Effect of temperature upon the fatty acid composition during seed development in oilseed rape (Brassica. napus L.)

by

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EFFECT OF TEMPERATURE UPON THE FATTY ACID COMPOSITION DURING SEED DEVELOPMENT IN OILSEED RAPE (*Brassica napus* L.)

BY

XINMIN DENG

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Xinmin Deng, M.Sc., The University of Manitoba .
Effect of temperature upon the fatty acid composition during seed development in oilseed rape (Brassica napus L.)
Major Professor, Dr. R. Scarth.

The influence of temperature during seed development on fatty acid composition of the harvested seed in oilseed rape (Brassica napus L.) was studied using two cultivars which differ in seed oil content of linoleic (C18:2) and linolenic (C18:3) acids. The cultivar Regent produces seed oil with 20% C18:2 and 8% C18:3. The cultivar Stellar is relatively high in C18:2 (25%) and low in C18:3 (2.5%). Plants of both cultivars were exposed to different temperature environments during the period of seed development under controlled conditions and in the field. Seed in the field was harvested at intervals during development and analyzed for fatty acid composition. The planting date study in the field did not provide distinct temperature environments in 1990 and 1991. Under controlled conditions, the content of the saturated fatty acids, the sum of the palmitic and stearic acids (C16:0+C18:0), and the monounsaturated fatty acid, oleic acid (C18:1) increased with longer exposure to high temperatures (30/25°C day/night) in the seed oil of cultivar Regent while the content of polyunsaturated fatty acids, C18:2 and C18:3 decreased. The content of both C18:1 and C18:3 did not change and the content of the saturated fatty acids increased with longer exposure to high temperatures in the seed oil of the cultivar Stellar. The C18:2 content was

the highest for both cultivars under the intermediate temperature treatment (25/20°C) . With longer exposure to low temperatures (15/10°C day/night) during seed development, the saturated fatty acids decreased while the polyunsaturated fatty acids increased in the seed oil of both cultivars. In the field environments provided by different locations in 1990 and 1991, the content of saturated and monounsaturated fatty acids was high in the seed oil of both cultivars when seed development occurred under high temperatures. The C18:3 was low in the seed oil although the response was not as great as was anticipated from the controlled environment studies. This may be due to compensation from lower night temperatures in the field. The low C18:3 trait of cultivar Stellar was relatively stable under both controlled environment and field conditions.

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The research that goes into the production of a thesis is rarely accomplished through individual effort. I would like to take this opportunity to express my gratitude to those who helped along the way.

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A final "thank you" goes out to the graduate students of Plant Science Department for making a Chinese student feel at home when the work was through.

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1.0. INTRODUCTION

The fatty acid composition of oilseed rape (Brassica napus L.), is influenced by aerial temperature during seed development (Canvin, 1965). A common phenomenon found in plants is the ability to adapt to lower environmental temperatures by increasing the level of unsaturation in the fatty acids of membrane diacylglycerols, and to higher temperatures by lowering the level of unsaturation of their membrane fatty acids (Williams et al, 1988).

The period of seed development for spring oilseed rape in western Canada coincides with high average daily temperatures. As specialty canola oil has a marketing advantage in its favourable fatty acid composition of the seed oil, it is of interest to determine the effect of temperature on the C18:3 content. Two oilseed rape cultivars with distinct fatty acid composition were used in this study. The cultivars were grown in different locations over two years in western Canada, with two planting dates at the University of Manitoba. A controlled environment study was conducted using different lengths of exposure to high and low temperature regimes during the seed development.

The objective of this study is to define the effect of different aerial temperatures during seed development on the fatty acid composition of the low linolenic spring oilseed rape cultivars.

2.0 LITERATURE REVIEW

2.1. Canola development and breeding

2.1.1. History of canola breeding in Canada

"Canola" is the term now generally applied to those oilseed rape cultivars (B. napus or B. rapa) with oil containing less than two percent erucic acid and seed meal containing less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxyl-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air dry, oil free solid (Canola Oil and Meal Standards and Regulations, 1990).

Oilseed rape may well have been among the earliest of the domesticated plants since some of the vegetable forms were in common use in Neolithic age, and the Indian Sanskirit writing of 1500 to 2000 BC directly refers to oilseed rape and mustard. Greek, Roman and Chinese writing of 200 to 500 BC also specifically mentions these crops and also describes their medical value. In Europe, domestication is believed to have occurred in the early Middle Ages. Commercial plantings of rapeseed were recorded in the Netherlands as early as the 16th century. At that time rapeseed oil was used primarily as a lamp oil and later as a lubricant for steam engines. Despite its wide acceptance for edible purposes in Asia, it is only through improved processing techniques after World War II and the success

in breeding for superior quality that oil and meal gained a major market share in Western nations (Downey and Robbelen, 1989).

In fact, it was the need for rapeseed oil as a lubricant in the engines of naval and merchant ships which brought rapeseed cultivation to Canada in 1942. Rapeseed oil was not used for edible purpose in western nations until the end of World War II. Rapeseed breeding began in Canada by Mr. H. G. Neufeld at Nipawin in 1943, when he made several selections from the seed stocks introduced from Argentina. Lacking facilities for plant breeding then he turned these selections over for evaluation to Dr.W. J. White at the University of Saskatchewan. Dr. White worked on rapeseed agronomic improvement, and the chemical analysis were performed by Prairie Regional Laboratory of the Nation (Canada). This work led to the release of the first Canadian rape cultivar "Golden" in 1954 (Stefansson, 1974).

In the early years plant breeding efforts were concentrated on improvements of the agronomic performance of rapeseed including yield, maturity and lodging resistance. In recent years there has been extensive research into improvement of the nutritional properties of rapeseed oil and rapeseed meal.

Rapeseed oil, in addition to the fatty acids found in other vegetable oils, also contained significant amounts of the long chain fatty acids eicosenoic and erucic. Oil high in erucic acid have been shown to have an adverse effect on experimental animals when fed as a large proportion of the diet (FAO, 1980; Sauer and Kramer, 1983). The significance of erucic acid in the human diet

is not well defined. McDonald (1983) has reviewed the limited number of relevant human studies. Erucic acid is well digested by humans (Vaisey-Genser et al., 1973) but it has been related to a reduction in blood platelet count in subjects fed diets containing rapeseed oil for a period of three weeks (McDonald, 1974). These results provided the motivation for the development of strains having low erucic acid content in the oil.

Plant breeders were able to isolate low erucic acid strains, and rapeseed oil with considerable less than 5% erucic acid has been extracted from Canadian oilseed rape varieties since the first low erucic variety, Oro, was licensed in 1968 (Stefansson, 1974). The Canadian rapeseed industry on December 1, 1973, voluntarily established the guideline that erucic acid would not constitute more than 5% of the total fatty acids in margarine, shortening, mayonnaise, salad oil and dressing and cooking oil produced in Canada (Canada's Canola , 1988).

More recently, low glucosinolate (< 30 μmol/g) canola cultivars have provided expanded markets for the meal. Although it had been known that glucosinolates caused enlargement of the thyroids of animals, it was not until 1955 that glucosinolates were identified in rapeseed meal. By 1967, plant breeders discovered that the Polish B.napus cultivar Bronowski contained about one-tenth the amount of glucosinolates found in standard varieties. This characteristic was transferred to Canadian strains and in 1974 the first low erucic acid, low glucosinolate canola variety, Tower, was released by Dr. Stefansson from the

University of Manitoba (Downey and Robbelen, 1989).

2.1.2. Breeding for special oil quality in canola.

a). Higher level of erucic acid (C22:1)

A limited but significant industrial oil market has developed for very high (>50%) erucic acid rapeseed oil. Although there are many minor applications for this oil, such as a lubricant for the cold rolling of steel and in jet engines, most of the oil is fractionated and the erucic acid converted to erucamide for use in the plastics industry. The erucamide is sprayed on plastic sheeting and mouldings to prevent their sticking together or to the extruding machinery (Downey and Robbelen, 1989).

"A very typical new use is the development of an alcohol ester of rapeseed oil (AERO) that holds promise as a clean-burning fuel. Researches at the University of Idaho were quoted as saying that the ignition characteristics are more than adequate and engine modifications for AERO usage would result in even better performance. The estimates are that one acre of rapeseed is capable of producing 100 gallons of oil, at a cost of $1.18 to $1.31 a gallon." (Scarth et al., 1992).

Several winter type industrial quality rapeseed cultivars including Bridger have been developed by the Idaho Agricultural Experiment Station at Moscow, Idaho, USA. Mature seed of the cultivar Bridger contains in excess of 45% oil (8% seed moisture basis) with a fatty acid composition that ranges from 47.2 to 55%

erucic acid. Glucosinolate concentration of the defatted meal has ranged from 14 to 28 umol/g dependent upon the production environment and / or the analytical procedure utilized in the determination (Auld et al., 1987). The four parental lines of Bridger were selected in the F_6 generation from cross between 'Indore' and 'Norde'. Indore is a low glucosinolate, high erucic acid rapeseed cultivar released by Oregon State University in 1983. Norde is winter-hardy, traditional rapeseed cultivar released by Swedish Seed Association at Svalof, Sweden in 1969 (Auld et al., 1987).

The University of Manitoba breeding program targeted the development of a high erucic cultivar with canola quality meal, to achieve the maximum economic benefit for producers and crushers (Scarth et al., 1992). The source of high erucic character was a B. napus summer rape strain introduced from Sweden. This strain was grown in isolation for 4 years at the University of Manitoba and single plants with high erucic acid content in the seed oil were selected. These lines were then crossed to the high erucic cultivar, Reston, followed by selection of high erucic-low glucosinolate plants in the F_2 and F_3 generations. F_4 lines were selected for high seed yield and high oil and meal protein contents. Line S82-4362, derived from a single F_3 plant selected in 1982, had the desired combination of high erucic acid (50.2%) and low glucosinolate levels in the meal (15 μmol g^{-1}). After four years regional testing, the strain was registered as Hero in 1989 (Scarth et al., 1992).

The University of Manitoba breeding program aims for further improvements in oil quality by introducing the low linolenic character into the high erucic acid background. Partial hydrogenation is the method of choice for removing linolenic acid, but it is an expensive process and causes the formation of unwanted by-products. Therefore, the reduction of linolenic acid would be of benefit to the industrial oil. Lower levels of linoleic acid are also desirable in applications that require very high stability. The reduction of factors such as the level of free fatty acids, chlorophyll and glucosinolates would all improve the industrial oil quality (Scarth et al., 1992).

b) Lower levels of linolenic acid (C18:3)

One of the breeding objectives in oil quality improvement is to reduce the percentage of C18:3 from the present level of 8 to 10% to less than 3% while maintaining or increasing the level of linoleic acid (C18:2).

The high C18:3 content is the source of oxidative rancidity and loss of flavour stability in storage. In some food applications such as industrial frying, the level of C18:3 has to be less than 3%. This level can be achieved by partial hydrogenation which reduces one of the three double bonds in C18:3 to produce two double bonds C18:2. Blending with low C18:3 oil generally improves the stability of canola oil under accelerated storage at 65°C. It was possible to blend 25 % canola oil and 75 % cottonseed or sunflower oil and still maintain the stability of the original parent sunflower and

cottonseed oil (Durance, 1986). The formation of trans fatty acids after hydrogenation raises some nutritional concerns.

The alternative to the processing or blending solutions is to breed canola cultivars which have a low level of C18:3, to improve the storage characteristics of the oil while a higher C18:2 content may be nutritionally desirable. It is well known that some plant families include species that do not synthesize linolenic acid but only linoleic acid in the oil. Scientists screened the available oilseed rape germplasm for the genetic block between linoleic acid and linolenic acid. The necessary variation was not available. Therefore, mutation experiments were initiated at the Institute of Plant Breeding, University of Gottingen in West Germany. Rakow (1973) used chemical mutagen ethyl methanesulfonate (EMS) to treat seeds of the Canadian spring rapeseed (B. napus L.) cultivar, Oro, then selected plants with an altered C18:2 : C18:3 ratio in the seed oil. One line, M11, with about 5% C18:3 and 20% C18:2 was selected (Robbelen and Nitsch, 1975).

Dr. B. Stefansson introduced the M11 mutant line as the source of variation in the University of Manitoba low linolenic breeding program. There was evidence of deleterious effects from the mutation treatment as the plants of M11 line were reduced in height and vigour and had a reduced seed set. After three years of selection within the M11 line for single plants accumulating seed oils with reduced C18:3 and increased C18:2 levels, lines with seed oil containing 3% of C18:3 and 28% of C18:2 were

obtained. These low C18:3-high C18:2 lines were crossed with the canola cultivar Regent, followed by further selection for oil quality and agronomic characteristic (Scarth et al. 1988). Line S81-2716 was a single plant selection in 1981, tested for quality and agronomic characteristics in Western Canadian Co-operative testing system for four years 1983 - 1986, and registered as Stellar in 1987 (Scarth et al., 1992).

The seed oil of Stellar has a very distinct fatty acid composition with the desired combination of low levels of C18:3 acid combined with high C18:2 content. The excellent stability of the low linolenic character has been demonstrated under different environments, as shown over a five years period of testing and seed increase 1981 to 1986 (Scarth et al., 1992).

c). Modification of other fatty acids.

Increased content of fatty acids with shorter chain lengths is also of interest. Sweden researchers have selected B. rapa lines with 10-12% of palmitic (C16:0) plus palmitoleic acids (C16:1) compared with 4-5% in the unselected population but the value and acceptability of such an oil composition has yet to be determined (Downey and Robbelen, 1989).

The development and commercialization of high oleic acid (C18:1) canola (B. napus L.) is currently under way in Canada. There are three major steps involved in the development of high C18:1 phenotype: production of lines containing high C18:1 (73-75%) through seed mutagenesis; combining high C18:1 (75-76%) with

low C18:3 (2-4%) through crossing and subsequent progeny selection; and identification of segregant with very high levels of C18:1 (>85%) and low levels of C18:3 (<3%) from (spring/spring)/winter crosses. According to Wong et al., (1991), results from two years of multiple-location field testing indicate that the fatty acid composition of the oil from the high C18:1 lines is stable under a wide range of environmental conditions.

