severe stress increased its level by 60%, compared to the control.

Concentrations of 3IME were 0.16, 0.20, and 0.26 μmoles under the control,

moderate, and severe stress treatments, respectively (Figure 1). Severe stress treatment resulted in only 38% compared to the control treatment.

The major individual glucosinolates were 3BUT, 3IM, and 3BOH. Concentrations of 3BUT were 1.49, 1.57, and 4.14 μmoles/g of defatted meal under the control, moderate, and severe treatments, respectively (Figure 2). Simple correlation analysis showed that 3BUT was highly correlated to the total glucosinolate content of seed with correlation coefficient of 0.956 ($P<0.01$) (Table 5). This indicates that 91% of the variation in total glucosinolate content is explained by a positive variation in 3BUT.

Concentrations of 3BOH were 3.21, 5.07, and 12.52 μmoles/g of defatted meal under the control, moderate, and severe stress treatments, respectively (Figure 2). The simple correlation coefficient between the total glucosinolate content and 3BOH was 0.988 ($P<0.01$) (Table 5), indicating that 97% in the variation in the total content could be explained by a linear variation in 3BOH. Severe stress increased the 3BOH concentration by almost 75%, compared to the control.

Concentrations of 3IM were 7.39, 9.22, and 8.84 μmoles/g of defatted meal under the control, moderate, and severe moisture stress treatments, respectively (Figure 2), with a 0.53 ($P<0.05$) simple correlation coefficient with total glucosinolate content (Table 5).

Both moderate and severe water stress treatments resulted in overall increase in relative concentration of the various individual glucosinolates. These results were

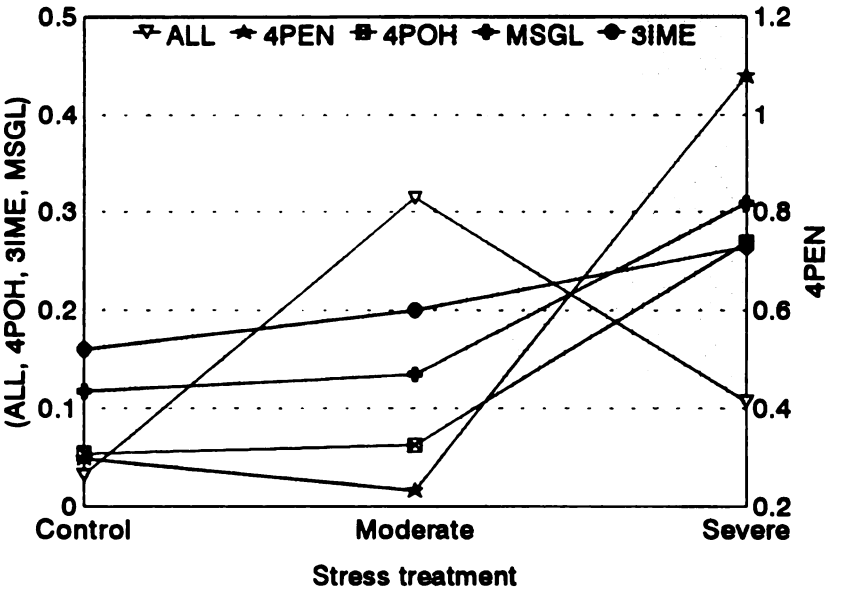

Figure 1. Profile of individual glucosinolates content of spring canola grown at moderate and severe water stress under greenhouse conditions.

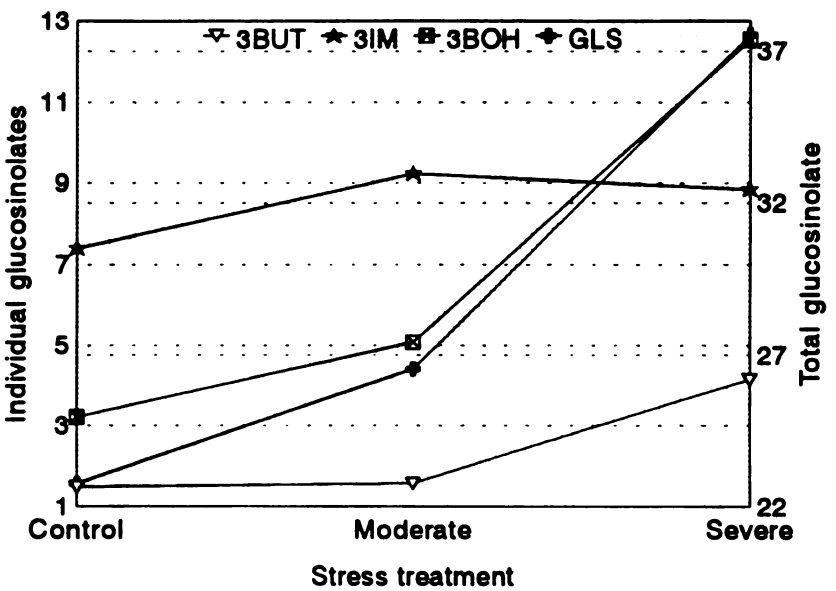

Figure 2. Total and individual glucosinolate content of spring canola grown at moderate and severe water stress under greenhouse conditions.

somewhat consistent with field data described in the previous chapter. Total glucosinolate content increased sharply under severe water stress conditions, however, under moderate water stress, the glucosinolate content of seed increased but did not exceed the canola standard of 30 μmoles/g of defatted meal. Under severe water stress, total glucosinolate content increased primarily as a result of a sharp increase in 2-hydroxy-3-Butenylglucosinolate. Other individual glucosinolates present at low concentrations also increased under stress conditions and contributed to the final seed glucosinolate content.

Table 5. Simple correlation coefficients between total glucosinolate content and individual glucosinolates of Delta canola grown at three water regimes under controlled greenhouse conditions.

Individual Glucosinolate	Correlation Coefficient	P[1]
Allylglucosinolate	0.859	**
3-Butenyl	0.956	**
4-Pentenyl	0.925	**
2-Hydroxy-3-Butenyl	0.988	**
2-Hydroxy-4-Pentenyl	0.831	**
Methylthio-but&pent	0.945	**
Indol-3-ylmethyl	0.340	ns
Methoxylindol-3-ylmethyl	0.530	*

[1] (* and **) significant at the 0.05 and 0.01 probability levels, respectively. (ns) not significant at the 0.05 probability level.

GENERAL CONCLUSIONS

* Under mid-Michigan field conditions, sulfur fertilization of three commercial spring canola cultivars did not result into significant crop morphological changes or differences in levels of protein, oil, and glucosinolates. When no sulfur was added, leaves had adequate sulfur content (0.5%) during flowering, suggesting that mid-Michigan soils, and perhaps atmospheric conditions, provided sufficient sulfate levels above the threshold needed for application of sulfur fertilizers to produce a significant effect.

* Under controlled greenhouse conditions, both vegetative and reproductive growth were significantly affected. Seed oil, protein, and glucosinolate concentration were all significantly influenced by sulfur application.

* Water stress treatments caused significant morphological changes in spring canola grown on both sandy and loam soils. Under water stress, plants tended to be smaller, with significantly fewer pods, and lower seed yield. Protein and oil content of seeds were also affected, as well as the overall fatty acid profile. Erucic acid was present in very low concentrations in the seed and, therefore relatively unaffected by water stress. Glucosinolate levels in the seeds were increased by water stress treatments and exceeded the canola standard of 30 μmoles/g of defatted meal.

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