

**EFFECT OF ELEVATED TEMPERATURE AND CO₂ ON THE LEAF AND
SEED FATTY ACID COMPOSITION OF *Arabidopsis thaliana* AND *Brassica
napus* FATTY ACID MUTANT LINES**

BY

DARREN CRAIG PLETT

**A Thesis Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the
Requirements for the Degree of**

MASTER OF SCIENCE

**Department of Plant Science
University of Manitoba
Winnipeg, Manitoba**

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FACULTY OF GRADUATE STUDIES

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ABSTRACT

Plett, Darren Craig. M.Sc., The University of Manitoba, May, 2003. Effect of elevated temperature and CO₂ on the leaf and seed fatty acid composition of *Arabidopsis thaliana* and *Brassica napus* fatty acid mutant lines. Major professor: Dr. Rachael Scarth.

Elevated temperature and CO₂ are two components of climate change which will impact crop production significantly. Atmospheric CO₂ level is increasing from the current level of 360 ppm at an increasing rate and global mean temperatures are predicted to rise 3.5°C over the next century. These factors will significantly affect crop growth and development, as well as the seed oil quality produced by oilseed rape (*Brassica napus*).

Controlled environment studies were undertaken to determine the effects of climate change on *B. napus* and *Arabidopsis thaliana* leaf and seed fatty acid composition. Fatty acid desaturase (*fad*) mutant *Arabidopsis* lines were chosen for study because of the temperature tolerance expressed by these lines as a result of their altered leaf membrane lipids. *B. napus* mutant lines were selected with a range of altered seed oil traits. The two sets of *Arabidopsis* and *B. napus* lines were grown at two day/night temperature levels (20/15°C and 30/25°C) and two CO₂ levels (360 and 720 ppm). Leaf samples were taken at the six-leaf stage (28 DAS) and seed samples at maturity. Samples were analyzed for fatty acid composition using gas chromatography.

The *B. napus* lines responded to elevated temperature with higher levels of saturation of the leaf lipid fatty acids. The *B. napus* lines were sorted into three clusters, based on average number of double bonds in an average fatty acid (ADB) values. The lines in each cluster shared a similar pedigree. The *Arabidopsis* lines responded differently from the *B. napus* lines as the *fad7* and *fad7fad8* mutant lines maintained a

relatively saturated leaf lipid profile in both temperature treatments. CO₂ played a less significant role in affecting ADB values of the *Arabidopsis* lines. The *B. napus* lines responded to elevated CO₂ with significant decreases in ADB value or more saturated leaf lipid profiles. The response of the seed oil profiles to temperature and CO₂ values was similar, despite the range of ADB values in the mutant *B. napus* lines.

The *B. napus* lines were also studied in the field, using two locations (Carman and Winnipeg) and two seeding dates (early and late) to provide different environments during leaf and seed development. Location had a greater influence on leaf lipid fatty acid composition data than temperature. However, leaf lipids were more saturated when the plants developed under higher temperature conditions [i.e. higher growing degree days (GDD)] compared to lower temperature conditions (lower GDD). Elevated temperatures during seed development also resulted in a more saturated fatty acid profile in the seed oil.

This study showed that *B. napus* and *Arabidopsis* leaf and seed lipids become more saturated in response to elevated temperatures. The *fad* mutations in *Arabidopsis* resulted in a relatively saturated leaf lipid profile independent of temperature, which provides an important adaptation to elevated temperature conditions. This mutation may be useful in the breeding of crops for climate change conditions. The effect of CO₂ on leaf and seed fatty acid profiles was more complex than that of temperature and the effect of temperature and CO₂ seem to be species specific.

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1. INTRODUCTION

Brassica napus is currently the primary spring oilseed rape species grown in Canada. Canadian farmers produced 4.93 million tonnes of seed, contributing 1.72 billion dollars to the Canadian economy in 2001 (Statistics Canada 2002). The seed oil of *B. napus* is composed primarily of medium and long chain fatty acids including palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0), erucic (C22:1), and lignoceric (C24:0). The levels of the fatty acids, and the ratios between them, determine the functional and nutritional aspects of the oil.

Plant scientists utilize the model plant, *Arabidopsis thaliana*, a member of the *Cruciferae* family, for physiological and genetic studies which can be applied to economically important crops, such as oilseed rape. *Arabidopsis* is short in stature and has a short maturation time (35 d.) making it a useful plant for laboratory studies. Additionally, *Arabidopsis* has numerous well-characterized mutations, and its genome is relatively small (~125 Mbp) and has been completely sequenced (The *Arabidopsis* Genome Initiative 2000).

Climate change, or global warming, has been attributed to the rising levels of greenhouse gasses in the atmosphere. These gasses trap heat and reflect it back to the earth's surface (Environment Canada 2002). Atmospheric CO₂ levels have risen to 360 ppm from the pre-Industrial Revolution level of 280 ppm, and continue to rise at an increasing rate. The result has been an increase in global mean temperatures. More substantial increases are predicted for more polar latitudes. The mean temperature in

Canada has risen 1°C in the past 100 years and is predicted to rise another 1°C to 3.5°C over the next century (Environment Canada 2002).

Increasing temperature and atmospheric CO₂ levels will significantly affect crop production. The integrity of leaf chloroplast membranes is an important determinant of plant adaptation to changing environmental conditions. Membrane fluidity, which is a function of the saturation state of the lipids incorporated in the membrane, must be maintained to allow cellular function at different temperatures (Harwood 1998a). Plants grown at elevated temperatures tend to have more relatively saturated leaf membrane lipids in order to maintain proper cellular function. *Arabidopsis* fatty acid desaturase (*fad*) gene mutants characterized by elevated dienoic (C16:2 + C18:2) and decreased trienoic (C16:3 + C18:3) fatty acid levels were able to withstand exposure to temperatures above 35°C for significantly longer periods than wild type plants (Murakami *et al.* 2000).

The effect of elevated CO₂ on plants is a general increase in growth rate (Raven *et al.* 1999), increased yield (Thompson and Woodward 1994) and increased seed size (Ferris *et al.* 1999). However, the effect of CO₂ on lipid biosynthesis are complex (Williams *et al.* 1998a, Williams *et al.* 1998b).

Increased temperature effects on oilseed fatty acid biosynthesis was studied in sunflower (*Helianthus annuus* L.) (Sarmiento *et al.* 1998), flax (*Linum usitatissimum*) (Tonnet and Green 1987), soybean (*Glycine max*) (Cheesebrough 1989) and canola (*B. napus*) (Deng and Scarth 1998). These studies found that higher temperatures during seed development were associated with an increase in the levels of saturated fatty acids or relative saturation levels in the seed oil. This may be due to the increased rate of plant

development and the resulting decrease in the time available for seed development and maturation and thus for fatty acid desaturation. Little research has been devoted to determining how elevated CO₂ may affect the fatty acid composition of oilseeds. Some research has been carried out in winter wheat (*Triticum aestivum* L.), however, the results were complex and difficult to interpret (Williams *et al.* 1994, Williams *et al.* 1995).

Since canola is an extremely valuable commodity to Canadian farmers and the economy, it is important to anticipate the potential impact of climate change on yield and oil quality in this crop. Canola breeders have successfully modified canola varieties to express such traits as low C22:1 (Stefansson and Kondra 1975), low C18:3 (Scarth *et al.* 1988), low saturate levels (Raney *et al.* 1999) and elevated C22:1 (McVetty *et al.* 2000). In order to successfully breed a *B. napus* variety adapted to climate change conditions, it must be understood how *B. napus* growth and development are impacted by environmental changes such as elevated CO₂ and temperature.

The first objective of this study was to determine the change in leaf and seed fatty acid composition of *B. napus* and *Arabidopsis* fatty acid mutant lines as a result of climate change conditions. This objective was studied in controlled environment conditions with ambient and elevated levels of temperature and CO₂.

The second objective of the study was to examine the leaf and seed fatty acid composition of the *B. napus* lines in the field environment. Two seeding dates at two locations were utilized to provide different temperature regimes for the crop development.

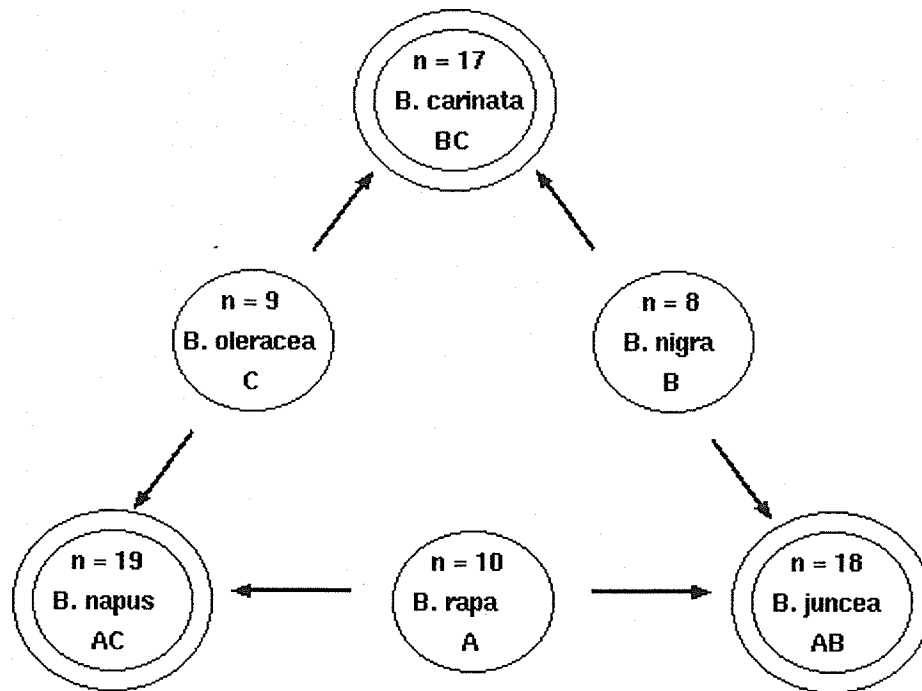
2. LITERATURE REVIEW

2.1 Oilseed rape (*Brassica spp.*) history and breeding

2.1.1 History of rapeseed

The vegetable *Brassica* species were cultivated in the Neolithic age, making them one of the earliest domesticated plants (Downey and Röbbelen 1989). The first mention of oilseed rape is found in Indian Sanskrit writing dating from 2000 to 1500 BC. Writings of Greek, Roman and Chinese origin from 500 to 200 BC describe the medicinal value of the *Brassica* species. European domestication of *Brassica* species did not occur until the Middle Ages, and these species were utilized for lamp oil and lubrication purposes, not for human consumption.