2.2. The effect of fatty acid composition on processing, storage and nutrition properties of canola oil

2.2.1. Processing and storage properties

The presence of 7 to 11 % of C18:3 in canola is a source of oxidative rancidity and loss of flavour stability during storage. The rate of oxidation of oils is determined by the presence of oxygen, antioxidants and proxidants, degree of unsaturation of the fatty acids and, the conditions of light and temperature. The susceptibility of individual fatty acids to oxidation is dependent on their degree of unsaturation. Thus the rate of oxidation of C18:3 is 25 times that of C18:1 and twice that of C18:2 (Tokarska et al., 1986).

Studies by Durance (1986) showed that reducing the level of C18:3 in canola oil, by blending with low C18:3 oils, generally improved the stability of canola oil to accelerated storage at 65°C. It was possible to blend 25 % canola oil with 75 %

cottonseed or sunflower oil and still maintain the stability of original parent sunflower or cottonseed oil. Eskin et al. (1989) showed that blending canola oil with either palm or palm-olein substantially reduced the degree of rancidity as measured by peroxide value (PV) and thiobarbituric acid values (TBA) whether subjected to heat storage or exposure to fluorescent light.

Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytiluene (BHT), tertiary butylhydroquinone (TBHQ) and anoxomer have been added to canola oil in order to retard oxidative change due to storage and heat (Hawrysh et al., 1988; Tokarska et al., 1986; Vaisey-Genser and Ylimaki, 1985). However, these antioxidants are either of little benefit in canola oil (BHA and BHT) or are not licensed for use in Canada (TBHQ and anoxomer). Reports suggest that addition of ascorbyl palmitate (AP) to oils such as soybean, corn and peanut was effective in promoting antioxidative stability during storage (Cort, 1974). AP may offer advantages over other antioxidants in terms of efficiency, safety, and positive labelling connotations. Results showed that 200 ppm AP is effective in delaying autoxidative changes in canola oil subjected to the Schaal Oven test (Hawrysh, 1990). Results obtained from the fluorescent light test showed that AP had little effect in protecting canola oil from photoxidation. According to Hawrysh et al., (1990), practical storage test results of canola oils stored in PVC bottles for up to 10 months indicated that the addition of either AP or BHA/BHT to canola oils was of little benefit in extending

storage stability.

The hydrogenation process adds hydrogen at the double bonds of the unsaturated fatty acids under conditions of high temperature and high pressure in the presence of a suitable catalyst, usually nickel. This process modifies the physical properties of the fat or oil as well as rendering it more resistant to oxidative and thermal damage. During the course of this process the double bonds of the fatty acid in the triglycerides become more saturated thus changing the fatty acid composition compared to the corresponding refined oils. Studies on the hydrogenation of canola oil showed that under selective conditions the trans-isomer levels reached over 50% and 38-45% under nonselective hydrogenation conditions (El-Shattory et al., 1981). The formation of the trans-isomer imposes physical changes to the oil. The trans triglycerides with double bonds are similar in physical properties to the saturated fatty acids, and trans fats are digested as saturated fats in animals.

These processes are expensive, unreliable and adversely affect nutritional properties of the oil. The alternative is to breed canola cultivars which have a reduced level of C18:3.

The production of low C18:3 canola cultivars would essentially eliminates the need for the copper-hydrogenated process. The oil from the low C18:3 cultivar Stellar has a significantly lower heated odour problem which is caused in part by C18:3 (Eskin et al., 1986). The low C18:3 canola oil showed greater stability to accelerated storage at 60°C up to 12 days

compared to the Westar canola oils (Eskin et al., 1989). Further evidence showed that there was a substantial improvement in the stability of low C18:3 canola oil to the development of heated odor during frying when compared to other conventional canola oils (Eskin et al., 1989).

A recent study in France compared room odour of the low C18:3 canola oil from Stellar to that of oil from the conventional canola Westar and French rapeseed cultivar Bienvenu (Prevot et al., 1990). France specifically excludes oils with more than 3% C18:3 from use for deep-frying and there is a perception that both rapeseed and soybean oil have an unpleasant odour when used in deep-frying. The score obtained by the low linolenic oil were significantly better than the other oils, and the difference persisted through eight fryings. The scores of the low linolenic oil were comparable to those obtained by sunflower (Prevot et al., 1990).

2.2.2. The role of individual fatty acids in human nutrition.

Canola oil is characterized by low level of saturated fatty acids (6%), a relatively high level of the monounsaturated fatty acid, C18:1 (55-60%), and an intermediate level of the polyunsaturated fatty acids, C18:2 (20-25%) and C18:3 (10%). Like its progenitor, rapeseed oil, canola oil contains high percentage of C18:3 in comparison to the common vegetable oils.

The content of polyunsaturated fatty acids (PUFA) in canola oil is appreciably higher than that of palm, coconut and olive

oils, but it is considerably lower than that of corn, cottonseed and sunflower oils. The interest in PUFA stems from their role as essential fatty acids and from the evidence of beneficial effects in reducing coronary heart disease.

The two PUFAs, C18:2 (n-6) and C18:3 (n-3) are known as the essential fatty acids. They cannot be synthesized by the human body and must be obtained from the diet. The two families (n-3 and n-6) derived from C18:2 and C18:3 have distinct nutritional and metabolic effects.

A major reason for the interest in polyunsaturated fatty acids related to the evidence linking dietary fat and coronary heart disease (CHD). High levels of blood cholesterol constitute a risk factor for coronary heart disease. The general recommendation for lowering blood cholesterol is to reduce the dietary intake of saturated fatty acids and increase the intake of PUFA (McDonald, 1987). Scientists have suggested that decreasing the intake of saturated fatty acid is about twice as effective as increasing the intake of PUFA in bringing about a lowering of serum cholesterol (Keys et al., 1965).

Linoleic acid (C18:2) is required in the diet of animals, including humans, because they are unable to produce it. However they are able to convert C18:2 to arachidonic and other higher homologous in the "n-6 family" of fatty acids. These long chain, highly unsaturated fatty acids are important in membrane structures and as starting materials for the synthesis of metabolically active substances such as prostaglandins and

related compounds (McDonald, 1987).

However, the most recent interest in dietary C18:2 in the world has focused on its ability to lower plasma cholesterol. The effects of long-chain saturated fatty acids and the PUFA of the n-6 series (mainly C18:2) on the plasma cholesterol concentration have been known for some time. The effect of a high P:S ratio diet in lowering plasma cholesterol is one of the most consistent finding in nutrition. The formation of VLDL (very low-density lipoprotein), which may compound the potentially undenied effect of lowering HDL, is less with C18:2-rich than with more saturated fatty acid diets (Cortese et al., 1983).

The C18:1 (55-60%) content of canola oil is only lower than that of olive oil (64%), but much higher than other vegetable oils. Until recently, C18:1 content has been considered "neutral" with respect to its effect on serum cholesterol. However, a study by Mattson and Grundy (1985) found that diets containing a high level of C18:1 were equivalent to diets containing a similar level of C18:2 in lowering the serum cholesterol of human subjects.

Evidence is accumulating that diets low in saturated fatty acids and high monounsaturated fatty acids are effective in controlling blood lipid levels. A likely consequence could be a beneficial effect on the risk of coronary heart disease. Studies have shown monounsaturated fatty acids or low-fat diets are effective in lowering blood low density lipoprotein cholesterol

(Mattson, 1989).

The monounsaturated fatty acids (mainly C18:1) apparently have the added advantage of not causing a decreasing in high-density-lipoprotein cholesterol or an increase in blood triglycerides, which can be a consequence of other dietary modification. In the past, olive oil was the only fat rich in monounsaturated fatty acid that was generally available in the United States. Recently, canola oil, is being promoted as an oil rich in monounsaturates in retail food outlets (Mattson, 1989).

2.3. The effect of temperature on the fatty acid composition of oil crops

The sensitivity of fatty acid composition to the temperature is very important in production of commercial oilseed species. In some locations and seasons, the levels of C18:2 in sunflower has been below the minimum industry standard for margarine production (62%) due to the occurrence of high night temperatures during the seed maturation period (Harris et al., 1978). In order to ensure that oil quality standards are consistently met regardless of thermal environment, it would be desirable to develop a genotype having a temperature-stable oleic desaturase enzyme system similar to that of safflower (Green, 1986).

The effect of temperature upon the fatty acid composition in mature seeds has been documented for several crop species. These studies usually show a negative correlation between the concentration of C18:1, C18:2 and C18:3 (if present) when seeds

are grown at different temperatures. However, the magnitude of such genotype by environment (G * E) interactions attributed to temperature may vary among crop species.

According to Nagao and Yamazaki's (1984) experiment using sunflower (Helianthus annuus L.) seeds produced at three different locations, there was a good correlation between the C18:2 percentage and average daily temperature at maturation (r=-0.801). There was no significant correlation between the C18:2 percentage and the average temperature of Stage-1 (11 days after flowering, DAF), while the C18:2 percentage showed a good correlation with those of Stage-2 (22 DAF) and Stage-3 (33 DAF). There was a higher correlation with the average temperature of Stage-3 (R=-0.927) than with that Stage-2 (R=-0.729).

The effect of temperature on fatty acid composition of traditional high-C18:3 flax genotype is well documented. Yermanos and Goodin (1965) found that an increase in post-flowering temperature resulted in a decrease in the C18:3 content and an increase in C18:1 content while C18:2 remained constant. Green (1985) studied the effect of temperature during seed maturation on the oil composition of low-C18:3 genotypes of flax (Lineum usiatissium L.). The effect of increased temperature on fatty acid composition was to decrease the level of unsaturation in all four genotypes, through a decrease in the proportion of the polyunsaturated fatty acids, C18:2 and C18:3, and an increase in that of the saturated fatty acids, palmitic (C16:0) and stearic (C18:0), and the monounsaturate C18:1.

Green (1986) studied the desaturation steps by employing two parameters-ODP (C18:1 desaturation proportion) and LDP (C18:2 desaturation proportion). The initial desaturation step, conversion of C18:0 to C18:1 , showed a slight temperature sensitivity, since in all four genotypes the total percentage of unsaturated fatty acids declined as temperature increased. However, the magnitude of this sensitivity was considerably less than that of the subsequent deasturation step, conversion of C18:1 to C18:2, as measured by ODP. By contrast, conversion of C18:2 to C18:3, measured by LDP, was remarkably insensitive to temperature treatments in low C18:3 genotypes.

Canvin (1965) demonstrated that the C18:1 desaturation step was temperature stable in the high-C18:2 safflower (Carthamus tinctorius L.) cultivar Lethbridge 25. There was no significant decrease in C18:2 content over the temperature range of 10°C to 27°C.

The fatty acid composition of seed oil from soybean grown at different temperatures showed the concentration of C18:1 to be negatively correlated with the concentration of C18:2 and C18:3 (Martin et al., 1986). During seed development, temperatures during growth significantly affected the expression of the high 18:1 trait in N78-2245 (high C18:1 type) seed, but had little effect upon the C18:1 content in Dare (typical soybean). After 45 DAF, there was essentially no further increase in the actual amount of C18:1 in either treatment. There was a greater proportion of highly unsaturated glycerolipids formed in N78-2245

seed at 22/18°C than 30/26°C, according to the rates of unsaturated fatty acid synthesis derived from the incorporation of [2-^{14}C] acetate. Within the range of 15°C to 35°C, there appeared to be a positive correlation between temperature and 18:1 synthesis.

Canvin (1965) found that the fatty acid composition of rapeseed (traditional high erucic rape Nugget) was more complex than of the other oil crops in his experiment and correspondingly the changes in composition that occur due to temperature were also complex. The highest level of erucic acid (C22:1) in the oil was obtained at 16°C; this increase in C22:1 was contrasted by a decrease in C18:1 content. At 21°C and especially at 26.5°C there were decrease in the levels of C22:1, C18:2 and C18:3 content in the seed oil. The decreases in these fatty acids were accompanied by an increase in C18:1.

Although several theories have been proposed to explain the relative increase in polyunsaturated fatty acids at low temperature, little is known about the biochemical mechanism(s) which elicit the response. Most notable among these theories is the concept that oxygen concentration or oxygen solubility in the cell cytoplasm increases at lower temperatures. Because oxygen is required in acyl desaturation reactions, greater amounts of available oxygen at low temperature may stimulate 18:1-desaturase activity (Harris and James, 1969). Another hypothesis contends that decreased membrane fluidity at low temperature alters the conformational structure of C18:1 or C18:2-desaturase enzymes.

Acyl desaturase activities could then increase as a result of greater exposure of the active sites to substrates (Shinitzky , 1979). Yet another proposal suggests that the C18:1-desaturase has a lower energy of activation(E_a) than the rate limiting reaction in fatty acid synthesis (Browse and Slack, 1983). Thus, the 18:1-desaturase may achieve relatively greater activity at low temperature compared to the overall rate of fatty acid synthesis.

Collectively, the wide range of opinions about the biochemical mechanism by which temperature causes changes in unsaturated fatty acid composition attests to the complexity of this biological phenomenon. It is doubtful that any single mechanism will fully account for the response of unsaturated fatty acid composition to temperature in all plant species. If several mechanisms are involved and can be identified within a given species, it is also highly probably that there will be genetic variability for the fatty acid composition (Martin et al., 1986).

3.0. EFFECT OF TEMPERATURE ON THE FATTY ACID COMPOSITION OF CANOLA - FIELD ENVIRONMENT

3.1. Introduction

The stability of the fatty acid composition of oil crops under different growing temperatures is an important determinant of the value of the crop. Investigators have used different seeding dates to provide distinct temperature regimes for comparison of oil quality. Seeding date had a considerable effect on sunflower (Helianthus annuus L.) seed yield, oil content of the seed and the fatty acid composition of the seed oil (Dedio, 1985). In the late seeding date trial, the final fatty acid composition of the achenes was affected by the temperature during physiological maturity. Cool temperatures increased the content of polyunsaturated fatty acids in the seed oil.

The relative concentration of certain fatty acids in the seed oil are of key importance for specialty oil production. The low linolenic (C18:3) oil quality has advantages in the improved oil stability and improved processing characteristics. These advantages are only of economic benefit if the low linolenic character is stable under different environments. The objective of this experiment is to understand the influence of temperature on the C18:3 content of the seed oil and obtain a better understanding of C18:3 accumulation in canola seed under different environments. This would be very useful for low

linolenic canola breeding programs and low linolenic canola production.

3.2. Materials and Methods

Two spring oilseed rape (Brassica napus L.) cultivars with differences in C18:2 and C18:3 content in the seed oil were used in this study. The seed oil of cultivar Regent has conventional canola composition with approximately 20% C18:2 and 8-10% C18:3. The seed oil of cultivar Stellar has approximately 25% C18:2 and 2-3% C18:3 (Scarth et al., 1988). Both cultivars were planted at two dates (May 7 and May 27 in 1990, May 7 and May 21 in 1991) at the Point field, Winnipeg, The University of Manitoba.

The experimental design was an Random Complete Block Design (RCBD) within cultivars with 6 replicates. The plots of each cultivar were isolated to ensure that there was no cross pollination. The recommended seeding rate for canola of 6 kg/ha was used. Each plot was made up of six rows 5 m long with 0.3 m row spacing. The maximum and minimum daily aerial temperatures were monitored and recorded during the period from flowering to physiological maturity, referred to as the period of seed development.

At flowering, the plants were tagged with short colored wires to identify plants at the same physiological stage. At 20, 30, 40 and 50 (maturity) days after flowering (DAF), approximately 30 plants were sampled randomly from each replicate of each cultivar. The pods were harvested from the main stems,

the seed were removed and mixed together to give a uniform sample. The oil seed content was determined using Nuclear Magnetic Resonance (Robertson et al., 1979) and fatty acid composition of seed samples was determined using Gas Chromatography (Hougen and Bodo, 1973).

Samples of 1/8 g were crushed in a carver press, then transferred into 10 ml flask. Heptane (5 ml) is added in the flask then left overnight. Part of clean solution was transferred into a clean flask and evaporated to a small volume for subsequent preparation of the methyl esters of the fatty acids. Sodium Methoxide (500 μl) was added, then left overnight. A 400 ul aliquot was removed and placed in a 1 ml crimp cap vial for Gas chromatographic analysis. A Perkin Elmer Capillary Gas Chromatography from J & W Scientific, model 8320B, was used with a flame ionization detector. A Capillary column (DB 225, 20 meters) was packed. With this column, the injection port, column and detector temperatures were 260, 220 and 280°C, respectively. For this column, the helium flow rate was 8 psi; the injection volume were usually 2-3 μl. Fatty acid composition was calculated as the relative peak areas for the methyl esters, without use of detector response factors.

Fatty acid compositions of seed oils are usually expressed on a percentage basis, a practice which is apt to mask patterns of synthesis. In percentage data, the tendency of one variable to influence the value of others may be substantial when a limited number of substances are considered (Stefansson and

Storgaard, 1969). To elucidate the patterns of fatty acid biogenesis during seed development more clearly, fatty acid levels were expressed on both a percentage and a weight basis (Rakow and McGregor, 1975).

Statistical analysis was carried out on the University of Manitoba mainframe computer using the SAS program (SAS, 1985). ANOVA of the content of each fatty acid was performed within cultivars. The Bartlett's test results showed homogeneity of error variances between the two planting dates over the two years for both cultivars. Thus, data was combined and analyzed as a split split plot with planting date as the main plot and sampling date as the subplot within cultivars. Data from two years was combined to test the effect of different environments on the C18:3 content. All graphs were generated by using Sigma-plot software package.

3.3. Results and Discussion:

3.3.1. The effect of year and planting date on the C18:3 content

* Cultivar Regent:

The ANOVA results of the C18:3 content of cultivar Regent are shown in Table 3.1. The individual ANOVA of other fatty acids are provided in the Appendix (Table A1).

Table 3.1 The ANOVA results for the C18:3 content of CV Regent for year (Year), planting date (Pldate) and the interactions between the year and planting date (Year*Pldate) and sampling date (Spdate) expressed as a % of total oil.

Source	DF	MS	F-Value	Sig.
Rep	5	0.53	0.56	--
Year	1	30.15	46.96	**
Error I	5	0.19	2.87	
Pldate	1	4.95	46.96	**
Year*Pldate	1	0.26	2.47	--
Error II [Rep*Pld(Year)]	10	1.05	1.58	
Sampdate	3	9.31	139.81	**
Error III	60	0.07		

** significant at P=0.05, -- non significant

ANOVA results showed there were significant differences on the C18:3 content between the two years. There was also a significant effect of planting date although the interaction between the year and the planting date was not significant.

A mean comparison was carried out for the C18:3 content (%) in the oil of mature seed of cultivar Regent over two years (Table 3.2).

Table 3.2 Mean comparison of the C18:3 content (%) of mature seed of cultivar Regent within each year in 1990 and 1991

Year	1990	1991
Planting Date I	7.35 A	9.41 B
Planting Date II	7.61 A	10.25 A

Values in columms followed by different letters indicate significant at the P=0.05 level.

There was no difference found in the C18:3 content of the mature seed of cultivar Regent between the two planting dates in 1990. However, the C18:3 content in the mature seed of the first planting date was significantly lower than that of the second planting date in 1991.

* Cultivar Stellar

The ANOVA results of the C18:3 content of cultivar Stellar are shown in Table 3.3. The individual ANOVA of other fatty acids are provided in the Appendix (Table A2)

Table 3.3 The ANOVA results for the C18:3 content of CV Stellar for year (Year), planting date (Pldate) and the interactions between the year and planting date (Year*Pldate) and sampling date (Spdate) expressed as a % of total oil

Source	DF	MS	F-Value	Sig.
Rep	5	0.51	0.75	--
Year	1	0.41	3.02	--
Error I (Rep*Year)	5	0.14	1.62	
Year*Pldate	1	0.86	67.11	**
Pldate	1	0.04	2.93	--
Error II [Rep*Pld(Year)]	10	0.01	0.15	
Sampdate	3	23.82	211.68	**
Error III	60	0.08		

** Significant at the P = 0.05 level
-- Not significant

There was no significant effect of year or planting date on the C18:3 content in the seed oil of cultivar Stellar. However, the interaction between the year and planting date was significant.

A mean comparison was carried out for the C18:3 content in the oil of mature seed of cultivar Stellar within each year (Table 3.4).

Table 3.4. Comparison of the means of the C18:3 content (%) in the mature seed of cultivar Stellar within each year in 1990 and 1991

Year	1990	1991
Planting Date I	2.43 A	2.72 A
Planting Date II	2.53 A	2.46 A

Values in columms followed by different letters indicate significant at the P=0.05 level.

There was no difference found in the C18:3 content in the seed oil of cultivar Stellar between two planting dates in either 1990 or 1991.

The maximum, minimum and mean daily temperature data during seed development in both 1990 and 1991 are presented in Table 3.5. The temperature patterns were very similar during the period of seed development in the two planting dates in 1990. In 1991, the temperatures during late seed development (from 30-40 DAF and 40-50 DAF) of the second planting date were slightly higher than those during the same period in the first planting date.

Table 3.5 Temperatures during seed development of 1990 and 1991 at The Point, Winnipeg, the University of Manitoba

Year	Planting date	Type	0-20DAF	20-30DAF	30-40DAF	40-50DAF
1990	I	MINIMUM	15.65	14.62	14.63	12.93
		MAXIMUM	26.36	27.09	28.45	26.19
		MEAN	21.01	20.86	21.45	19.56
	II	MINIMUM	14.69	14.13	14.12	12.29
		MAXIMUM	27.00	27.50	28.90	27.18
		MEAN	20.85	20.82	21.51	19.74
1991	I	MINIMUM	15.88	16.18	12.66	14.80
		MAXIMUM	24.43	27.51	27.57	30.31
		MEAN	20.16	21.85	20.12	22.56
	II	MINIMUM	15.42	13.28	14.99	15.66
		MAXIMUM	27.25	25.71	30.29	30.47
		MEAN	21.34	19.50	22.64	23.07

The two planting dates in 1990 provided two very similar temperature regimes. This is consistent with the C18:3 content in the mature seed of both cultivar Regent and Stellar as there were no differences found in C18:3 content between the two different dates for either cultivars in 1990. The two planting dates in 1991 provided some differences in temperature during the late seed development. The C18:3 content in the mature seed of cultivar Regent of the second planting date was significantly higher than that of the first planting date (Table 3.2). However, the C18:3 contents between two planting dates in the mature seed of cultivar Stellar were not significantly different under this slightly temperature differences in 1991.

The two planting dates of both 1990 and 1991 did not provide the desired distinct temperature environments during the seed development. Such as occurred in the study conducted by Dedio in

1985. According to Dedio (1985), the frost and cool temperature that often occur in the fall at the time of physiological maturity of the late seeded plants resulted in an increase in the polyunsaturated fatty acids.

In summary, the relationship between the C18:3 content and the temperature during seed development could not be clearly defined in this field study, due to the lack of the distinct temperature environments. However the C18:3 content of cultivar Stellar was very stable under different planting dates and years.

3.3.2. The patterns of accumulation of the major fatty acids and the oil content for cultivar Regent and cultivar Stellar.

The patterns of accumulation of each major fatty acid and the oil content were plotted using both percentage of fatty acids in the oil (fatty acid compositions) and seed weight-concentration (mg/g seed^{-1} = 1 g seed x oil content % x fatty acid content %) within each cultivar (Figure 3.1 - 3.5). Means of each of the major fatty acids were plotted against the four sampling dates in each of two planting dates.

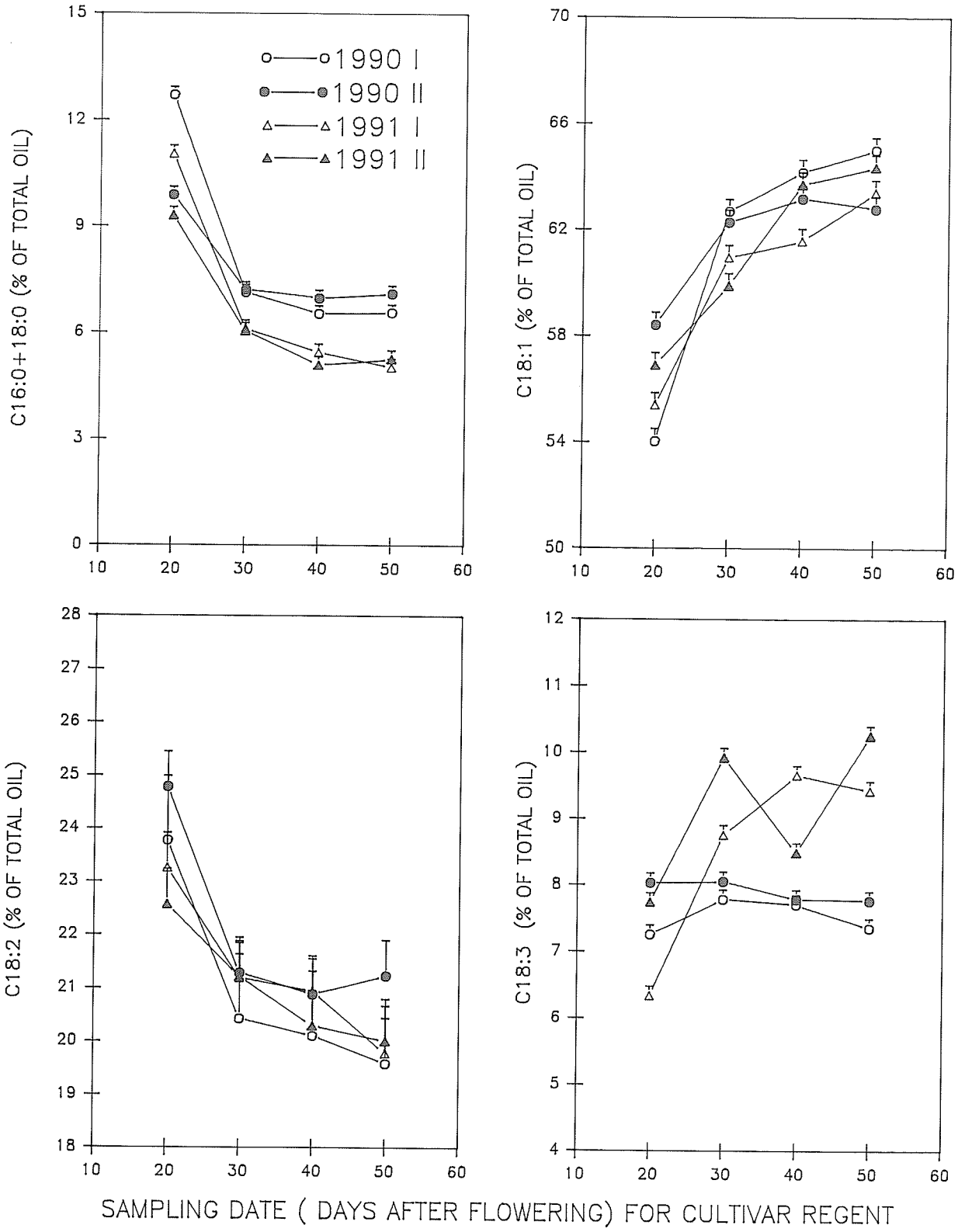
* Cultivar Regent:

The C18:3 content was constant over the period of seed sampling in 1990 (Figure 3.1). The C18:3 content increased until seed maturity was reached in 1991. The relative compositions of the other fatty acids in the seed oil also changed during seed development of the cultivar Regent. The C18:1 content in the

seed oil increased as seed development progressed, while the C16:0+18:0 and C18:2 decreased in both 1990 and 1991. These results from both years' experiments are very much in agreement with the changes in fatty acid composition of M364 (a high linolenic 20%) during the seed maturation reported by Rakow and McGregor (1975).