The species *B. rapa* and *B. oleracea* are the diploid ancestors of *B. napus* (Figure 2.1). *B. rapa* had the widest original distribution of the *Brassicaceae*, found from the Atlantic islands to the eastern parts of Korea and China, and from Norway to Northern India. The primary center of origin for *B. rapa* is the Himalayan region, with secondary centers of origin in the Mediterranean and Asian regions. *B. oleracea* was originally found only in the Mediterranean region, thus the interspecific cross that created *B. napus* probably occurred in the southern European region (Downey and Röbbelen 1989).



Note: *B. campestris* = *B. rapa*

Figure 2.1: U's triangle. (U 1935).

B. napus, *B. rapa* and *B. juncea* are the predominant forms of oilseed rape grown in Canada. However, the acreage grown of *B. juncea* is small and *B. rapa* acreage has declined from 40 to 60% to below 20% of oilseed rape acreage. (Canadian Grain Commission 1998). Although winter and spring forms exist in *B. napus* and *B. rapa*, the extreme winter conditions in Western Canadian production regions preclude the growth of the winter types in all areas except southern Ontario. Therefore, spring varieties are grown in Canada despite being lower yielding than the winter varieties.

2.1.2 Development of Canola quality oilseed rape

Rapeseed oil was used extensively in Europe upon the development of steam power as it was found to be an excellent lubricant that would cling to water and steam-washed metal surfaces (Adolphe *et al.* 2002). Prior to World War II, small research plots of rapeseed had been grown in Canada. A farmer from Shellbrook, Saskatchewan

increased a *B. rapa* accession from Poland in 1936 to meet seed supply demands in anticipation of the wartime embargos on petroleum products. This is the origin of the common name, "Polish rapeseed", for *B. rapa* in Canada.

The shortage of available seed led to the import of *B. napus* seed from the United States in 1943. The original accession had been obtained from Argentina and thus the variety became known in Canada as "Argentine rapeseed."

Following World War II, the use of oilseed rape as human food and animal feed began to be explored. The seed contained nearly 40% oil, which was attractive to potential processors, and the seed oil contained significant levels of the essential fatty acids linoleic acid (C18:2) and linolenic acid (C18:3). However, high levels of the long chain fatty acid erucic acid (C22:1) in the seed oil and glucosinolates in the seed meal were viewed as serious limitations to the widespread use of *B. napus* and *B. rapa* seed.

In 1968, the first variety of low C22:1 *B. napus*, Oro, was developed by Dr. Keith Downey at the Agriculture Canada research station in Saskatoon, Saskatchewan and Dr. Baldur Stefansson at the University of Manitoba in Winnipeg, Manitoba. In 1974, the first low C22:1, low glucosinolate ("double low") variety of *B. napus*, Tower (Stefansson and Kondra 1975), was developed by Dr. Stefansson, and the term 'canola' was coined in 1978 to describe the combined oil and meal quality. The canola trademark was registered by the Western Canadian Oilseed Crushers' Association, now the Canadian Oilseed Processors Association and became the property of the Canola Council of Canada in 1980 and was amended in 1986 by the Trademarks Branch of Consumer and Corporate Affairs. The official definition of canola oil is, "an oil that must contain less than 2% C22:1, and the solid component of the seed must contain less than 30 micromoles of any

one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Adolphe *et al.* 2002). Canola oil has become Canada's leading vegetable oil, and is exported largely to the United States, Japan and Mexico. In 2001, Canadian farmers grew 3.82 million hectares of canola varieties, which produced 4.93 million tonnes of seed worth 1.72 billion dollars in cash receipts to the Canadian economy (Statistics Canada 2002). Canola oil is used primarily in products like salad oils, margarines and shortenings and canola meal is used in the feed rations of dairy, beef, swine and poultry.

2.2 *Arabidopsis thaliana* history and development

2.2.1 History and usage of *Arabidopsis*

The *Arabidopsis* genus is a member of the mustard or crucifer family (*Brassicaceae* or *Cruciferae*), which consists of approximately 340 genera and 3350 species (Price *et al.* 1994). The family contains the greatest abundance of genera and species found in the northern hemisphere (Al-Shehbaz 2002). Centers of origin for the *Arabidopsis* genus are found in southwestern and central Asia and in the Mediterranean region. Secondary centers of diversity are located in the arctic, western North America and the mountains of South America (Price *et al.* 1994). Presently, *Arabidopsis* species are found across Asia and Europe with new distribution in areas where it has been introduced by human migration. Like other Crucifer species, *Arabidopsis* is an annual herb characterized by a cross-shaped corolla, tetradynamous stamens (four long and two short), and capsule shaped fruit (silique or silicle) which consist of two valves that

separate at maturity for seed distribution (Price *et al.* 1994). A member of the *Brassicaceae* family, *Arabidopsis* seed contains glucosinolate compounds, which give the oil a pungent mustard flavor.

A. thaliana was discovered by Johannes Thal in the Harz mountains in the 16th century. At the time he called the plant *Pilosella siliquosa* and it went through a number of name changes before its present classification (Meyerowitz 1998). The first botanical literature appearance of *Arabidopsis* was in 1873 in a paper by Alexander Braun who described a mutant plant. The correct haploid number of chromosomes ($n = 5$) was identified in 1907 by Friedrich Laibach (Meyerowitz 2001). In 1943 the use of *Arabidopsis* as a model organism for the study of plants was proposed by Laibach who was studying the first mutants generated by x-ray mutagenesis (Meyerowitz 2001). Very little research was done on *Arabidopsis* in the 1950s and early 1960s, but interest began to grow as shown by the convening of an International Congress of *Arabidopsis* in April, 1965 (Pigliucci 2002). It wasn't until the 1980s that there was recognition of the potential role of *Arabidopsis* as an important model plant species by scientists such as George Redei, Chris Somerville, Elliot Meyerowitz and Maarten Koorneef (Meyerowitz 2001).

A. thaliana, also known as mouse-eared cress, has a relatively small genome (125 Mb) for higher plant species and the complete sequence has recently been published (The *Arabidopsis* Genome Initiative 2000). Extensive physical and genetic maps exist for all 5 of its chromosomes. *Arabidopsis* has a small growth habit, is a prolific seed producer and, for higher plants, requires a relatively short maturation period. In addition, a large number and wide variety of mutations exist in *Arabidopsis*, and it is easily transformed

via *Agrobacterium tumefaciens*. All of these factors contribute to its use by plant physiologists, geneticists, pathologists, molecular biologists and breeders, as a model for flowering higher plants.

2.2.2 *Arabidopsis* and *Brassica* species relationship

Arabidopsis thaliana, *B. rapa* and *B. napus* are all members of the *Cruciferae* (*Brassicaceae*) family. As such, the species have similar centers of origin, many physiological similarities and extensive genetic similarities.

Comparative genetic mapping studies have been performed between *Arabidopsis* and the three diploid *Brassica* species (*B. rapa*, *B. oleracea*, *B. nigra*). Genetic synteny refers to the presence of two or more loci on the same chromosome, or more specifically, it refers to the gene order on chromosomes being conserved over wide evolutionary distances (Melcher 2000). Knowing the synteny between species and the rate of genetic change in species allows estimations of evolutionary divergence time. Estimations of the evolutionary divergence date of *Arabidopsis* and the *Brassica* species range from 12.2 – 19.2 M years (The *Arabidopsis* Genome Initiative 2000) to 35 M years (Lagercrantz 1998) ago.

Studies of collinearity of genomes have indicated that the diploid *Brassica* genomes result from a triplication of the *Arabidopsis* genome (O'Neill *et al.* 2000). Further evidence to support this hypothesis has been provided through measurements of genome weights (Lagercrantz 1998), comparative physical mapping by fluorescence *in situ* hybridization (FISH) (Jackson *et al.* 2000) and genetic marker studies (O'Neill *et al.* 2000).

Since the diploid *Brassicac*s have three copies of each gene in the *Arabidopsis* genome, it follows that the amphidiploid *B. napus* should have six copies of each *Arabidopsis* gene. It is estimated that the 125 Mb *Arabidopsis* genome was triplicated and expanded with non-coding DNA 1.5 times to account for the 600 Mb genome size of the diploid *Brassica* species and the 1200 Mb genome size of the amphidiploid *Brassica* species (O'Neill *et al.* 2000). This is plausible due to the much higher level of non-coding DNA found in the *Brassica* genome. The recently sequenced *Arabidopsis* genome has been estimated to contain approximately 25,000 genes (The *Arabidopsis* Genome Initiative 2000). By extension, the diploid *Brassicac*s would contain 75,000 genes and amphidiploid *Brassicac*s could contain as many as 150,000 genes.

The significant genetic relationship between *Arabidopsis* and *Brassica* species provides a powerful tool for the breeding and development of *Brassica* crop species. Genetic studies may be performed in the simpler *Arabidopsis* model before analysis of the more complex *Brassica* species is undertaken. Genetic synteny between species will be useful in understanding *Brassica* species and *Arabidopsis* is a potential gene bank for future transformation of *Brassica*.

2.3 Climate change

2.3.1 Introduction

The Earth's climate is subject to change. Environment Canada (2002) describes climate change, or global warming, as, "Human activities are altering the chemical composition of the atmosphere through the build-up of greenhouse gasses that trap heat

and reflect it back to the earth's surface. This is resulting in changes to our climate, including a rise in global temperatures and more frequent extreme weather events.”