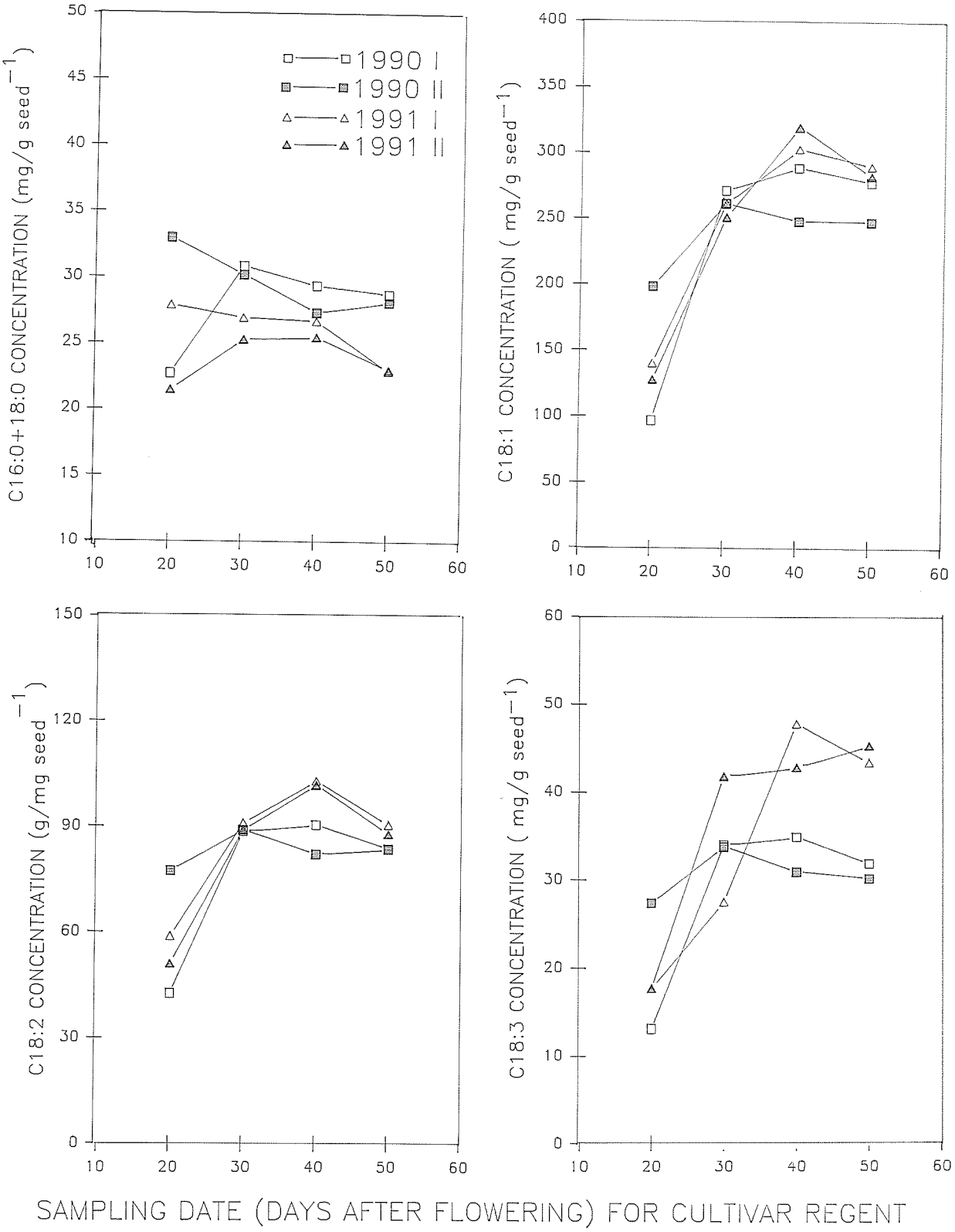
Figure 3.1 The patterns of accumulation of the major fatty acid composition of cultivar Regent in two planting dates in 1990 and 1991.

(SE on C16:0+C18:0 is too small to be represented on the graph)


C16:0+18:0 (% OF TOTAL OIL)
1990 I
1990 II
1991 I
1991 II
C18:1 (% OF TOTAL OIL)
C18:2 (% OF TOTAL OIL)
C18:3 (% OF TOTAL OIL)
SAMPLING DATE (DAYS AFTER FLOWERING) FOR CULTIVAR REGENT

When fatty acid content was expressed as a concentration (mg/g seed^{-1}), the C18:3 concentration in the seed oil of cultivar Regent increased to a peak at 30 - 40 DAF then remained constant until maturity in both planting dates of both years. Both the concentrations of C18:1 and C18:2 showed similar accumulation pattern in both planting dates of the two years. The accumulation pattern of C16:0+18:0 concentration showed a small decrease in both years (Figure 3.2). The accumulation patterns of most fatty acids are very comparable with the previous observations made by Rakow and McGregor (1975), that the weight (μg/seed) of all fatty acids were increased during seed maturation. However, the decrease in the C16:0+C18:0 concentration during seed development in both planting dates of two years was not observed by Rakow and McGregor in 1975.

Figure 3.2 The accumulation patterns of the major fatty acid concentration of cultivar Regent across sampling date during the seed development in 1990 and 1991

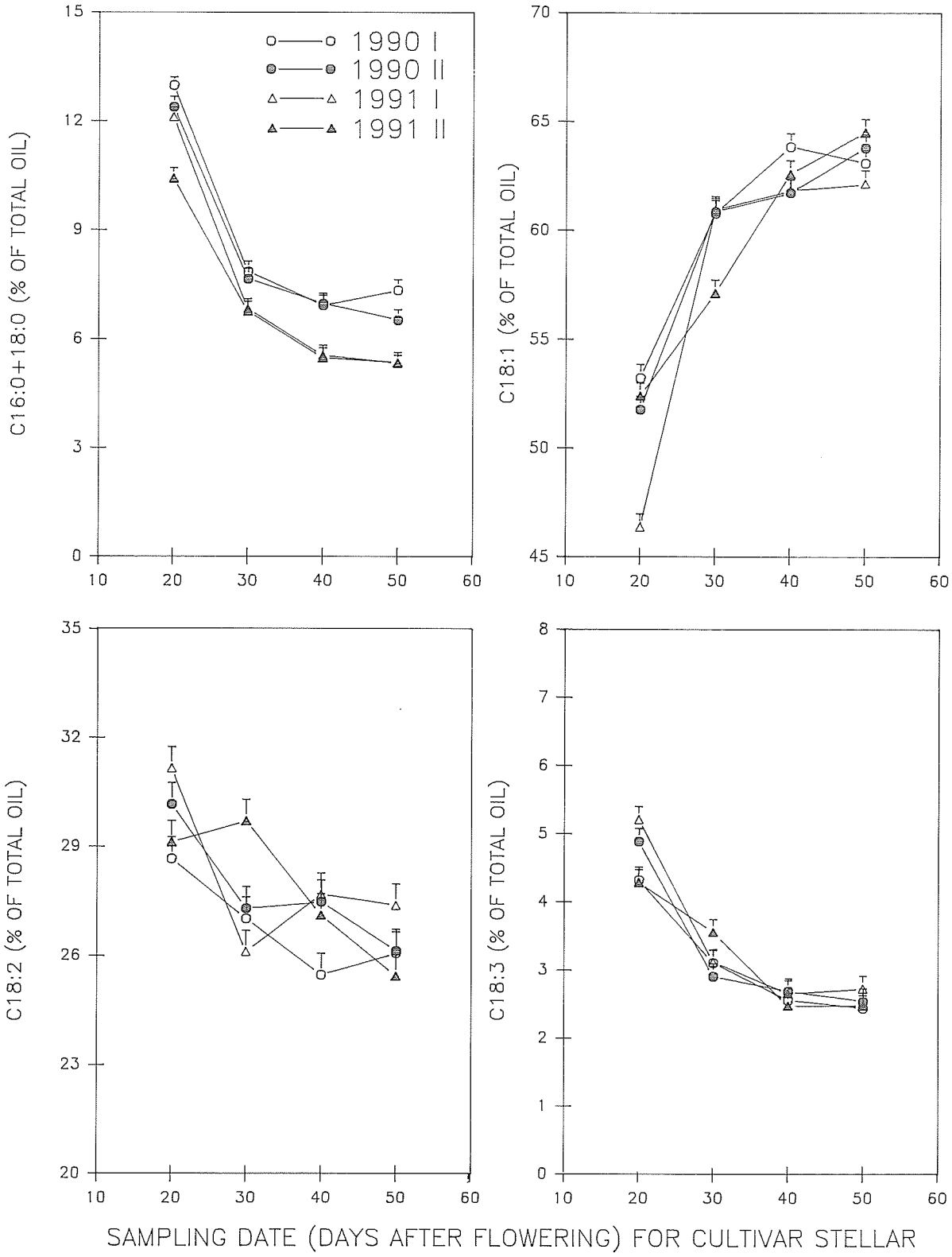

C16:0+18:0 CONCENTRATION (mg/g seed^{-1})
1990 I
1990 II
1991 I
1991 II
C18:1 CONCENTRATION (mg/g seed^{-1})
C18:2 CONCENTRATION (g/mg seed^{-1})
C18:3 CONCENTRATION (mg/g seed^{-1})
SAMPLING DATE (DAYS AFTER FLOWERING) FOR CULTIVAR REGENT

* Cultivar Stellar:

The C18:3 content of cultivar Stellar decreased over the period of seed development in both planting dates of two years (Figure 3.3). The C18:1 content increased throughout the sampling period accompanied by decreases in the C16:0+C18:0 and C18:2 contents in the seed oil in both years. There was considerable variation in the C18:2 content observed over the sampling period in planting dates of each year. This may be caused by sampling temperature changes immediately prior to seed sampling.

The accumulation patterns of the C18:3, as well as other fatty acid concentrations of cultivar Stellar are very much similar to that of M57 - a low linolenic (5%) line used in the Rakow and McGregor study (1975). The weight of the C18:3, C18:2 and C16:0+C18:0 decreased during seed maturation while C18:1 increased (Rakow and McGregor, 1975).

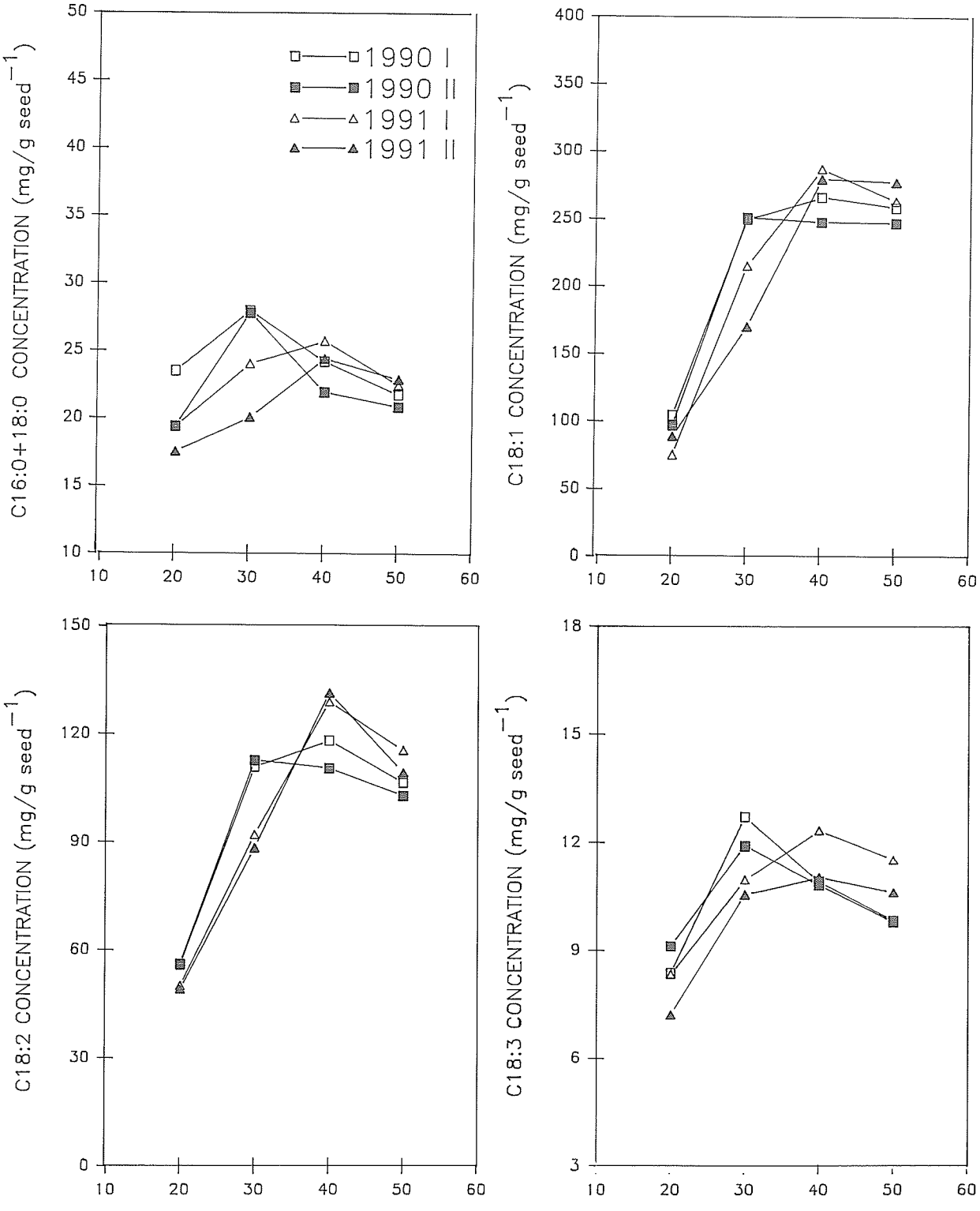
Figure 3.3 The patterns of the major fatty acid composition of cultivar Stellar across sampling dates during the seed development in 1990 and 1991


1990 I
1990 II
1991 I
1991 II
C16:0+18:0 (% OF TOTAL OIL)
C18:1 (% OF TOTAL OIL)
C18:2 (% OF TOTAL OIL)
C18:3 (% OF TOTAL OIL)
SAMPLING DATE (DAYS AFTER FLOWERING) FOR CULTIVAR STELLAR

The concentration (mg g seed^{-1}) of C18:3 in the seed of cultivar Stellar increased to a peak at 30 DAF, then decreased until seed maturity in 1990 (Figure 3.4). The C18:3 concentration reached a peak at 40 DAF, then decreased until seed maturity in 1991. The C18:1 and C18:2 concentration remained constant for the rest of the sampling period after reaching a peak at 30-40 DAF in 1990 and, reached a peak at 40 DAF, then decreased slightly in 1991. The concentration of C16:0+18:0 decreased slightly after an early increase prior to 30 DAF.