The “greenhouse effect” is responsible for creating the current global temperature mean of 15°C. The greenhouse gasses (methane, water vapor, nitrous oxide, halocarbons and carbon dioxide) reflect the radiant energy from the Earth's surface back toward the Earth, causing the atmospheric temperature to warm significantly. Without this layer of gasses the global temperature mean would be -18°C and life would not exist on Earth. Since the Industrial Revolution (1850), atmospheric levels of CO₂, the most abundant greenhouse gas, have increased significantly. For tens of thousands of years prior to the Industrial Revolution, the atmospheric CO₂ concentration was around 280 parts per million (ppm). The current level of CO₂ in the atmosphere is approximately 370 ppm, an increase of 30%. Global mean temperature has increased 0.6°C over the past 100 years. More northern latitudes are seemingly more affected by the change, including Canada where mean temperature has risen 1.0°C in the past 100 years. If greenhouse gas emissions continue to rise exponentially, mean atmospheric temperatures will also continue to rise (Environment Canada 2002). Climate models such as a General Circulation Model predict increases of 1°C to 3.5°C over the next century.

Climate change has many possible impacts on the Earth and its biosystems. Generally warmer and drier conditions on the Prairies may increase the length of the growing season, but may also create drought, pest infestations and disease outbreaks. Drought conditions could also impact fish stocks, hydroelectric generating potential, wetland populations and a variety of systems which depend on natural resources (Environment Canada 2002).

2.3.2 Effects of climate change on plant growth and development

As with any other biosystem, climate change will significantly impact the growth and development of plants. In particular, plants will be most significantly affected by the elevation of CO₂, the increase in temperature and water deficit components of climate change. Plants are a vital component of the global Carbon cycle. They fix inorganic carbon (CO₂), via photosynthesis, into many forms of organic carbon (sugars, cellulose, etc.). As atmospheric levels of CO₂ are increasing, the ability of plants to flourish will be extremely important to continuation of life on Earth. Realizing the potential impact of climate change on plants, researchers began to study the interactions in the early 1990s.

Unlike animals, plants are unable to significantly change their location in order to avoid temperature extremes. Temperature plays a vital role in plant growth through its effect on biochemical processes which affect resource acquisition, growth and leaf energy budgets (Coleman and Bazzaz 1992). Elevated temperatures will stimulate plant growth due to increased enzyme activity (Raven *et al.* 1999). Warmer temperatures will also increase photosynthetic rates due to the increase in rates of electron transfer through electron transport chains and increased demand for photosynthate by the plant (Salisbury and Ross 1992). However, elevated temperatures also lead to increased transpiration rates which may result in drought stress (Coleman and Bazzaz 1992). Additionally, temperature may exceed the optimal temperatures of enzymes (~30°C) resulting in decreased activity and, therefore, lower plant growth rates. Membrane structure and function is disrupted at elevated temperature and this severely alters cellular function and growth potential for the plant (Coleman and Bazzaz 1992).

Photorespiration and CO₂-concentration mechanisms play a vital role in determining the response of plants to CO₂ levels. As a result, the influence of elevated CO₂ on the growth of C₃ and C₄ plants differs dramatically. The photosynthetic rate of C₃ plants is affected more significantly by photorespiration, which is the light dependent evolution of CO₂ and uptake of O₂ (Canvin and Salon 1997). O₂ competes with CO₂ for fixation by the enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco). At low CO₂ concentrations, C₃ plants are severely limited photosynthetically. Conversely, C₃ plants display linear photosynthetic rate increases with increasing CO₂ concentration. C₄ plants avoid the photorespiration losses using biochemical and structural mechanisms which concentrate CO₂ in the presence of the Rubisco enzyme (Canvin and Salon 1997). As a result, C₃ plants show linear increases in photosynthetic rate to CO₂ levels of 1,000 to 1,200 ppm, at which point CO₂ can become toxic or cause increased stomatal closure. C₄ plants which employ CO₂ concentration techniques can survive at levels much lower than C₃ plants (to nearly 0 ppm). However, C₄ plants reach CO₂ saturation point at levels around 400 ppm, which is just above current atmospheric levels. C₃ plants will benefit from increasing atmospheric CO₂ levels in terms of increased dry matter production, yield, water-use efficiency and leaf area (Lawlor and Mitchell 1991). As an example, wheat grown at 700 ppm CO₂ had 30% more grain yield than did wheat grown at 350 ppm CO₂ (Thompson and Woodward 1994). Elevated CO₂ could also produce selection pressure against plants or genotypes of species which are poorly adapted to CO₂ increase, which may lead to genetic shifts within populations (Andalo *et al.* 2001).

Researchers at the Plant Environment Laboratory in Reading have conducted studies to determine the effects of environmental stresses associated with climate change

on soybean. Ferris *et al.* (1999) examined the main effects and interactions of ambient CO₂ (aCO₂) (360 ppm) versus elevated CO₂ (eCO₂) (700 ppm), ambient temperature (temperature outside glasshouse) versus elevated temperature (HT) (+15°C outside temperature) and water deficit (WD) on soybean (C₃) dry matter, harvest index, yield, seed number, pod number and individual seed mass.

Total plant dry weight increased an average of 37% in eCO₂ versus aCO₂ conditions. Harvest index, however, was relatively unaffected by the change in CO₂ level. Seed yield, seed number, pod number and individual seed mass were all significantly increased under eCO₂ conditions. Seed yield increased an average of 32% over all eCO₂ treatments compared to aCO₂ treatments. HT and eCO₂ interacted to create a yield increase while HT and aCO₂ interacted to create a yield decrease. Individually, HT and WD resulted in decreased seed yield and seed number, but did not affect pod number or individual seed mass. HT + WD interacted to create a much larger decrease in the yield components than the combined individual effects of HT and WD.

Eighty-five percent of the variation in seed yield per plant between the treatments was explained by differences in seed number as opposed to differences in seed size. Seed size was increased by the HT and WD stress treatments, but pod and seed number were reduced. This response indicates that the stress treatments cause a sink limitation to final seed yield. However, depending on the timing of the stress treatment, the reduction in seed yield could have resulted from a source limitation imposed by the HT and WD conditions. Leaf photosynthetic rate during pod filling is another important factor affected by the environmental stress conditions imposed by climatic change and cannot be ignored as a factor affecting final seed yield in plants. This study provided useful

information for the modification of crop models and for the selection of crop cultivars adapted to climate change conditions.

The study also included the assessment of photosynthetic rate (A_{\max}), stomatal conductance (g_s), transpiration rate, instantaneous water use efficiency (IWUE), leaf water potential and leaf stomate potential under climate change (Ferris *et al.* 1998). It was found that the short-term stresses reduced A_{\max} at either aCO₂ or eCO₂. Associated with the decreased A_{\max} was a decreased leaf water potential. More specifically, the response of plants in both CO₂ conditions to HT was a recovery of A_{\max} to pre-stress levels. However, after WD stress, plants at eCO₂ fully recovered to pre-stress A_{\max} levels, while plants at aCO₂ could not recover. The authors hypothesized that this may be a result of the decreased water potential of the plants at aCO₂ after the WD episode. Additionally, plants at eCO₂ would have been able to reduce g_s during the WD episode thereby slowing transpiration rate during the stress period. They further hypothesize that under eCO₂ conditions of future climates, short-term WD episodes may be less detrimental to plant growth than under present conditions.

The effect of the interaction between temperature and CO₂ on plant growth is extremely complex. Morrison and Lawlor (1999) warn that the response of plant development, growth and biomass accumulation to increased CO₂ concentration at different temperatures cannot be estimated by analysis of photosynthetic processes alone. They contend that different ecosystems, genotypes and acclimatization state make it impossible to generalize about the interaction of temperature and CO₂. Additionally, carbon balance, meristem initiation and expansion rates, senescence, fertilization,

carbohydrate storage and mobilization, water status, stage of growth cycle and weather conditions would significantly interact with temperature and CO₂ to alter plant growth.

2.4 Fatty acid biosynthesis in plant leaves and seed

2.4.1 Introduction

The characteristics of the lipids of plant leaves and seed are vital in determining the adaptability of the plant to various growth environments and the usefulness of the plant as a crop species. The lipids of leaves are associated with the membranes of the various cell types found within the leaf. However, the majority (~75%) of leaf lipids are found within the membranes of chloroplasts (Browse and Somerville 1994). The lipids found in the chloroplast thylakoid membranes include monogalactosyldiacylglycerol [MGD(51%)], digalactosyldiacylglycerol [DGD(26%)], sulphoquinovosyldiacylglycerol [SL(7%)], phosphatidylcholine [PC(3%)], phosphatidylinositol [PI(1%)] and phosphatidylglycerol [PG(9%)] (Harwood 1998a). The predominant fatty acid species found within these lipids are palmitic acid (C16:0) and linolenic acid (C18:3), which are found in all membrane lipids, hexadecatrienoic acid (C16:3), found in the lipids of some species, and to a smaller extent, palmitoleic acid (C16:1), hexadecadienoic acid (C16:2), oleic acid (C18:1) and linoleic acid (C18:2) (Figure 2.2).

Two major pathways exist by which the acyl-ACP products of plastid fatty acid synthesis are utilized in plant cells for the biosynthesis of glycerolipids and associated production of polyunsaturated fatty acids. Fatty acids synthesized by *de novo* synthesis in plastids may be used directly for production of chloroplast lipids by a pathway in the plastid (the "prokaryotic pathway") or may be exported to the cytoplasm as free fatty

acids that are first converted to CoA esters, which are then incorporated into lipids in the endoplasmic reticulum by an independent set of acyltransferases (the “eukaryotic pathway”) (Browse and Somerville 1994). The essential features of the two pathways are depicted in Figure 2.2

Two categories of plants exist based on relative contents of C16:3 and C18:3, “C16:3” plants contain significant levels of C16:3 and C18:3 within their glycosylglycerides, while “C18:3” plants contain no C16:3 and high levels of C18:3 within their membrane lipids (Harwood 1998a).