The changes in the concentrations of C18:3 and other fatty acids of cultivar Stellar are very similar to that of M57 in Rakow and McGregor study. The weights (μg/seed) of the C16:0+C18:0, C18:1, C18:2 and C18:3 increased until 49 DAF, then followed by a slightly decrease before seed maturity (Rakow and McGregor, 1975).

Figure 3.4 The accumulation pattern of the major fatty acid concentrations of cultivar Stellar across sampling dates during the seed development in 1990 and 1991

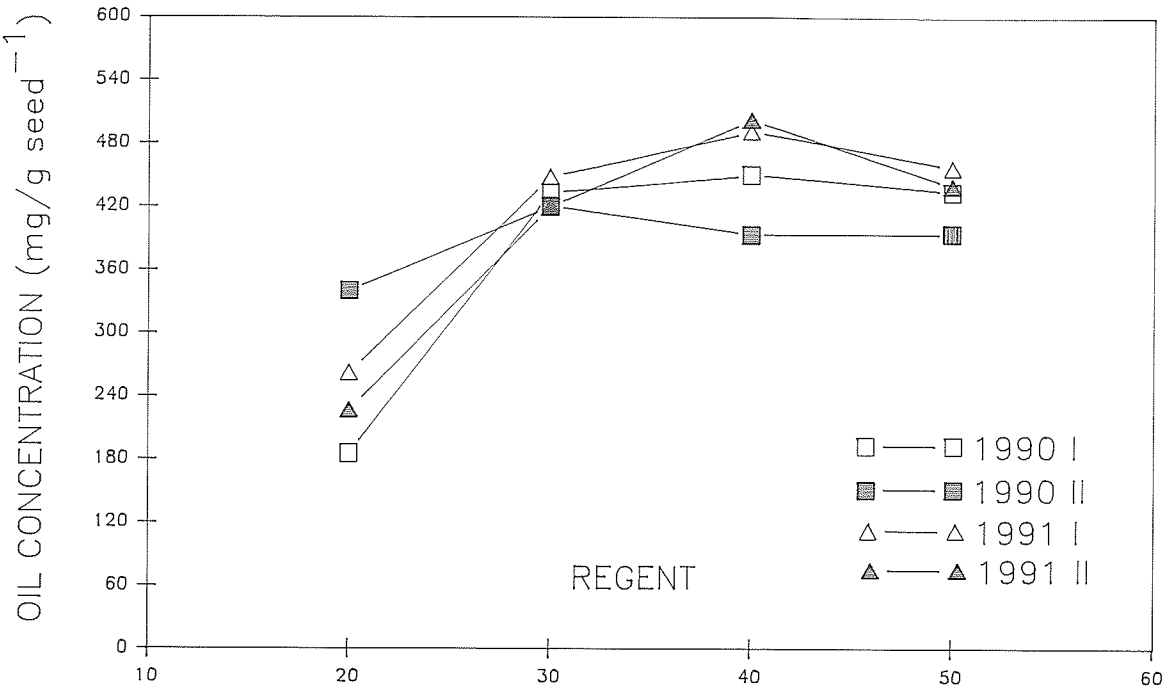

C16:0+18:0 CONCENTRATION (mg/g seed^{-1})
1990 I
1990 II
1991 I
1991 II
C18:1 CONCENTRATION (mg/g seed^{-1})
C18:2 CONCENTRATION (mg/g seed^{-1})
C18:3 CONCENTRATION (mg/g seed^{-1})
SAMPLING DATE (DAYS AFTER FLOWERING) FOR CULTIVAR STELLAR

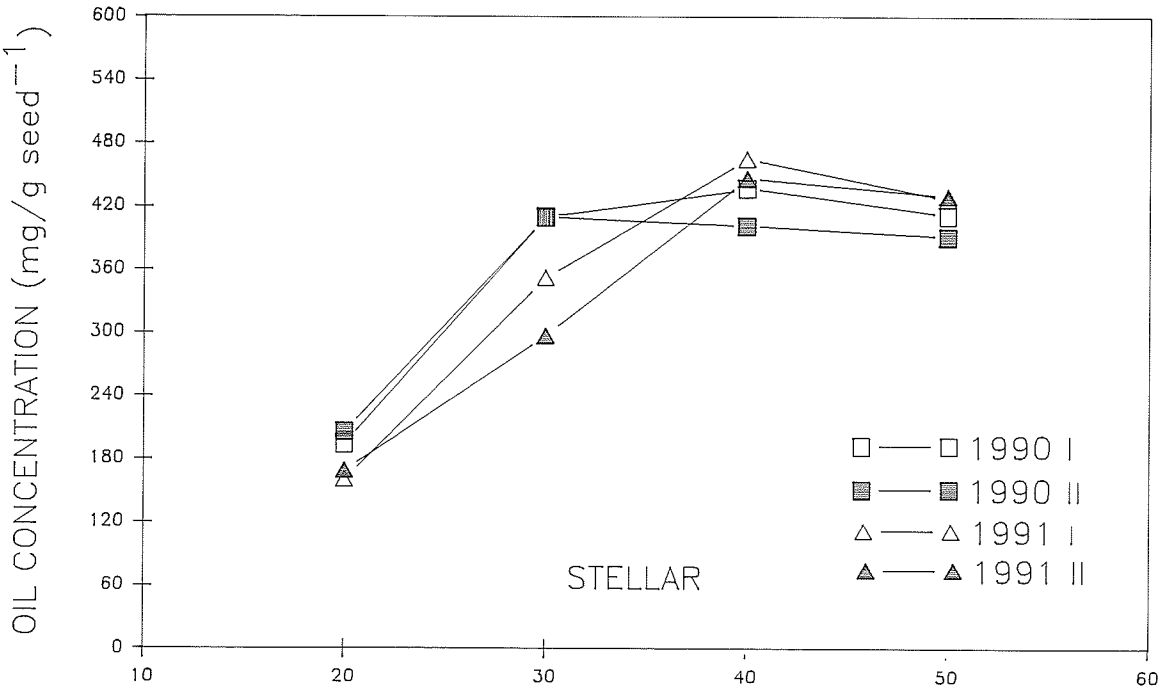
The accumulation patterns of the oil were the same for both cultivars in each year (Figure 3.5). The oil concentration of both cultivars in both planting dates rapidly increased between 20 and 30 DAF, then continued to increase at a reduced rate until maturity in 1990. In 1991, the oil concentration of both cultivars increased to a peak at 40 DAF in both planting dates, then slightly decreased to maturity. The differences between the patterns of accumulation of the major fatty acids and oil concentration were much greater between years than between planting dates within each year.

The accumulation patterns of oil concentration of both cultivars in this study are comparable to the results provided by Fowler and Downey in 1969. Their study showed that oil content expressed as the weight of oil (mg per 100 seed^{-1}) increased very rapidly between 14 and 21 DAP (date after pollination). The oil content continued to increase at a slow rate until maturity (Fowler and Downey, 1969).

In summary, oil accumulation continues actively until seed maturation. The pattern of fatty acid accumulation varies depending on genotypes. The low C18:3 character of cultivar Stellar is relatively stable under field conditions over two years. However, it is difficulty to ensure significantly distinct environments in field studies.

Figure 3.5 The accumulation patterns of oil concentration of both cultivars across sampling dates in 1990 and 1991





SAMPLING DATE (DAYS AFTER FLOWERING)

4.0. EFFECT OF DIFFERENT TEMPERATURES AND DURATION OF TEMPERATURE TREATMENTS ON THE FATTY ACID COMPOSITION OF CANOLA

4.1. Introduction

The effect of temperature upon the fatty acid composition in mature seeds has been documented for several crop species. The studies usually show a negative correlation between the monounsaturated C18:1 fatty acid and the polyunsaturated C18:2 and C18:3 fatty acids (Brett et al., 1985). However, the magnitude of genotypic and environmental (G*E) interaction varied among species.

Within species such as oilseed rape (B. napus), the influence of the environment may vary among genotypes. The low linolenic (C18:3) genotype was the result of a mutation treatment which produced an altered pattern of fatty acid accumulation (Rakow and Mcgregor 1975). It is of interest to determine whether the low C18:3 genotype responds differently to temperature in terms of fatty acid composition. In this investigation, two canola cultivars Regent and Stellar were used. The seed oil from the cultivar Regent is representative of conventional canola cultivars which normally contain 8-10% of C18:3. Stellar is a cultivar with seed oil low in C18:3 content selected from cross between Regent and a mutation line from the cultivar Oro carrying the low C18:3 character. Stellar seed oil contains 2-3% C18:3 (Scarth et al., 1988).

The objectives of this investigation were to define the

response of the C18:3 content as well as the other fatty acids of these two cultivars to different temperature regimes and to different durations of the temperature treatments during the seed development.

4.2. Materials and Methods:

This experiment consisted of three controlled environment temperature treatments (30/25Ĉ, 25/20Ĉ and 15/10Ĉ) and five durations (0, 10, 20, 30 and 40 days) within each temperature treatment. Plants of the two canola cultivars Regent and Stellar were direct sown and grown under growth conditions of 20/15Ĉ day/night aerial temperature and 16/8 h day/night photoperiod. The light (1/3 Gro-lux wide spetrum VHO, 2/3 Cool white VHO) intensity at the top of the plants was 20.0 klx (ASHS Committee on Growth Chamber Environments, 1972). At the beginning of flowering, 40 plants of each cultivar were selected at the same stage of growth. Eight of the 40 plants of each cultivar were designated as controls and were maintained in the growth room (20/15° C) until maturity. The remaining 32 plants were moved into growth chambers (1/4 Gro-lux wide spectrum VHO, 3/4 Cool white VHO) maintained at the one of three temperature treatments (30/25°C, 25/20°C and 15/10°C) with a 16/8 h day/night temperature cycle. After 10, 20, 30, 40 days duration of each temperature treatment, 8 plants of each cultivar were moved back to original growth room (20/15Ĉ) and maintained until maturity. Self-pollination was assisted by brushing each plant with a feather

duster. Seeds were harvested from each single plant at maturity stage 5.5 (Harper and Berkenkamp, 1975) and analyzed for fatty acid composition (Hougen and Bodo, 1973). Two runs of each temperature and duration treatment were conducted. These runs were treated as replicates.

Statistical analysis was performed on the U of M mainframe computer using the SAS program (SAS, 1985). Temperature and duration of each temperature regime were analyzed as treatments with 2 and 12 degree of freedom respectively in the analysis. ANOVAs and Duncan's mean comparison tests were run on the data.

4.3. Results and Discussion

The two cultivars showed a distinct responses to the different temperature regimes and the duration of each temperature treatment (Table 4.1).

Table 4.1. The effect of temperature and duration of temperature treatment on C18:3 content in the seed oil of two canola cultivars Regent and Stellar

CV Regent:	DF	MS	F-Value	Sig.
Replicate	1	2.49	1.69	-
Temperature	2	13.60	9.49	**
Duration(Temp)	12	7.16	5.00	**
Error	14	1.43		

CV Stellar:	DF	MS	F-Value	Sig.
Replicate	1	0.24	2.03	--
Temperature	2	1.59	13.62	**
Duration(Temp)	12	0.17	1.43	--
Error	14	0.12		

** significant at P=0.01 - non significant

The C18:3 content in the seed oil of both cultivars showed a significant response to the temperature treatment. The duration within each temperature treatment had a significant effect only on the C18:3 content of the cultivar Regent, but not on the C18:3 content of cultivar Stellar.

The summary of the ANOVA results of the other major fatty acids are presented in Table 4.2. Detail ANOVA results are presented in Appendix (Table A3-A5).

Table 4.2 Summary of the effect of temperature and duration of temperature treatment on the major fatty acid content in the seed oil of two canola cultivars Regent and Stellar

Cultivar	Treatment	C16:0+C18:0	C18:1	C18:2
Regent	Temperature	--	**	**
	Duration	--	--	--
Stellar	Temperature	**	**	**
	Duration	**	--	--

** significant at P=0.05
-- non significant

Neither temperature treatment nor the duration within each temperature treatment had an effect on the saturated fatty acids (C16:0+C18:0) content of cultivar Regent. However, both temperatures and the durations within each temperature treatment had significant effects in the C16:0+C18:0 content of cultivar Stellar (Table 4.2).

The C18:1 content in the seed oil of the two cultivars responded to the temperature treatment and the duration within each temperature treatment in a similar way (Table 4.2). Temperature treatment had a significant effect, but there was no

significant effect of the duration of the temperature treatment.

The C18:2 acid content in seed oil of both cultivars was significantly influenced by the temperature treatment but not by the duration of each temperature treatment(Table 4.2).

Duncan's mean comparison tests were carried out for C18:3 and the other fatty acids of both cultivars when a significant temperature effect was found (Table 4.3).

Table 4.3. Duncan's mean comparison test results of individual fatty acid composition of cultivars Regent and Stellar under three temperature regimes

CV Stellar

Temperature(℃)	C16:0+18:0 Mean (%)	Group
15/10	5.96	B
25/20	5.94	B
30/25	6.59	A
	C18:1 Mean (%)	
15/10	64.65	A
25/20	60.98	B
30/25	65.34	A
	C18:2 Mean (%)	
15/10	23.66	B
25/20	27.52	A
30/25	22.54	B
	C18:3 Mean (%)	
15/10	3.09	A
25/20	3.10	A
30/25	2.40	B

CV Regent

Temperature(℃)	C18:1 Mean (%)	
15/10	64.43	A B
25/20	62.52	B
30/25	66.06	A
	C18:2 Mean (%)	
15/10	18.16	B
25/20	20.40	A
30/25	19.07	A B
	C18:3 Mean (%)	
15/10	8.26	A
25/20	8.43	A
30/25	6.33	B

A-B Means within cultivar between different temperatures which are followed by same letter are not significantly different at the level of 0.05 of significant using Duncan's test.

The highest C18:3 content was found in both cultivars under the intermediate and low temperature treatments, while the lower C18:3 content was produced in the seed oil under the high temperature treatment. These results are consistent with the observation made by Tremolieres et al., (1978). In their study, the change in temperature conditions during seed maturation largely modified fatty acid desaturation in the seeds. It was observed that exposing oilseed rape plants to low temperatures (12-17Ĉ) during seed maturation induced higher oleate and linoleate desaturation in the seed. An increase of poly-unsaturated fatty acid production at low temperature was observed (Tremolieres et al., 1978).

The effect of high temperature on fatty acid composition was to decrease the level of desaturation, through a decrease in the proportion of the C18:2 and C18:3 in all four flax genotypes used by Green (1986). This was accompanied by an increase in saturated and monounsaturated fatty acids.

The effect of the duration of the temperature treatment on the major fatty acids was relatively minor. Only the saturated fatty acids (C16:0+C18:0) in the Stellar and the C18:3 content of Regent showed significant responses. Duncan's mean comparison tests were performed on the C16:0+18:0 content of the cultivar Stellar and C18:3 content of the cultivar Regent (Table 4.4).

The C16:0+18:0 content of cultivar Stellar under long (30 and 40 days) durations of 30/25°C treatment was significantly higher than under short (10 days) and 0 day (control). The

C16:0+18:0 acid content was also significantly higher under intermediate (20 days) duration than that under short and control durations (Table 4.4).

Table 4.4. Duncan's mean comparison test results for C16:0+C18:0 of the cultivar Stellar under five durations of each temperature treatment

Duration(DAF)	30/25Ĉ	25/20Ĉ	15/10Ĉ
00	5.41 C	5.59 A	5.95 A
10	5.77 C	5.79 A	5.68 A
20	6.94 B	6.05 A	5.85 A
30	7.45 A	6.17 A	6.06 A
40	7.39 AB	6.18 A	6.30 A

A-C Means between durations within each temperature treatment which are followed by same letter are not significantly different at the level of 0.05 significance using Duncan's test.

The C18:3 content of cultivar Regent under the 30/25Ĉ treatment also showed a significant effect of duration, with significantly lower C18:3 content under the long and intermediate durations in comparison to that under the short and control duration (Table 4.5). The C18:3 content of cultivar Regent was also significantly lower under the long durations of intermediate temperature treatment (25/20Ĉ) in comparison to the control treatment. However, the highest C18:3 content in the seed oil of cultivar Regent was found in the longest durations of exposure to low temperature. The lowest C18:3 contents were found at the intermediate and short durations (20 and 10 DAF) of the low

temperature treatment (Table 4.5).

Table 4.5. Duncan's mean comparison test results for C18:3 of the cultivar Regent under five durations of each temperature treatment

Duration (DAF)	30/25℃	25/20℃	15/10℃
00	8.70 A	9.90 A	6.58 C
10	9.30 A	9.38 AB	7.25 C
20	5.52 BC	8.30 B	7.53 C
30	4.48 CD	7.46 B	9.13 B
40	3.68 D	7.10 B	10.82 A

A-D Means between durations within each temperature treatment which are followed by same letter are not significantly different at the level of 0.05 significance using Duncan' test.

The effects of both short and long duration of temperature on the fatty acids of oilseed rape have been observed. There was a higher percentage of polyunsaturated fatty acids in the seeds at the lowest growth temperature (Tremolieres et al., 1978).

Temperature and duration of temperature both had influence on fatty acid composition of seed oil of the two cultivars in this study. The saturated (C16:0+C18:0) and monounsaturated (C18:1) fatty acid contents were higher when seed developed under high temperatures. Intermediate temperature conditions during seed development resulted in a high C18:2 content. Both intermediate and low temperature conditions resulted in a high C18:3 content in the mature seed of cultivar Regent. The C18:2

and C18:3 contents of these two cultivars responded to temperature differently. The quantitative differences of the C18:3 content of cultivar Stellar was not so great as the C18:3 content of the cultivar Regent.

Duration of temperature treatment also affected the fatty acid composition in this study. The C16:0+18:0 content of Stellar was the highest in the mature seeds of plants exposed to long duration of the high temperature treatment. The C18:3 content of cultivar Regent responded to duration of high temperature treatment with the lowest C18:3 occurring at the longest durations.

The effect of temperature on the unsaturated fatty acids can be explained by examining the fatty acid desaturation pathway (Jaworski, 1987):

```
     18:0-ACP desaturase  18:1-desaturase    18:2-desaturase
C16:0+C18:0---------->C18:1------------>-C18:2------------>C18:3
```

The lowest C18:3 content accompanied by the highest C18:1 content of both cultivars occurred under highest temperature treatment. The high temperature appears to have an inhibitory effect on the 18:1-desaturase and 18:2-desaturase enzymes. The desaturation of C18:1 to C18:2 and C18:2 to C18:3 is slowed and resulting in the accumulation of C18:1 in the seed oil.

These observations are consistent with the Tremolieres et al., (1978) report. At high temperatures, the C18:1 biosynthesis is stimulated and desturation is relative slow and as this C18:1

is relatively less desaturated. It follows with enrichment of the rapeseed triglycerides in C18:1.

The highest level of C18:2 content of both cultivars was reached under the 25/20Ĉ treatment, accompanied by the highest C18:3 and the lowest C18:1 content. For the conventional canola Regent, this may be due to higher activity rate of the 18:1-desaturase and 18:2-desaturase under 25/20Ĉ compared to the 30/25Ĉ temperature treatment. This results in higher amounts of C18:1 desaturated to C18:2 and then further desaturated to C18:3.

In the seed oil of the low C18:3 cultivar Stellar, most of the desaturation occurred at the step which converts C18:1 to C18:2. The mutation treatment which created the low C18:3 trait may have resulted in an altered C18:2 desaturase with lower rate of desaturation of C18:2 to C18:3. This would explain the high C18:2 content and the low response to temperature in the C18:3 content of Stellar. This is supported Rakow and McGregor (1975). Their report showed that significant differences in C18:1 and C18:3 content in the oil of mature seeds of M57 (C18:3 5%) and M364 (C18:3 20%) were not caused by differences in the length of time during which accumulation occurs, but rather due to the different rates of accumulation. Further support is available in the research in flax genotypes which differ in levels of C18:3 (Green, 1986). The Zero genotype which approximately 2% of C18:3 showed a linear decline in C18:2 from 70% at 15/10 to 47% at 30/25 while C18:1 increase from 12 to 34% over the same temperature. The C18:3 content showed very little variation from

1.8 to 3.1%. However, the other three high C18:3 genotypes Glenelg, M1589 and M1722 (C18:3 30-50%) showed a decrease in C18:2 and C18:3 approximately equivalent to the increase in C18:1 under high temperature (Green 1986).

It was also observed that the duration of seed maturation was much longer (usually 10 - 15 days) when plants were grown under low temperature (15/10Ĉ) in comparison to plants grown under intermediate and high temperature treatments. This is in agreement with study done by other workers (Tremoleries et al., 1975), who also found at 12 and 17Ĉ, the rate of seed maturation was greatly reduced and siliqua and seed remained rich in chlorophyll. The content of polyunsaturated fatty acids was still high after 8 week of flowering: 29 % C18:2 and 13% C18:3 in the seed of oilseed rape (winter type). The period of 8 weeks after flowering corresponds to the end of maturation time for 22Ĉ growing temperature.

In conclusion, the fatty acid composition of the two cultivars responded to temperature in the same way. The lowest C18:3 content was produced at the highest temperature treatment while the C16:0+C18:0 and C18:1 content was very high in the seed oil. Only the C18:3 content in the seed oil of cultivar Regent and the C16:0+C18:0 content of cultivar Stellar responded to duration of temperature treatment. The highest C18:3 content in the seed oil of cultivar Regent was found when the seed development occurred under the longest duration of low temperature. The highest C16:0+C18:0 content was found in the

seed oil of Stellar under longest duration of the high temperature treatment. The low C18:3 trait was relative stable in the controlled envrionment studies. The C18:3 content was about 3 % even under the low temperature treatment and the long duration of the low temperature did not increase the C18:3 content in the seed oil of Stellar.

5.0. EFFECT OF ENVIRONMENT (LOCATION) ON FATTY ACID COMPOSITION IN CANOLA

5.1. Introduction

The temperature during seed development in oil crops is recognized as the most important environmental factor affecting fatty acid composition (Howell and Collins, 1957, Slack and Roughan, 1978 and Dedio, 1985). Stability of fatty acid composition across environments is considered essential to the production and utilization of specialty oils. Recently, canola genotypes have been developed with low linolenic acid (C18:3) levels. This change in fatty acid composition may result in a distinct response to the environmental conditions. The purpose of this study was to evaluate the stability of the low C18:3 trait using a low linolenic canola cultivar grown under different environments.

5.2. Materials and Methods:

Two B. napus genotypes with distinct fatty acid composition were used in this study - the cultivar Regent with conventional canola quality (C18:3 8-10% of the seed oil) and the cultivar Stellar with low level of C18:3 (2-3% of the seed oil). Cultivar Regent was the backcross parent used in the development of Stellar (Scarth et al., 1988). In 1990, the cultivars Regent and Stellar were grown in Saskatchewan Wheat Pool trial sites at Canora, Paddockwood, Tisdale and North Battleford in Saskatchewan

and at the University of Manitoba campus in Winnipeg, Manitoba, for a total of five locations. In 1991, the cultivars Regent and Stellar were planted in Denholm and Beatty in Saskatchewan, Portage La Prairie and Winnipeg in Manitoba, with four replicates for each cultivar. All experiments consisted of three replicates.

The recommended seeding rate for canola of 6 kg/ha was used, and each plot was made up of six rows 5 m long with 0.3 m row spacing. The two sets of plots at each Saskatchewan Wheat Pool site were separated by plots of B. rapa to reduce pollen exchange between the B. napus genotypes. The maximum and minimum daily aerial temperature during seed development were obtained from the Environment Canada Weather Stations close to each site. The plots were harvested at maturity and seed samples obtained for oil content by NMR (Robertson and Morrison, 1979) and fatty acid composition by GC (Hougen and Bodo, 1973) analysis.

5.3. Results and Discussion:

The major fatty acid compositions (C16:0+18:0, C18:1, C18:2 and C18:3) fatty acids were determined for each cultivar at each location in each year (Table 5.1).

Table 5.1. The major fatty acid content expressed as % in the mature seed of cultivars Regent and Stellar over nine locations in 1990 and 1991

Regent

1990:

	C16:0+18:0		C18:1		C18:2		C18:3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Canora	5.20	0.10	61.57	0.73	20.07	0.52	10.03	0.18
Paddockwood	5.27	0.09	61.43	0.22	19.33	0.20	10.63	0.27
Tisdale	5.13	0.07	61.67	0.26	19.70	0.21	10.23	0.33
N. Batt.*	5.10	0.06	62.30	0.21	20.83	0.23	9.07	0.09
Winnipeg	6.00	0.20	64.40	0.21	19.96	0.27	7.40	0.30

1991:

	C16:0+18:0		C18:1		C18:2		C18:3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Winnipeg	5.05	0.03	63.50	0.19	19.83	0.10	9.33	0.11
Portage	5.45	0.03	64.53	0.17	18.93	0.06	8.08	0.23
Denholm	6.30	0.07	67.83	0.09	16.93	0.05	6.15	0.12
Beatty	6.85	0.10	61.50	0.17	21.43	0.21	6.75	0.10

Stellar

1990:

	C16:0+18:0		C18:1		C18:2		C18:3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Canora	5.80	0.02	60.13	0.18	27.03	0.23	3.70	0.10
Paddockwood	5.43	0.09	61.53	0.15	25.90	0.15	3.73	0.09
Tisdale	5.07	0.19	62.20	0.28	25.93	0.19	3.00	0.58
N. Batt.*	5.37	0.15	62.87	0.88	25.70	0.19	2.83	0.03
Winnipeg	5.76	0.08	63.10	0.64	26.30	0.55	2.40	0.58

1991:

	C16:0+18:0		C18:1		C18:2		C18:3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Winnipeg	5.40	0.04	61.19	0.09	27.50	0.15	2.48	0.05
Portage	5.25	0.13	65.43	0.51	24.35	0.65	2.68	0.08
Denholm	6.28	0.03	65.55	0.29	23.05	0.25	2.50	0.04
Beatty	6.95	0.13	61.88	0.40	24.80	0.51	3.08	0.19

* N. Batt. = North Battleford

ANOVA results for each of the major fatty acids for each cultivar over location are presented in Table A6 to A9 (Appendics). The data was analyzed as a RCBD with location as treatment. The results are summarized in Table 5.2.

Table 5.2. Summary of the environment (location) effect on the fatty acid composition of cultivars Regent and Stellar in 1990 and 1991

Cultivar	Year	C16:0+18:0	C18:1	C18:2	C18:3
Regent	1990	**	**	**	**
	1991	**	**	**	**
Stellar	1990	**	**	NS	**
	1991	**	**	**	**

** Significant at the P = 0.05 level
NS Not significant.

The C18:3 contents showed significant difference among the locations in both years for both cultivars, so did the C16:0+18:0 and C18:1 contents. The C18:2 content of cultivar Regent was significantly different among five locations while no significant difference was shown in C18:2 content of cultivar Stellar over locations in 1990. In 1991, the levels of C18:2 content of both cultivars showed significant differences among locations.