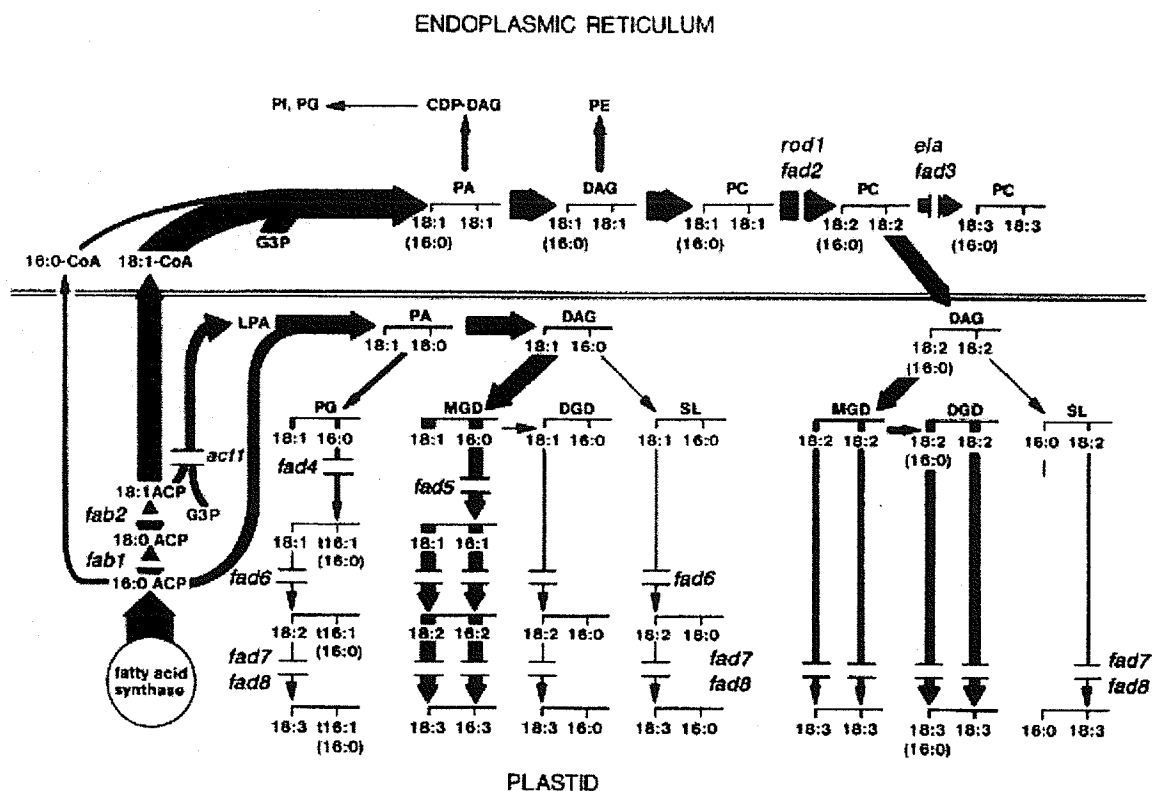


Figure 2.2: Fatty acid biosynthetic pathways of *Arabidopsis* leaves. Abbreviations: PA, phosphatidic acid; PG, phosphatidylglycerol; DAG, diacylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SL, sulpholipid; MGD, monogalactosyl diacylglycerol; DGD, digalactosyl diacylglycerol. (Browse and Somerville 1994).

Seed lipids are primarily composed of triacylglycerol (TAG) species, which can contain any combination of 3 acyl types (Figure 2.3). Temperate oilseed species produce

oil containing primarily the C18 fatty acids, while tropical oils are comprised of high levels of shorter chain fatty acids (C8:0 – C16:0) with lower levels of the C18 fatty acids.

De novo fatty acid biosynthesis takes place in the plastid (Figure 2.3) and involves the addition of 2-carbon units to a growing acyl chain bound to acyl carrier protein (ACP). A series of ketoacyl-ACP synthases (KAS) with specialized substrate specificities catalyze the elongation of the fatty acyl chain (Knutson and Knauf 1998). In *Brassica*, as in most seeds, the major end product of *de novo* fatty acid synthesis is stearic acid (C18:0). C18:0 is then desaturated in the plastid by the soluble stearyl-ACP desaturase to produce C18:1, which is cleaved from the ACP carrier by an oleoyl-ACP thioesterase (TE). C18:1 is then transported to the cytoplasm and esterified to CoA to become available for further elongation or incorporation into glycerolipids and storage TAG. Seed storage TAG are synthesized via the Kennedy pathway (Knutson and Knauf 1998) in the ER from acyl-CoA and glycerol-3-P in a series of reactions catalyzed by glycerol-3-P acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), phosphatidic acid phosphatase (PAP) and diacylglycerol acyltransferase (DAGAT). Other modifications that the C18:1 may undergo in the cytoplasm include incorporation into the *sn*-2 position of PC and further desaturation to C18:2 and C18:3 by the membrane-associated Δ -12 and Δ -15 desaturases. The C18:1-CoA molecules can also serve as a substrate for a microsomal fatty acid elongation (FAE) system (Knutson and Knauf 1998). Although distinct from the soluble FAS system in plastids, the FAE system is believed to consist of a four-step mechanism analogous to that of FAS. The first step, involving condensation of malonyl-CoA with a long chain acyl-CoA, is catalyzed by a β -ketoacyl-CoA synthase (KCS) (Knutson and Knauf 1998).

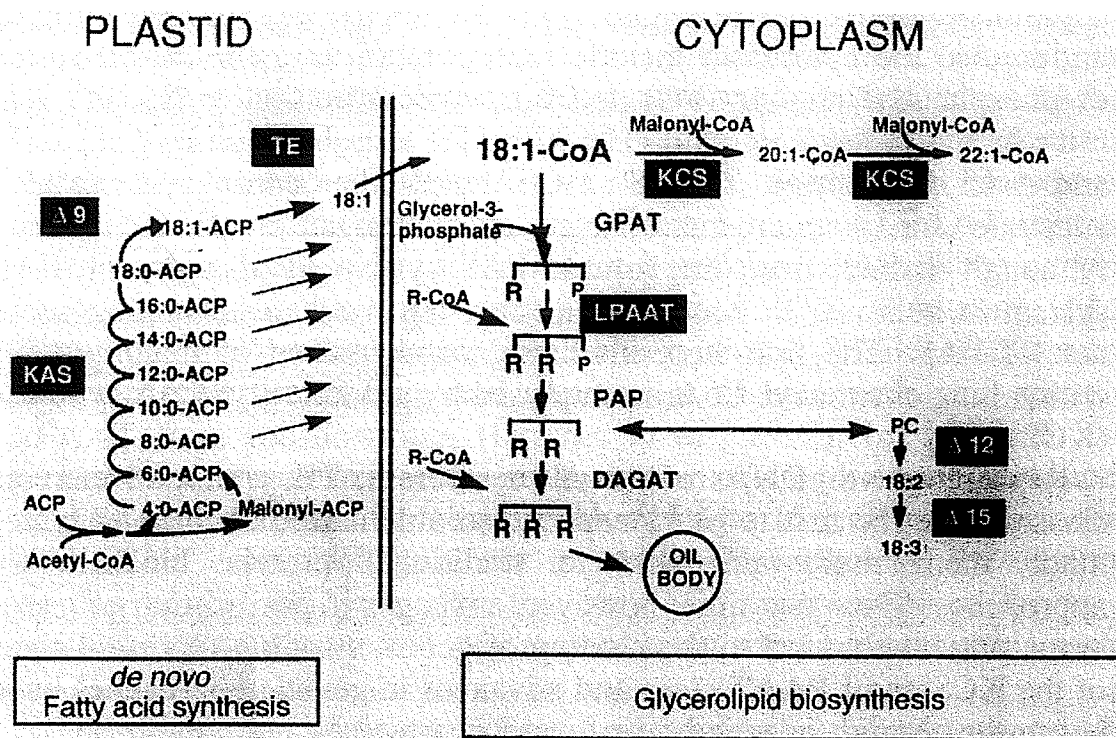


Figure 2.3: Fatty acid biosynthesis in oilseeds. Abbreviations: ACP, acyl carrier protein; DAGAT, diacylglycerol acyltransferase; GPAT, glycerol-3-P acyltransferase; KAS, ketoacyl-ACP synthase; KCS, ketoacyl-CoA synthase; LPAAT, lysophosphatidic acid acyltransferase; PAP, phosphatidic acid phosphatase; PC, phosphatidyl choline; TE, acyl-ACP thioesterase; Δ9, Δ9 desaturase; Δ12, Δ12 desaturase; Δ15, Δ15 desaturase. (Knutzon and Knauf 1998).

Very different lipid species and fatty acid compositions are produced in leaf and seed lipid biosynthesis, despite being based on the same plastid-based biosynthetic pathways (Somerville *et al.* 2000). However, little is known about the method utilized by plants to keep membrane and storage fatty acids distinct. Subcellular compartmentalization and differential enzymes within separate compartments is one explanation, but there is no significant evidence to substantiate it.

2.4.2 Effects of climate change on leaf fatty acid biosynthesis

Temperature effects on leaf fatty acid biosynthesis

The lipid biosynthesis of plants may be affected by temperature in several ways. The rate of growth and development of a plant may be altered by changes in temperature because of its influence on metabolic activities. Changes in the rate of growth and development of a plant will alter the rate of lipid biosynthesis and resulting fatty acid profile of the plant tissue. Additionally, extreme temperature results in stress response and lipid biosynthesis may be altered as an adaptation strategy (heat or chilling/freezing stress).

Membrane lipids of leaves respond to temperature stress in a variety of ways (Harwood 1998b). Changes in cellular membrane fluidity or alteration of subcellular morphology can provide sufficient adaptation to allow the plant to survive stress events. The molecular species which comprise the membrane lipids may be altered to remodel the lipid structure and to alter membrane fluidity. The fatty acids may be altered in chain length as longer fatty acid chains create lipids which resist phase changes at elevated temperatures. The saturation may also be altered because increased saturation provides the same resistance to elevated temperatures as increased chain length. Degree of saturation can be altered in a number of ways: increasing the turnover of molecular species, altering desaturase substrate supply, changing the gene expression of desaturase enzymes, changing the desaturase enzyme stability or by activating/deactivating preexisting enzymes. The entire proportion of lipid classes comprising the membrane may be altered to provide a membrane structure more suited to the prevailing

temperature. Finally, a change in the protein to lipid ratio in the membrane can also alter membrane fluidity.