Duncan's means comparison tests were performed to compare the seed harvested from different locations in each year of the study, using the means of C18:3 content and other fatty acid for the two cultivars (Table 5.3 to 5.6).

1990 Trial:

The C18:3 content was the lowest in the seed sampled in Winnipeg for both cultivar Regent and Stellar and significantly lower than other locations. The seed harvested from Paddockwood and Canora of both cultivars and also cultivar Regent harvested from Tisdale were significantly higher in C18:3 content in comparison with other locations (Table 5.3).

Table 5.3. Duncan's mean comparison test results for the C18:3 content (%) of cultivars Regent and cultivar Stellar over five locations in 1990

Location	Regent	Stellar
Paddockwood	10.63 A	3.73 A
Tisdale	10.23 A	3.00 B
Canora	10.03 A	3.70 A
N. Batt.*	9.07 B	2.83 B
Winnipeg	7.40 C	2.40 C

A-C Means with cultivar between locations which are followed by the same letter are not significantly different at the 0.05 level of significance using Duncan's test.

* N. Batt. = North Battleford

The C16:0+C18:0 and C18:1 contents of both cultivar Regent and cultivar Stellar were the highest in the seed harvested at Winnipeg site. Both C16:0+C18:0 and C18:1 contents of cultivar Regent at Winnipeg location were significantly higher than the other four locations.

The C16:0+18:0 content of cultivar Stellar in the seed harvested at Canora and Winnipeg was significantly higher than that of North Battleford and Tisdale locations. The C18:1

content in the seed of cultivar Stellar harvested in Winnipeg was significantly higher than that of Paddockwood and Canora locations (Table 5.4).

Table 5.4. Duncan's mean comparison test results for major fatty acids of cultivars Regent and Stellar over five locations in 1990

CV Regent

Location	C16:0+18:0 (%)	C18:1 (%)	C18:2 (%)
Winnipeg	6.00 A	64.40 A	19.97 AB
Paddockwood	5.27 B	61.43 B	19.33 B
Canora	5.20 B	61.57 B	20.07 AB
Tisdale	5.13 B	61.67 B	19.70 B
N. Batt.*	5.10 B	62.30 B	20.83 A

CV Stellar

Location	C16:0+18:0 (%)	C18:1 (%)	C18:2 (%)
Winnipeg	5.77 A	63.10 A	26.30 A
Paddockwood	5.43 A B	61.53 B	25.90 A
Canora	5.80 A	60.13 C	27.03 A
Tisdale	5.07 B	62.20 AB	25.93 A
N. Batt.*	5.37 B	62.87 A	25.70 A

A-C Means with cultivar between locations which are followed by the same letter are not significantly different at the 0.05 level of significance using Duncan's test.

* N. Batt. = North Battleford

The C18:2 content in the seed of cultivar Regent harvested at North Battleford was significantly higher than that of Paddockwood and Tisdale. The C18:2 content of cultivar Stellar was not affected significantly by location.

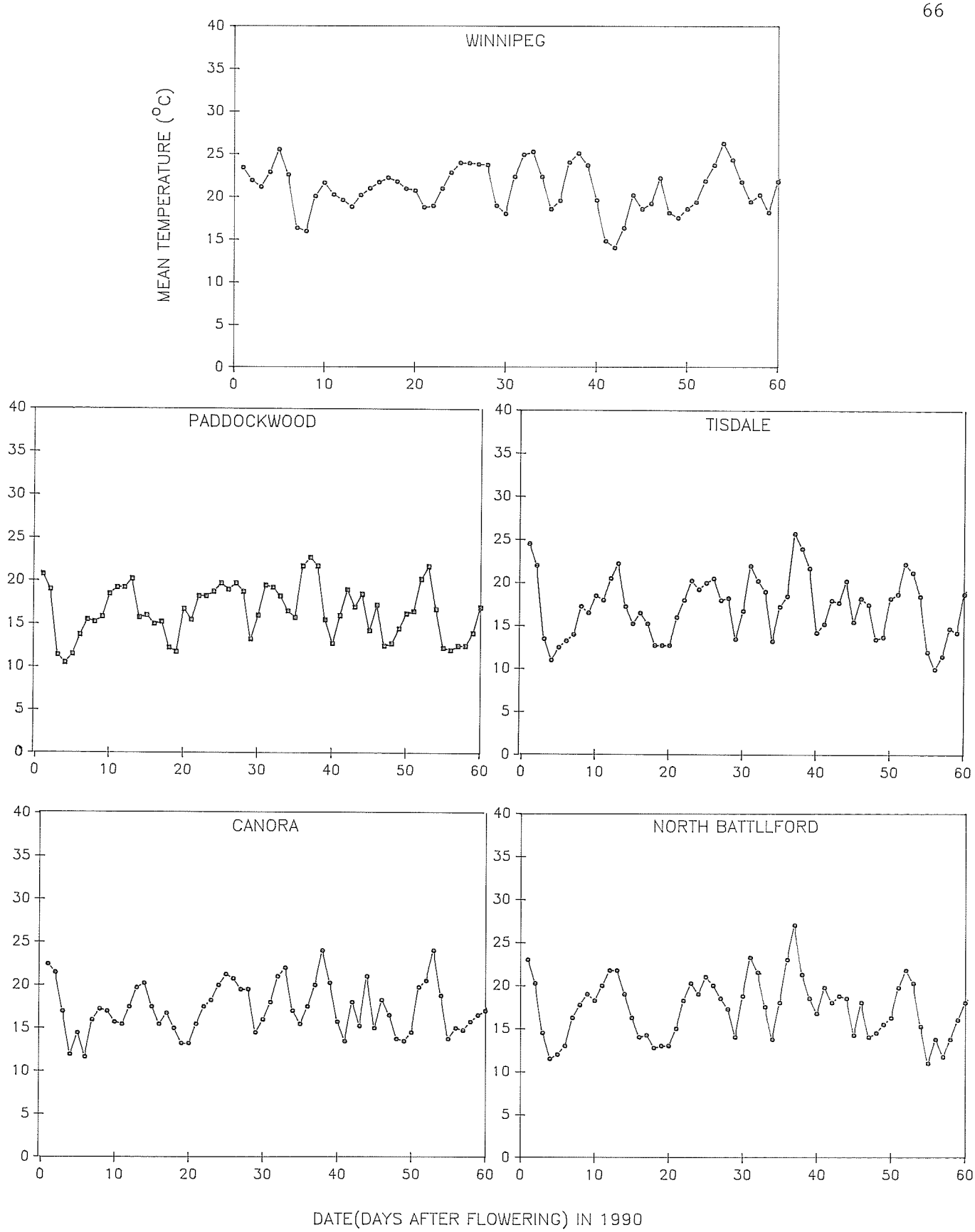
In 1990, the variation of the C18:3 and other fatty acids

cross locations may reflect the fact that Winnipeg location had the highest daily mean temperatures of the five locations (Figure. 5.1) with temperature ranging from 15 - 25Ĉ during the period of seed development (flowering to physiological maturity). The other four locations had daily mean temperature ranging from 10Ĉ -20Ĉ during the seed development period.

The C18:3 content in the seed of both cultivars was the lowest at Winnipeg (South) site accompanied by the highest C16:0+C18:0 and C18:1 content in 1990 multiple location experiment. These results are in agreement with the observations reported by Cherry et al., in 1985. They found that the fatty acid compositions of mature soybean were significantly influenced by the area in which the seed were produced. The two lines selected for low C18:3 content produced significantly less C18:1 and more C18:2 if they were grown in the north than if they were grown in south and all lines produced significantly more C18:3 and less C16:0 if they grown in north (Cherry et al., 1985).

The variation of C18:3 content in the seed oil of cultivat Stellar above 3% may have been caused by environment difference. It is also possibly it was caused by contamination by close by conventional canola plots or volunteer canola. An isolated growing condition is needed to ensure the C18:3 content below 3% for stably production of low C18:3 canola oil.

Figure 5.1 Temperature distribution of five locations during canola seed development in 1990 summer


WINNIPEG
PADDOCKWOOD
TISDALE
CANORA
NORTH BATTLLFORD
MEAN TEMPERATURE (°C)
DATE(DAYS AFTER FLOWERING) IN 1990

1991 Trial:

The highest C18:3 content in the seed of cultivar Regent was harvested at Winnipeg location, which was significantly higher than that of other three locations. However, seed of cultivar Stellar harvested at Winnipeg location had the lowest of C18:3 content among the four locations, significantly lower than Beatty location (Table 5.5).

Table 5.5 Duncan's mean comparison test results of C18:3 content of cultivar Regent and cultivar Stellar over four locations in 1991

Location	Regent	Stellar
Beatty	6.72 C	3.08 A
Denholm	6.15 D	2.50 B
Portage	8.08 B	2.67 B
Winnipeg	9.33 A	2.48 B

A-D Means with cultivar between locations which are followed by the same letter are not significantly different at the 0.05 level of significance using Duncan's test.

For both cultivar Regent and cultivar Stellar, the C16:0+18:0 content in the seed harvested from Winnipeg and Portage La Prairie locations were significantly lower than that of the other two locations.

The C18:1 content in the seed of cultivar Regent harvested at Winnipeg location also was significantly lower than Denholm and Portage but higher than that of Beatty location. The C18:1 content in seed of cultivar Stellar harvested from Winnipeg and Beatty locations were the lowest and significantly lower than at

the other two locations (Table 5.6).

Table 5.6. Duncan's mean comparison test results for major fatty acids of cultivar Regent and cultivar Stellar over four locations in 1991

CV Regent

Location	C16:0+18:0 (%)	C18:1 (%)	C18:2 (%)
Beatty	6.85 A	61.50 D	21.43 A
Denholm	6.30 B	67.83 A	16.93 D
Portage	5.48 C	64.53 B	18.93 C
Winnipeg	5.05 D	63.50 C	19.83 B

CV Stellar

Location	C16:0+18:0 (%)	C18:1 (%)	C18:2 (%)
Beatty	6.95 A	61.88 B	24.80 B
Denholm	6.28 B	65.55 A	23.05 C
Portage	5.25 C	65.43 A	24.35 B C
Winnipeg	5.40 C	61.98 B	27.50 A

A-D Means within cultivar between locations which are followed by the same letter are not significantly different at the 0.05 level of significance using Duncan's test.

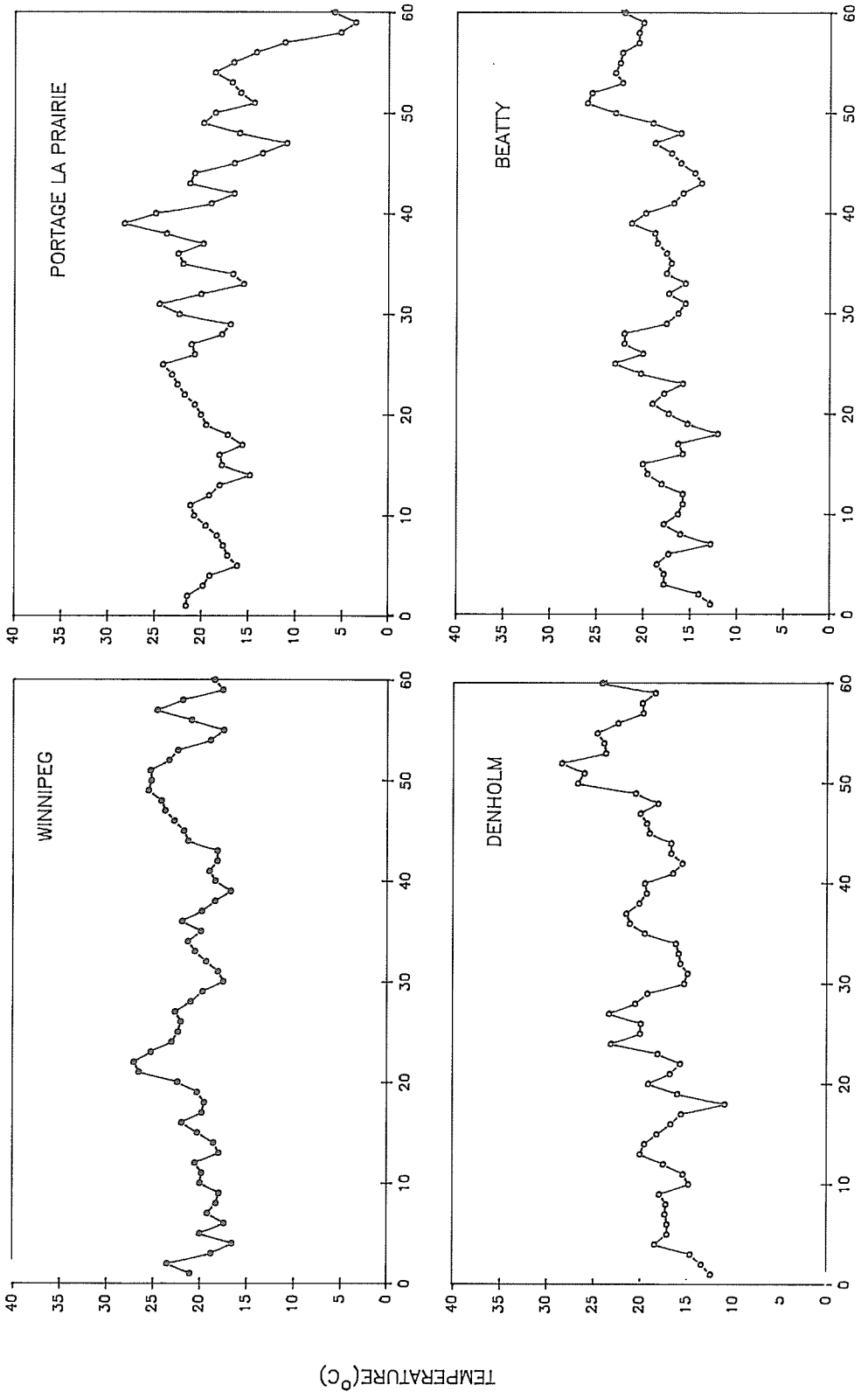
The C18:2 content in the seed of cultivar Regent harvested from Winnipeg was the second highest, which was significantly higher than seed harvested from Portage La Prairie and Denholm locations. The C18:2 content in the seed of cultivar Stellar harvested at Winnipeg was significantly higher than that at the other three locations.