The effect of temperature on lipid biosynthesis and stress tolerance of leaf lipid mutations has been studied in several crop species. The fatty acid profile of leaf lipids in peas (*Pisum sativum* L.) was higher in saturates in summer compared to winter conditions (Chapman *et al.* 1983). The leaves of corn (*Zea mays*) grown at 30°C contained approximately 30% unsaturated fatty acids while the leaves of plants grown at 20°C contained 60% unsaturated fatty acids (Hawke *et al.* 1974). A soybean mutant with high C16:0, low C16:1 and C18:3 displayed a higher temperature of 50% photosynthetic inactivation than wild type plants (Alfonso *et al.* 2001). *B. napus* leaves were found to have a higher rate of desaturation in the cytosolic and chloroplastic lipid biosynthetic pathways at 5°C than at higher temperatures (Williams *et al.* 1992). The study also concluded that this rapid desaturation at low temperatures was supplementary to a basal level of desaturation and that C16 and C18 fatty acids were desaturated by different pathways.

Research on the effect of temperature on leaf membrane lipid biosynthesis has been done in the model plant species *A. thaliana*. Many mutation lines have been created in *Arabidopsis* including lines with elevated C18:0 (Lightner *et al.* 1994), elevated C16:0 (Wu *et al.* 1994) and elevated C16:1 and C18:1 (Table 2.1) in the leaf lipids. These mutations have been associated with decreased tolerance of chilling temperatures (5°C) due to the increased saturation state. Many of the mutations have resulted in deficiencies in fatty acid desaturases (FAD). These desaturases are responsible for the desaturation of C16 and C18 fatty acids by inserting double bonds in the fatty acid carbon chain.

Chilling sensitivity has also been examined in *Arabidopsis* mutant lines which possess low levels of polyunsaturated fatty acids in their leaf membrane lipids. Mutant lines containing elevated levels of C16:2 and C18:2 and decreased levels of C16:3 and C18:3, due to a mutation in the *FAD7* gene, have been identified as being more sensitive to chilling (Somerville *et al.* 2000). Tobacco lines engineered to express the *FAD7* gene displayed elevated C16:3 and C18:3 levels and displayed decreased chilling tolerance (Kodama *et al.* 1994).

Table 2.1: Fatty acid compositions of leaf lipids of *Arabidopsis thaliana* altered biosynthetic pathway mutants.

Mutant Line	Fatty Acid								
	16:0	16:1c	16:1t	16:2	16:3	18:0	18:1	18:2	18:3
Wild Type	15	tr	3	tr	14	1	3	14	48
<i>fab1</i>	23	1	4	tr	17	1	3	11	39
<i>fab2</i>	14	tr	2	tr	6	14	3	18	42
<i>act1</i>	10	tr	2	tr	1	1	8	23	54
<i>fad4</i>	18	tr	0	tr	12	1	3	19	47
<i>fad5</i>	24	1	3	tr	tr	1	3	17	50
<i>fad6</i>	14	11	4	tr	tr	1	16	17	37
<i>fad7^a</i>	17	3	3	6	2	1	9	39	19
<i>fad2</i>	12	1	3	tr	17	1	21	4	41
<i>fad3</i>	15	tr	3	tr	16	1	3	21	41
Multiple mutants									
<i>fad7fad8^b</i>	15	1	2	10	0	1	4	49	17
<i>fad2fad6</i>	11	16	4	1	0	1	60	5^c	0
<i>fad3fad7fad8</i>	15	1	2	9	0	1	6	65	0

Data are in mole %

^aPlants grown at 28°C

^bThe fatty acid composition of *fad8* alone is indistinguishable from wild type

^cProbably the $\Delta 9$, $\Delta 15$ isomer (Browse and Somerville 1994)

Arabidopsis mutants have been isolated which could be useful in understanding plant species adapted to climate change conditions. These mutants were created by seed mutagenesis using ethylmethansulphonate (EMS) (Feldman *et al.* 1994). Murakami *et al.* (2000) examined the *Arabidopsis* double mutant *fad7fad8*, which lacks two chloroplast

localized ω -3 fatty acid desaturases. The chloroplast lipids contain small amounts of trienoic fatty acids (C16:3 & C18:3) and large amounts of dienoic fatty acids (C16:2 & C18:2). A relationship between trienoic fatty acid level and the thermal stability of the photosynthetic machinery of the chloroplast was established. Wild type and mutant plants grown at a cool temperature (15°C) and at a regular growth temperature (25°C) were similar in aerial tissue fresh weight. However, at high temperatures (35°C) the growth of the *fad7fad8* mutants was unaffected while the growth of the wild type plants was significantly reduced with leaf wilting.

Despite the effect of trienoic fatty acid level on the growth of plants at high temperatures, the effect on growth at temperatures lower than 10°C appears to be minimal. The *Arabidopsis* mutant *FAD7* overexpresses the ω -3 desaturase gene resulting in elevated trienoic fatty acid levels. These elevated trienoic levels did not appear to aggravate chilling-induced damage in leaf tissue. The *Arabidopsis* mutant *fad3fad7fad8* (*fad3* mutants lack ω -3 desaturase in the endoplasmic reticulum) lacks trienoic acids in all membranes, but can grow at temperatures as low as 6°C with minor reduction in photosynthetic capacity.

Murakami *et al.* (2000) hypothesized that increased levels of saturated fatty acids in membrane lipids such as monogalactosyldiacylglycerol (MGD), creates thermal stability in the membrane. Theoretically, these lipids phase-separate into nonbilayer structures much more slowly at high temperatures when there is a high saturated fatty acid level in the membrane lipids. Thus, reducing fatty acids of lower saturation might increase the resistance of plants to high temperatures. The *fad7fad8* mutant is a useful

model to use in the study of this trienoic fatty acid level interaction with high temperatures associated with climate change.

CO₂ effects on leaf fatty acid biosynthesis

While the effects of elevated atmospheric CO₂ levels have been studied in terms of plant growth and development, little is known about the potential effects increased CO₂ levels on lipid metabolism in plants. Some research has been done in winter wheat (*Triticum aestivum* cv. Hereward) to determine the effects of elevated CO₂ on various physiological components of the plant and on lipid metabolism. The total lipids of the first leaves of 7-d-old wheat plants grown at either 350 or 650 ppm CO₂ levels were examined for changes in lipid class quantity (Robertson *et al.* 1995). Total lipid quantities decreased at the elevated CO₂ level due to a large drop in the nonpolar fraction of leaf lipids. Conversely, phosphatidylcholine (PC) and phosphatidylglycerol (PG) amounts increased in the plants subjected to elevated CO₂ conditions. As well, diphosphatidylglycerol, a lipid uniquely found in mitochondrial tissue, increased by a factor of six as a result of the increased CO₂ treatment, which reflects the rapid increase in the number of mitochondria.

Wheat was grown under the same CO₂ levels as in Robertson *et al.* (1995) and changes in lipid classes and fatty acid profile of total leaf lipids were examined including a separate measurement of the basal portion of the leaf. The leaves of young wheat plants showed significant increases in diacylglycerol (DAG) and PC and significant decreases in waxes/hydrocarbons and pigments/non-polar lipids at elevated CO₂ levels (Williams *et al.* 1998a). Basal leaf segments additionally showed a significant decrease in phosphatidylethanolamine (PE) and significantly lower levels of total leaf lipid content

than the entire primary leaf. Significant, but complex changes were noted in the fatty acid composition of DAG, PC and PE lipid classes with exposure to elevated CO₂ levels, with the most significant changes occurring in the unsaturated C18 fatty acids.

A similar study was performed on mature wheat plants, 35 days after sowing (Williams *et al.* 1998b). Significantly lower levels of lipid were found in the basal segment of the leaf in the MGD, PG and total leaf lipid fractions than the whole primary leaf sample. Significant increases were noted in PC lipid quantities under elevated CO₂ conditions. More significant changes were noted in lipid classes in the fifth leaves of the wheat plants with significant decreases noted in several lipid classes at elevated CO₂. As with the young wheat leaf study, the mature wheat leaves displayed significant fatty acid profile changes in several of the lipid classes at elevated CO₂. Notably, the basal leaf segment displayed less change in fatty acid profile with the change in CO₂ concentration than did the entire leaf sample.

2.4.3 Effects of temperature and CO₂ on seed fatty acid biosynthesis

The effects of temperature on seed lipid fatty acid biosynthesis have been studied in a number of different crop species. A flax (*Linum usitatissimum*) cultivar, "Zero", low in C18:3 (2%), was grown under temperature regimes of 24°C days and 19°C nights and 15°C days and 10°C nights (Tonnet and Green 1987). The total lipid extract of the elevated temperature treatment showed significantly higher levels of C16:0, C18:0 and C18:1 than the lower temperature treatment. Also, the levels of C18:2 and C18:3 were significantly higher in the lower temperature treatment than that of the elevated temperature treatment. This data suggests the decreased activity of the C18:1 desaturase under elevated temperatures creates a more saturated fatty acid profile.

In sunflower (*Helianthus annuus* L.) the temperature during seed development strongly influences the profile of the seed lipids (Sarmiento *et al.* 1998). The seed from plants 16 days after flowering was analyzed after day/night temperature conditions of 12/12°C, 20/10°C, 15/18°C and 24/24°C. The level of C18:0 and C18:1 increased and C18:2 decreased with higher temperature. This indicated that the C18:1 desaturase was activated or synthesized in low temperature environments to create a more unsaturated fatty acid profile and reversibly inhibited at high temperatures to decrease desaturation.

Similar results were reported in oilseed rape cultivars grown in both field and controlled environment studies (Deng and Scarth 1998). In the field study, seed which developed under higher temperatures contained higher levels of saturated (C16:0 and C18:0) and C18:1 fatty acids than did seed which developed under lower temperatures. The seed developed under the higher temperature environment also contained lower levels of C18:3 compared to seed developed in the low temperature environment. The controlled environment study showed similar results, with the plants subjected to day/night temperatures of 30/25°C having higher levels of saturated fatty acids and a lower level of C18:3 than in seed in temperature regimes of 25/20°C and 15/10°C. Again, this indicates a decreased level of desaturation at elevated temperatures, resulting in a more saturated fatty acid profile.