In 1991, the major fatty acids of these two cultivars generally had the same pattern of response to the environment as in 1990. Both cultivars had lower saturated fatty acids (C16:0+18:0) content and monounsaturated fatty acid (C18:1)

content, and higher polyunsaturated fatty acids (C18:2 and C18:3 for Regent and only C18:2 for Stellar) at the Winnipeg location. Seed harvested at Denholm had high C16:0+18:0 and C18:1 contents, relatively low C18:2 and C18:3 contents. However, the lowest C18:3 content of the Stellar occurred in seed at the Winnipeg location, which was only significantly lower than that in Beatty location.

These variations of the major fatty acid composition in 1991 may due to the different temperature conditions during seed development at the four locations (Figure. 5.2). The Portage la Prairie site had a consistently high mean daily temperatures ranging from 15 - 25Ĉ during the seed development period, while the daily mean temperatures during the late seed development period (30 to 40 DAF) in Winnipeg location was relatively low (15 - 20Ĉ) although the daily mean temperatures varied from 15 - 25Ĉ in the early seed development stage. In Denholm and Beatty locations, the mean daily temperatures ranging from 10 - 20Ĉ during early seed development period, but the temperatures at 45 - 55 DAF were higher (20 - 25Ĉ) than that in the early stage. Fatty acid composition of the harvested sunflower seed was largely influenced by the temperature about 10 days before harvesting (Nagao and Yamazaki, 1984).

Figure 5.2 Temperature distribution of four locations during canola seed development in 1991 summer.



MEAN DAILY TEMPERATURES OF FOUR SITES IN SASKATCHEWAN AND MANITOBA (1991)

These results are generally in agreement with the reports for oil crops such as soybean (Brett et al., 1985), flax (Green, 1985) and sunflower (Robertson et al., 1979). When seed development occurs under higher temperatures, the proile of fatty acid is higher in saturated fats and mono-unsaturated fatty acids and lower in polyunsaturated fatty acids compared to seed development under lower temperatures.

Generally, both the genotype of plant and the environment in which canola seed development occurred contributed significantly to the C18:2 and C18:3 content in the mature seeds. Location affected the content of all the major fatty acids. Although the stability of C18:3 content of the cultivar Stellar was greater than that of the cultivar Regent in both years of study, it is very important to carry out a multiple-location trial when selecting for a specific oil quality in canola. The C18:3 content of cultivar Stellar was above 3% in some locations. It is possible that this was caused by pollen contamination from close by conventional canola plots or volunteer canola.

6.0. GENERAL DISCUSSION AND CONCLUSIONS

The temperature conditions during the period of seed development affect the fatty acid composition of canola oil. Generally, the response of C18:3 and other fatty acid composition of both cultivar Regent and cultivar Stellar to the temperature treatments followed the same pattern. When seed development occurred at high temperatures, the fatty acid profile was high in saturated fatty acids (C16:0+18:0) and monounsaturated fatty acid (C18:1) and low in polyunsaturated fatty acids (C18:2 and C18:3) compared to distribution of fatty acid composition under low temperature treatments.

The results of the planting date experiment did not give a clear indication of the effect of temperature during seed development. This was due to the very similar environments provided by four planting dates over two years. The patterns of oil and fatty acid accumulation of both cultivar were much more different between the two years than between the two planting dates within each year. However, the C18:3 accumulation during seed development were very different between two cultivars. The C18:3 concentration in the seed of cultivar Regent increased at a much higher rate than the rate in cultivar Stellar.

The growth cabinet results showed that both the temperature and the duration of the temperature treatment affected the fatty acid composition in the developing seed. Both the intermediate and low temperature treatments resulted in a higher C18:3 content

in both cultivars. The C18:3 content in the seed oil of cultivar Regent increased with the longer duration of low temperature. The lowest C18:3 content occurred under the longest duration of high temperature treatment. As the duration of the high temperature treatment increased, the saturated fatty acid contents in the seed oil of cultivar Stellar increased, and the C18:3 content decreased in the seed oil of cultivar Regent. The intermediate temperature treatments resulted in a high C18:2 content in the seed oil of both cultivars.

The duration of the seed development period was also affected by temperature. The results of planting date and controlled environmental studies showed that seed development was completed at 40 days under warmer temperatures (daily mean temperatures 15 - 25Ĉ), while the period was 10 to 15 days longer under cooler temperatures (daily mean temperatures 10 - 20Ĉ). This demonstrates the close relationship between the accumulation of growing degree days and the progress of seed maturation.

The relationship between temperature and fatty acid composition was supported by the study of canola seed development from different locations. A high saturated and monounsaturated fatty acid content occurred in the mature seed harvested at sites which had relatively high temperatures during the seed development period. The temperature conditions are particularly critical during the late stages of seed development, approximately 10 days before canola seed reaches physiologically maturity.

The 18:1 and 18:2 desaturase enzyme activities were not measured in this study. If a biochemical method of enzyme assay was available, it would help to improve our understanding of the mechanism(s) which are involved in the response of fatty acid composition to the temperature. The measurement of 18:2-desaturase expression and the time of expression during the seed development would help to explain the C18:3 response to temperature during the seed development.

The controlled environment can provide a more accurate and reliable assessment of the stability of fatty acid composition. The stability of low C18:3 (3%) and low C16:0+C18:0 (6%) traits are economically important to the promotian of low C18:3 canola oil in the market place.

The analysis of fatty acid composition over different years and different locations help to demonstrate the stability of the specialty fatty acid composition, which is very critical for specialty oil canola breeding and production.

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APPENDICES

Table A1. ANOVA Results for Major Fatty acid Compositon of Cultivar Regent under Two Planting Dates over Two Years

C16:0+C18:0

Source	DF	MS	F-Value	Sig.
Rep	5	0.22	3.07	--
Year	1	40.94	577.60	**
Error I (Rep*Year)	1	0.02	0.50	
Pldate	1	4.12	107.79	**
Year*Pldate	1	0.02	0.62	--
Error II [Rep*Pld(Year)]	10	0.04	0.81	
Sampdate	3	130.32	2757.25	**
Error III	60	0.05		

C18:1

Source	DF	MS	F-Value	Sig.
Rep	5	1.97	2.36	--
Year	1	17.34	20.76	**
Error I (Rep*Year)	5	0.84	1.10	
Pldate	1	5.61	0.93	--
Year*Pldate	1	2.16	2.66	--
Error II [Rep*Pld(Year)	10	2.11	2.77	
Sampdate	3	298.49	392.46	**
Error III	60	0.76		

C18:2

Source	DF	MS	F-Value	Sig.
Rep	5	1.07	0.66	--
Year	1	3.05	1.87	--
Error I (Rep*Year)	5	1.63	3.07	
Pldate	1	3.96	1.57	--
Year*Pldate	1	10.87	4.32	--
Error II [Rep*Pld(Year)]	5	4.73	2.22	
Sampdate	3	57.75	44.78	**
Error III	60	1.29		

C18:3

Source	DF	MS	F-Value	Sig.
Rep	5	0.53	0.56	--
Year	1	30.15	46.96	**
Error I	5	0.19	2.87	
Pldate	1	4.95	46.96	**
Year*Pldate	1	0.26	2.47	--
Error II [Rep*Pld(Year)]	10	1.05	1.58	
Sampdate	3	9.31	139.81	**
Error III	60	0.07		

** significant at P=0.05, -- non significant

Table A2. ANOVA Results for Major Fatty Acid Composition of Cultivar Stellar under Two Planting Dates over Two Years

C16:0+18:0

Source	DF	MS	F-Value	Sig.
Rep	5	0.09	0.06	--
Year	1	44.42	142.15	**
Error I (Rep*Year)	5	0.31	2.48	
Pldate	1	4.29	11.02	**
Year*Pldate	1	0.02	0.05	--
Error II [Rep*Pld(Year)]	10	0.39	3.10	
Sampdate	3	183.43	1458.11	**
Error III	60	0.13		

C18:1

Source	DF	MS	F-Value	Sig.
Rep	5	2.26	1.08	--
Year	1	47.04	22.35	**
Error I (Rep*Year)	5	2.11	1.17	
Pldate	1	2.47	1.33	--
Year*Pldate	1	24.81	13.32	**
Error II [Rep*Pld(Year)]	5	2.10	1.01	
Sampdate	3	2331.76	433.71	**
Error II	60	1.79		

C18:2

Source	DF	MS	F-Value	Sig.
Rep	5	1.50	1.51	--
Year	1	10.87	8.28	**
Error I (Rep*Year)	5	1.31	1.28	
Pldate	1	3.19	3.23	--
Year*Pldate	1	8.70	8.80	**
Error II [Rep*Pld(Year)]	10	0.99	0.96	
Sampdate	3	55.87	54.42	**
Error III	60	1.02		

** significant at P=0.05, - non significant

Table A3. The effect of temperature and duration of temperature treatment on C16:0+18:0 content in the seed oil of two canola cultivars Regent and Stellar

CV Regent	DF	MS	F-Value	Sig.
Replicate	1	0.07	0.34	--
Temperature	2	0.18	0.83	--
Duration(Temp)	12	0.36	1.72	--
Error	14	0.21		

CV Stellar	DF	MS	F-Value	Sig.
Replicate	1	0.09	0.60	--
Temperature	2	1.35	9.47	**
Duration(Temp)	12	0.68	4.74	**
Error	14	0.14		

** significant at P=0.05
-- non significant

Table A4. The effect of temperature and duration of temperature treatment on C18:1 content in seed oil of two canola cultivars Regent and Stellar

CV Regent:	DF	MS	F-Value	Sig.
Replicate	1	0.10	0.02	--
Temperature	2	31.27	6.60	**
Duration(Temp)	12	11.71	2.47	--
Error	14	4.74		

CV Stellar:	DF	MS	F-Value	Sig.
Replicate	1	10.01	1.89	--
Temperature	2	54.74	10.32	**
Duration(Temp)	12	11.55	2.18	--
Error	14	5.31		

** significant at p=0.05
-- non significant

Table A5. The effect of temperature and duration of temperature treatment on C18:2 acid content in the seed oil of two canola cultivars Regent and Stellar

CV Regent:	DF	MS	F-Value	Sig.
Replicate	1	0.11	0.06	-
Temperature	2	12.71	6.47	*
Duration(Temp)	12	4.23	2.15	-
Error	14	1.96		

CV Stellar:	DF	MS	F-Value	Sig.
Replicate	1	15.04	3.10	--
Temperature	2	68.34	14.11	**
Duration(Temp)	12	8.98	1.85	--
Error	14	4.84		

** significant at P=0.05
-- non significant

A-B Means within cultivar between different temperatures which are followed by same letter are not significantly different at the level of 0.05 of significant using Duncan's test.

Table A6. ANOVA Results of the C16:0+C18:0 content for Cultivar Regent and Cultivars Stellar over Locations in 1990 and 1991

Regent:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.01	0.31	-
Locations	4	0.42	9.21	**
Error	8	0.05		

1991

Source	DF	MS	F-Value	Sig.
Replicates	3	0.04	3.51	-
Locations	3	2.62	246.49	**
Error	9	0.01		

Stellar:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.07	1.75	-
Location	4	0.28	7.23	**
Error	8	0.04		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.08	3.39	-
Location	3	2.53	113.45	**
Error	9	0.02		

* ** significant at P=0.05 and P=0.01 respectively
- non significant

Table A7. ANOVA Results of the C18:1 content for Cultivar Regent and Cultivar Stellar over Locations in 1990 and 1991

Regent:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.22	0.47	-
Location	4	4.57	9.42	**
Error	8	0.49		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.13	1.52	-
Locations	3	27.93	317.23	**
Error	9	0.09		

Stellar:

1990

Source:	DF	MS	F-Value	Sig.
Replicate	2	0.66	1.93	-
Location	4	4.27	12.48	**
Error	8	0.34		

1991

Source:	DF	MS	F-Value	Sig.
Replicate	3	0.14	0.22	-
Location	3	16.94	26.84	**
Error	9	0.63		

* ** significant at P=0.05 P=0.01 respectively
- non significant

Table A8. ANOVA Results of the C18:2 content for Cultivar Regent and Cultivar Stellar over Locations in 1990 and 1991

Regent:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.57	2.58	-
Location	4	0.92	4.21	*
Error	8	0.22		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.03	0.37	-
Locations	3	14.09	206.24	**
Error	9	0.07		

Stellar:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.27	1.01	-
Location	4	0.83	3.05	-
Error	8	0.27		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.17	0.18	-
Location	3	13.99	14.73	**
Error	9	0.95		

* ** significant at P=0.05 and P=0.01 respectively
- non significant

Table A9. ANOVA Results of the C18:3 content of Cultivar Regent and Cultivar Stellar over Locations in 1990 and 1991

Regent:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.01	0.07	-
Location	4	0.81	33.36	**
Error	8	0.15		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.11	1.38	-
Location	3	8.09	101.00	**
Error	9	0.08		

Stellar:

1990

Source	DF	MS	F-Value	Sig.
Replicate	2	0.02	1.48	-
Location	4	1.00	71.07	**
Error	8	0.01		

1991

Source	DF	MS	F-Value	Sig.
Replicate	3	0.03	0.61	-
Location	3	0.31	6.14	*
Error	9	0.05		

* ** significant at P=0.05 and P=0.01 respectively
- non significant