Analysis of *Arabidopsis* seed lipid fatty acid mutants has lagged behind that of leaf lipid fatty acid mutant analysis as oilseed research groups have only more recently utilized the model plant. However, seed lipid fatty acid mutant lines exist in *Arabidopsis* with a variety of phenotypes including deficiencies in C18:1 desaturation, C18:2 desaturation, C18 elongation, elevated C16:0 levels, elevated C18:0 levels, elevated

>C18 levels and elevated eicosenoic acid (C20:1) and total FA content (James Jr. and Dooner 1990). The fatty acid profiles of the seed of these mutants sometimes reflect the fatty acid profiles of the leaf material, presumably because there are common desaturases mutated in both pathways. However, the degree of difference between mutant and wild type fatty acid profiles often differs between leaf and seed (Lemieux *et al.* 1990).

Very little research has been done to examine the effect of CO₂ changes on the TAG content of seeds and grains. It is largely unknown how the oilseed species will respond to the rising CO₂ levels associated with climate change. However, some work has been done to examine the influence of the greenhouse effect on the grain lipids of wheat (*Triticum aestivum* L.). Williams *et al.* (1994, 1995) studied the lipid composition of non-starch and starch lipids in response to growth regimes of combinations of two CO₂ levels (350 and 700 ppm), two temperatures (ambient and 4°C above ambient) and two nitrogen fertilizer levels (high and low). Temperature and CO₂ levels were found to be opposite in their effects on total seed lipid accumulation. Elevated temperature tended to increase the rate of physiological development of the wheat, thus limiting the time for seed storage lipid accumulation and resulted in decreased levels of accumulated lipids of all lipid classes. Elevated CO₂ conditions tended to increase the rate of growth of the wheat plants and to extend the development time of seed storage lipid, resulting in increased amounts of total grain lipids for both starch and non-starch lipids. Additionally, elevated temperature was found to reduce the total non-starch lipids while elevated CO₂ levels created more complex changes in the various lipid classes.

Changes in the quality of the wheat grain lipids were observed as well in response to the different growth regimes. In terms of non-starch lipids, elevated temperature

caused a significant decrease in C18:1 and a significant increase in C18:2 in neutral lipids (primarily TAG). In the DGD fraction, elevated temperature caused significant decreases in C18:1 and C18:3 while causing a significant increase in C18:2. Elevated CO₂ caused a significant decrease in the C16:0 level of the same fraction. Elevated temperature caused a significant decrease in the C18:1 level in MGD fraction while causing a significant increase in C18:2. In the starch related lipids, lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE), elevated temperature caused a significant decrease in C18:1 level and a significant increase in C18:2 level. Elevated temperature also caused a significant decrease in C18:3 in LPC and in C18:0 in LPE (Williams *et al.* 1995).

2.5 Breeding objectives for *Brassica napus*

2.5.1 Introduction

Fatty acid composition of vegetable oil determines the functional and nutritional aspects of the oil. Oil processors and refiners can modify the oil, using hydrogenation, to meet specific end-use markets. However, it is preferable for the oil to be as close to the desired end quality as possible in order to minimize time, cost and impact on the environment. In order to maintain or increase the market share of canola oil, the oil quality must be maintained or improved to produce new specialty oils. Plant breeders have been able to modify the oil composition of canola seed through conventional breeding techniques as well as through biotechnological techniques such as cell culture or genetic engineering.

Brassica breeders utilize several conventional techniques to accomplish their specific goals. The most frequently used technique for self-pollinating species, such as *B. napus* and *B. juncea*, is backcrossing in combination with pedigree selection (Downey and Röbbelen 1989). *Brassica* cultivars possessing desirable traits are crossed to cultivars adapted to Canadian growing conditions. The progeny are crossed to the adapted cultivar for several generations and each generation is tested to select for lines possessing the desired trait. This process continues until the adapted cultivar is essentially recovered along with the desired trait (Downey and Röbbelen 1989).

In cross-pollinating species, such as *B. rapa*, recurrent selection is used to avoid problems with inbreeding. This technique involves growing a superior population of mixed genotypes followed by selection and intermating of superior genotypes to produce new improved character expression (Poehlman and Sleper 1995).

If germplasm with the desired traits cannot be located for use in conventional breeding techniques, new technology exists to create variability. Ionizing radiations, such as x-rays, and chemical mutagens, such as EMS, are used to create mutations in the DNA of a plant (Poehlman and Sleper 1995). These mutations are generally deleterious, however, some mutations are beneficial in creating a desirable new trait. Useful mutant lines may be grown out and crossed with adapted cultivars in a conventional breeding program.

Anther culture is routinely used to create variability in germplasm. Anthers are cultured on specific nutrient media to induce the growth of microspores into haploid plants. The microspores are usually exposed to a mutagen, such as EMS, to induce DNA mutation. The microspores are grown into plantlets and are subjected to a chromosome

doubling agent, such as colchicine. These plants are referred to as doubled haploids and can possess useful traits as only one set of chromosomes needs to be exposed to the mutagenic agent (Poehlman and Sleper 1995). Due to chromosome doubling, any induced mutation, including recessive mutations, will automatically be present in homozygous condition, which is particularly advantageous to the plant breeder.

A final technology, utilized by some plant breeders, is genetic engineering. Foreign DNA, coding for a trait of interest to the plant breeder, may be transferred to a recipient plant via a bacterial plasmid, a virus or other vector. The desired DNA is often incorporated into the bacterium *Agrobacterium tumefaciens*, which can infect a plant and transfer the desired DNA into the plant cell genome. These plant cells are cultured on nutrient media and grown into whole plants. Plants possessing the useful trait are incorporated into a breeding program for cultivar development. Genetic engineering is useful as the plant breeder is no longer limited to using species that are cross-fertile since the donor DNA may be from any species (Poehlman and Sleper 1995).

Crop plants face a wide variety of biotic and abiotic stresses, including diseases, insects, temperature, salinity and heavy metal concentration. In order to maintain high levels of production and good quality product, breeders have selected lines and developed germplasm which is tolerant of several types of stress.

2.5.2 Breeding for modified fatty acid composition

Low erucic acid

The first fatty acid modification in *Brassica* species that plant breeders undertook was to lower the level of C22:1 in the seed oil. It was understood that the nutritional value of the *Brassica* seed oil would be substantially increased if the C22:1 content could

be reduced to <5% of the total fatty acid content (Downey and Röbbelen 1989). Dr. Keith Downey at Agriculture Canada in Saskatoon and Dr. Baldur Stefansson at the University of Manitoba in Winnipeg located *B. napus* plants producing seed with essentially no C22:1. These lines were crossed with an adapted cultivar to produce the first low C22:1 cultivar, Oro, in 1968. In 1974, the first low C22:1, low glucosinolate cultivar, Tower, was released by Dr. Stefansson for production (Stefansson and Kondra 1975).

In *B. napus*, the amount of C22:1 was found to be controlled by the genetic makeup of the developing embryo and not that of the maternal parent. Two genes, with multiple alleles, combine additively to produce levels of C22:1 from essentially zero to >50% (Downey and Röbbelen 1989). These genes will be discussed further in the high erucic acid rapeseed (HEAR) breeding section.

Low linolenic acid

C18:3 is an essential fatty acid, meaning animals require the fatty acid, but cannot synthesize it. C18:3 is recognized as having a significant role in reducing the levels of plasma cholesterol (Eskin *et al.* 1996). However, for oil applications requiring stability, such as frying, the relatively high C18:3 content of canola requires that the oil be partially hydrogenated leading to the formation of *trans* fatty acids. These *trans* fatty acids are associated with detrimental serum cholesterol changes (Fitzpatrick and Scarth 1998).

The breeding objective to lower the C18:3 content of canola was designed to produce an oil with significantly improved stability under conditions of accelerated storage, levels of free fatty acids after frying and no changes in odor. Germplasm screening did not identify the required variation. Researchers at the University of

Göttingen used EMS induced seed mutagenesis to produce altered C18:2/C18:3 ratio in the variety Oro (Röbbelen *et al.* 1975). This mutation line (M11) was used as the source of the low C18:3 variation at the University of Manitoba to breed the world's first low C18:3 (<3%) canola variety, Stellar (Scarth *et al.* 1988). The low C18:3 cultivar trait was shown to be controlled by three unlinked loci (Somers *et al.* 1998). Two Δ -15 desaturases responsible for C18:3 production, encoded by *FAD3* genes, were located on two linkage groups using amplified fragment length polymorphism (AFLP) analysis (Somers *et al.* 1999).

High oleic acid

Human nutritional studies have shown that the "Mediterranean diet" has health benefits such as reduction in low-density lipoprotein cholesterol, reduction in the formation of atherosclerotic plaques, increased life expectancy and lowered rates of cardiovascular disease and breast cancer (Kiritsakis 1999). The "Mediterranean diet" is rich in the consumption of monounsaturated olive oil.

Several canola breeding programs have targeted higher C18:1 and reduced C18:3 levels (>86% C18:1, <7% C18:2, <2.5% C18:3) to mimic the olive oil profile. Using seed mutagenesis, followed by crossing and selection, *B. napus* lines were developed with >85% C18:1 and low levels of the polyunsaturated fatty acids (Wong *et al.* 1991). Using EMS mutation, the University of Göttingen has produced winter oilseed rape lines with over 90% C18:1 (Shierholt and Becker 1999). Seed specific inhibition of microsomal C18:1 and C18:2 desaturase genes has been used to produce transgenic *B. napus* lines. Plants were produced which contained over 80% C18:1 and single seeds of the first transgenic generation contained 89% C18:1 (Stoutjesdijk *et al.* 1999). The total

polyunsaturate (C18:2 + C18:3) content was reduced to 4% and the total saturated fatty acid content (C16:0 + C18:0 + arachidic acid (C20:0) + behenic acid (C22:0)) was similar to control lines.

Low saturated fatty acids

Current dietary recommendations are that 30% or less of total caloric intake be derived from fat and less than 10% should be from saturated fat. This is based on the link between saturated fat and elevated blood cholesterol and risk of cardiovascular (Eskin *et al.* 1996). While C18:0 is apparently neutral in its effect on cholesterol, lauric (C12:0), myristic (C14:0) and C16:0 are associated with increasing cholesterol levels (Eskin *et al.* 1996).

The total saturated fat content of canola oil must be less than 7.1% (or 1g in 14g of oil) in order to be labeled as low in saturated fatty acids. This low level of saturated fatty acids in canola oil has been key in the establishment of the dominant market share in Canada and the substantial market to the United States and other countries (Adolphe *et al.* 2002).

The lowering of the level of saturated fatty acids to less than 4% in canola oil is a long-term objective for plant breeders. Development of a low saturated fatty acid soybean (<5% C16:0) has begun in the United States and will challenge the status of canola oil as the lowest saturated fat vegetable oil (McVetty and Scarth 2002). The decline in Canadian production of *B. rapa*, which has a lower saturated fatty acid level than *B. napus*, has led to an increase in the overall saturate levels in canola oil as (DeClerq *et al.* 1997). Elevated temperatures during seed development produces higher total saturated fat levels in *B. napus* seed oil (Deng and Scarth 1998), with the result that

saturated fat levels in commercial production can rise above the 7% level (DeClerq *et al.* 1997).

Interspecific crosses amongst *Brassica* species and mutagenesis of *Brassica* species have been used to create low saturate germplasm. Doubled haploid lines from interspecific crosses have shown saturate levels as low as 5.4% in field trials (Raney *et al.* 1999). *B. rapa* doubled haploid lines have been developed using microspore mutagenesis at the Plant Biotechnology Institute in Saskatoon. Lines with less than 4% saturates are being crossed to *B. napus* lines to produce low saturate germplasm at the University of Manitoba (McVetty and Scarth 2002).

Higher saturated fatty acids

Vegetable oils containing elevated saturated fatty acid levels have specific applications in solid fat products. Use of these oils reduces the need for extensive hydrogenation in the production of shortening and margarine. The development of canola lines with higher saturated fat would reduce the dependence on animal fats and high saturated fatty acid tropical oils in these applications (Del Vecchio 1996).

B. napus plants were transformed using the California Bay tree (*Umbellularia californica*) lauroyl C12:0- ACP TE gene. These plants produced between 55 and 60% C12:0 with very little decline in overall oil content (Voelker *et al.* 1996). However, production of C12:0 appears to be limited as the TAG produced was lacking any C12:0 at the *sn*-2 position as a result of the C18 fatty acid preference of the LPAAT, the enzyme responsible for *sn*-2 position acyltransferase reactions (Cao and Huang 1987). Laurical™ cultivars from Monsanto typically produce an oil profile containing 39% C12:0 and 46% saturated fatty acids (Del Vecchio 1996).

A TE gene and its seed specific promoter, isolated from *Cuphea lanceolata*, were used to transform the spring oilseed rape cultivar "Drakkar" (Rudolff and Wehling 1997). The resulting lines produced 12 to 14% C14:0, elevated C16:0 levels of 20% and a reduced level of C18:1. The expression of the altered fatty acid levels was found to be stable in field trials and no detrimental phenotypic effects were observed.

Elevated levels of C16:0 and C18:0 have been produced in transgenic lines transformed with TE genes from soybean (*Glycine max*) (Hitz *et al.* 1995), mangosteen (*Garcinia mangostana*) and *Cuphea lanceolata* (Rudolff *et al.* 1999).

High erucic acid

The traditional application of rapeseed oil in industrial applications, such as lubricants, was based on the high C22:1 level. In recent years, renewed interest in the industrial applications of rapeseed oil have led to programs designed to enhance the C22:1 level above the 45% normally encountered in oilseed rape germplasm. C22:1 may be used directly in some industrial applications or further processed into erucimide. High fluidity lubricants, slip agents, fuels, polymers, paints, inks, cosmetics and pharmaceuticals are some of the applications of C22:1 (Marcou 1996).

HEAR varieties, containing up to 55% C22:1, have been developed using pedigree selection techniques (McVetty 1999). Two genes, *E1* and *E2*, have been found to control the production of C22:1 in an additive manner (Thormann *et al.* 1996). The genes have been mapped and homology has been found between the genes and *FAE1*, which codes for a seed specific fatty acid elongase in *Arabidopsis thaliana* (Fourmann *et al.* 1998).

C22:1 content in the oil of rapeseed (*B. napus*) has not exceeded 66%. This limit results from the inability of *B. napus* to esterify C22:1 to the *sn*-2 position of seed TAG molecules (Taylor *et al.* 1992). Some genotypes of *B. oleracea* and *B. rapa* have been found which do esterify C22:1 to the *sn*-2 position. However, interspecific crosses and resynthesis of *B. napus* from its progenitors have not been successful in exceeding the 66% barrier.

Several breeding groups worldwide have the objective of developing HEAR, including the University of Manitoba (McVetty and Scarth 2002). The University of Manitoba released Hero, the first HEAR cultivar for production in Western Canada (Scarth *et al.* 1991), in 1991 and more recently MilleniUM03 was released with a C22:1 content of approximately 55% (McVetty *et al.* 2000). While these varieties are approaching the 60% C22:1 level, several other organizations are attempting to break the 66% barrier in *B. napus* to produce super high erucic acid rapeseed (SHEAR). Most groups are using interspecific hybridization to combine *B. oleracea* and *B. rapa* germplasm capable of esterifying C22:1 to all three positions on the glycerol molecule (McVetty and Scarth 2002). Efforts to break the 66% barrier through transgenic modification are underway at the Plant Biotechnology Institute in Saskatoon.

2.5.3 Stress resistance

As with all crop plants, stresses of various types cause significant yield loss and limit suitable production areas for *Brassica* species. In order to increase yield and increase production area, it is necessary to research the physiological and genetic basis of stress tolerance. A major problem for production of stress tolerant varieties is the

availability of useful germplasm. The application of biotechnology techniques has been useful in producing sources of resistance to various stresses.

Biotic stress accounts for severe yield loss in oilseed *Brassica* species. Pathologists, entomologists and agronomists conduct the basic research required to identify the germplasm which plant breeders can use in the development of varieties tolerant of these biotic stresses.

Several major diseases cause major yield loss in *B. napus*. These include blackleg (*Leptosphaeria maculans*), *Sclerotinia sclerotiorum*, white rust (*Albugo candida*), soil borne organisms (*Fusarium spp.*, *Rhizoctonia solani*), blackspot (*Alternaria spp.*), club root (*Plasmodiophora brassicae*), light leaf spot (*Pyrenopeziza brassicae*), bacterial rot (*Xanthomonas campestris*), aster yellows, phyllody (*mycoplasmas*), broomrape parasite (*Orobanche*) and several viral diseases (Downey and Röbbelen 1989). Of these, blackleg and *Sclerotinia* are the major research priorities in western Canada.

Insects including flea beetles, diamond back moth, midge, *Lepidoptora spp.*, aphids, root maggot, and nematodes (Bertha army worm) are primarily responsible for damage caused to *B. napus* in Western Canada.

Competition with weedy species for resources is another factor in yield reduction. Herbicide tolerant *B. napus* varieties have been bred for the purpose of providing a system for highly efficient control of weed species. Varieties have been produced which are resistant to the herbicides glyphosate, glufosinate, bromoxynil and imazidolinone, using traditional and biotechnology related breeding techniques.

Abiotic stress accounts for major yield loss in *B. napus*, and equally importantly, limits the suitable production zone for the crop. Plant physiologists perform the basic

research for the breeding of varieties which will be tolerant of abiotic stresses. In the northern temperate regions, tolerance of cold temperatures during early spring growth and maturation is important. Limited growing degree days and frost-free period are challenges for the adaptation of crops in northern production regions. After the seeding of *B. napus* in spring, night temperatures can reach freezing levels, severely affecting young plants. Somatic hybridization of *B. rapa* and *B. oleracea* has produced resynthesized *B. napus* plants with the objective of developing cold tolerant germplasm (Heath and Earle 1996). Other attempts have been made to develop cold tolerant germplasm including intertribal somatic hybridization of *B. rapa* with both *Barbarea stricta* and *B. vulgaris* (Oikarinen and Ryöppy 1992), and of *B. napus* with *B. vulgaris* (Fahleson *et al.* 1994). These attempts also produced sterile and male sterile lines which were difficult to evaluate and maintain. Research into cold acclimation has indicated that a cold-response pathway found in *Arabidopsis* is also found in *B. napus* and other flowering plants (Jaglo *et al.* 2001). The engineering of cold tolerant *B. napus* germplasm is a prospect in the near future.

Drought tolerance in *Brassica* crops is a major concern of the producers in western Canada. Alberta, Saskatchewan and Western Manitoba have light textured soils which are prone to water deficit during low rainfall years. Water stress causes a broad spectrum of plant responses depending on the nature of the stress. Quick onset and extended period of water stress causes injury, permanent wilting and plant death (Morillon and Lassalles 2002). A slow onset and less severe water stress may result in adaptive mechanisms such as metabolic pathway alterations or early flowering. The genes involved in the production of aquaporin (water channels in cell membranes) have

been discovered and their use in transgenic plant production may increase the tolerance of *B. napus* to drought conditions.

Hypoxic (O₂ limiting) and anoxic (O₂ elimination) soil conditions result from flooding of soil during prolonged heavy rain conditions. In western Canada, these conditions are more likely to occur in the heavy clay soils of eastern Manitoba and the Red River Valley. Very little research has been undertaken in *Brassica* crops to develop flooding tolerance.

Several crop production areas in Western Canada are affected by increased levels of salinity in the soil. Intense irrigation or naturally elevated salt levels in the soil create production areas which require growth of salt tolerant crops. Various methods are used to produce salinity tolerance in crops in order to expand the region of successful production and increase yields in marginal soil zones. Genetic engineering has been used to alter levels of the osmoprotectant, betaine, in order to minimize plant damage in saline conditions (Huang *et al.* 2000). Additionally, transgenic *B. napus* plants have been used to study the role of Na⁺/H⁺ antiporters in the exclusion of salt from the roots of the plant and the possibility of production of salinity tolerant *B. napus*.

Metallic pollutants can be present in natural and agricultural environments from sources such as the use of industrial and municipal wastes for fertilization, the combustion of fossil fuels and the use of various agro-chemicals. The pollutants are taken up by plant roots and can accumulate in the leaves and grain. Thus, these pollutants are potentially damaging to the plant and the consumer of the food or feed developed from the plant material (Baryla *et al.* 2001). Aluminum tolerance is required in many crop production regions and has been studied in transgenic *B. napus* plants

which overexpress an aluminum-induced mitochondrial manganese superoxide dismutase cDNA (Basu *et al.* 2001). *B. napus* plants have also been used to study the effects of cadmium on photosynthesis and growth (Baryla *et al.* 2001). Using studies of the physiological effects of heavy metals on plant growth, the identification of tolerant plant germplasm and the discovery of genes which provide tolerance mechanisms to plants, breeders should have the tools necessary to develop heavy metal tolerant *B. napus* varieties.

The conditions associated with climate change include elevated temperatures and atmospheric carbon dioxide levels. As discussed in previous sections, these factors will significantly affect all biosystems, including crop plants. The potential influence of these conditions on plant growth and development will be extremely important to understand when designing plant breeding goals for oilseed rape varieties. In order to ensure stable oil quality and yield, it should be determined how climate change will affect leaf biosynthetic pathways and processes which alter the fatty acid composition of seed oil. Once this is understood it may be possible to breed for traits which will provide increased adaptability and tolerance to the crop to changing environmental conditions.

3. THE EFFECT OF ELEVATED TEMPERATURE AND CO₂ ON THE LEAF AND SEED FATTY ACID COMPOSITION OF *Arabidopsis thaliana* AND *Brassica napus* FATTY ACID MUTANT LINES.

3.1 Introduction

Climate change, with rising temperatures and increasing levels of atmospheric CO₂, has the potential to significantly impact growth and development of plants (Environment Canada 2002). Lipid biosynthetic pathways in the leaves have been shown to respond to environmental influences (Harwood 1998b). Lipids play a vital role in the function of cell membranes of leaves (Somerville *et al.* 2000), thus the ability of a plant to alter the fatty acid makeup of these lipids is crucial to survival in changing abiotic conditions.

The properties of fatty acids are determined by length of the carbon chains and the number and position of double bonds, which then contribute to the function of the lipid and cell membrane into which they are incorporated (Harwood 1998b). Lipids containing fatty acids with short chain length and low numbers of double bonds have a higher melting point than do those containing fatty acids with longer chain length and high numbers of double bonds. It follows that membranes will be more stable under elevated temperatures when they contain shorter or more saturated fatty acids in their lipids (Browse and Somerville 1994). *Arabidopsis thaliana* leaf lipid mutants containing lower levels of trienoic fatty acids and elevated levels of dienoic fatty acids have increased tolerance of elevated temperatures compared to the wild-type (Murakami *et al.* 2000).

The effect of CO₂ levels on leaf lipids has been studied in wheat plants and results have been more varied and less easily understood than the temperature effects (Williams *et al.* 1998a). Additionally, the potential interaction between temperature and CO₂ on the lipid metabolism of leaves will be crucial to understand how plants will react to the two major components of climate change.

The effects of climate change on the seed lipid metabolism of oilseed crop plants is particularly important because of the value of these crops to agriculture and human nutrition. The effect of elevated temperature during reproductive development on seed lipid fatty acid profile has been studied in several crop species including wheat (Williams *et al.* 1995), sunflower (Sarmiento *et al.* 1998), soybean (Cheesebrough 1989) and oilseed rape (Deng and Scarth 1998). Elevated temperatures during seed development generally result in the seed oil containing higher levels of saturated fatty acids in a relatively more saturated profile (i.e. lower trienoic fatty acid levels). However, extreme weather events may occur during early vegetative growth and the effect on seed oil quality has not been determined.

It is known that leaf membrane lipids are affected by both elevated temperature and CO₂ levels (Williams *et al.* 1998b; Harwood 1998b), but whether these leaf lipid changes would result in a “carry-over” effect on seed lipid makeup is unknown. It has also been determined that elevated temperature is a detriment to seed yield and physical qualities such as seed size, while plants grown in elevated CO₂ environments generally yield higher and produce better quality seed (Ferris *et al.* 1999).

B. napus breeders have used techniques, such as mutation induction via ethyl methanesulfonate (EMS), to alter the enzymes involved in the biosynthetic pathways of

seed oil production in order to alter its fatty acid makeup. *B. napus* varieties have been developed that express seed oil profiles with low levels of saturated fatty acids, low linolenic acid (C18:3) levels, high oleic (C18:1) acid levels and high erucic acid (C22:1) levels. The biosynthetic pathways involved in seed oil production are similar to those involved in leaf lipid production despite leaf lipids being primarily diacylglycerols and seed lipids being predominantly triacylglycerols. Thus, with knowledge of how climate change will alter leaf and seed lipid biosynthesis, it will be possible for plant breeders to produce varieties which will be better adapted to climate change conditions.

In this study, a selection of *Arabidopsis thaliana* and *Brassica napus* mutant and wild type lines were chosen for controlled growth chamber trials. The effect of climate change on the fatty acid profile of the leaf lipids and seed oil was examined using ambient and elevated levels of temperature and CO₂.

3.2 Methods and Materials

3.2.1. Growth of *Arabidopsis thaliana* mutant lines

Seven lines of *Arabidopsis* were chosen for the experiment including wild-type lines of both Columbia and Landsberg *erecta* backgrounds. The other five lines all contained different fatty acid biosynthetic pathway mutations (Appendix A: Table 1). The *Arabidopsis* leaf mutants were chosen because they contain higher levels of dienoic fatty acids (C16:2 + C18:2) and lower levels of trienoic fatty acids (C16:3 + C18:3) than do wild type lines (i.e. a more saturated profile), which improves high temperature tolerance in the mutant lines (Murakami *et al.* 2000).

The *Arabidopsis* lines were seeded into moist Metromix in 3" pots. The pots were placed in a clear plastic covered box to maintain humidity for germination. Watering occurred from a shallow pool of water in each box through absorption into the pot of soil. The boxes were placed in a 5°C growth chamber for 5 days to enhance germination rate and ensure even germination. The boxes were then moved to a 20°C growth chamber until the 2-leaf stage (14 days after seeding [DAS]) when they were moved into the treatment chamber.

The treatment chamber (Conviron Model CMP4030) was specially designed to maintain CO₂ and temperature at set levels, controlled by a touch-screen computer. The chamber also maintained light ($625 \mu\text{mol m}^{-2} \text{s}^{-1}$) levels with incandescent and fluorescent bulbs as well as relative humidity (50%). Four treatments were examined using ambient and elevated temperatures (20/15°C and 30/25°C, respectively) and ambient and elevated levels of CO₂ (360 and 720 ppm, respectively). The four treatments were comprised of ambient CO₂ (aCO₂) + ambient temperature (aT), aCO₂ + elevated temperature (eT), elevated CO₂ (eCO₂) + aT, and eCO₂ + eT treatments. The chamber settings for temperature and CO₂ levels were confirmed, mid-way through each treatment, using a hand-held carbon dioxide and temperature monitor (Telaire, Model 7001 – Spectrum Technologies).

Each of the four treatments was replicated for a total of 8 growth chamber runs. Each of the 7 *Arabidopsis* lines was replicated 10 times per treatment for a total of 70 plants per experiment. The plants were placed in random positions within replicates in the chamber at the 2-leaf stage (14 DAS) and were removed from the chamber just before bolting occurred (28 DAS). At the time of removal from the chamber the plants were

sampled (described below) and placed in the 20°C growth chamber where they remained until seed maturity. Harvesting of the plants occurred when the lowest pods were brown and mature. The plants were allowed to dry in paper envelopes and then were harvested by crushing the dried plants and screening the remains.

3.2.2. Growth of *Brassica napus* mutant lines

Twenty-four *Brassica napus* lines were chosen to include unique fatty acid profiles in the seed oil, with high and low saturated fatty acid (C16:0 + C18:0 + C20:0 + C22:0) levels, high oleic (C18:1)/ low linolenic acid (C18:3) and high erucic acid (C22:1) levels (Appendix A: Table 2).

The *B. napus* lines were seeded into moist Metromix in 6" pots. Two replicates of each line were grown for a total of 48 pots. The pots were placed in random locations within replicates in the 20°C growth chamber until the 2-leaf stage (14 DAS) when they were moved to the treatment chamber. Treatments were the same as described for the *Arabidopsis* lines. The *B. napus* plants remained in the treatment chamber until bolting was about to occur (6th leaf stage/28 DAS). At this time the leaves were sampled (see below) and the plants were moved back to the 20°C chamber to be grown to maturity. The plants were bagged during flowering to obtain selfed seed. Harvesting of the plant occurred by restricting watering when the lower pods were brown, then allowing the plants to dry and collecting the pods.

3.2.3. Chamber sampling procedure

The sampling method of leaf tissue was similar for *Arabidopsis* and *B. napus*, with some key differences. Two *Arabidopsis* plants were grown per pot to provide sufficient leaf tissue for sampling. Just prior to the bolting stage (6 leaf stage/28 DAS),

the four leaves that had emerged while the plant was in the treatment chamber were removed from the plant and frozen on dry ice to halt cellular processes. The four leaves were then placed in a vial in a -20°C freezer until lipid extraction. The other plant in the pot was grown to maturity in order to obtain a seed sample for fatty acid analysis to compare to the leaf fatty acid profiles.

One *B. napus* plant was grown per pot. Just prior to the bolting stage (28 DAS), a hole punch was used to punch a single hole in the five leaves that had emerged while the plant was in the treatment chamber. The samples were frozen on dry ice to stop cellular processes and were placed in vials to be stored in the -20°C freezer until lipid extraction. The plant was then moved to the 20°C growth chamber until maturity when a seed sample was obtained for fatty acid analysis.

3.2.4. *Arabidopsis* and *B. napus* leaf lipid extractions

Frozen leaf samples were placed in a freeze dryer (Labonco Freeze Dryer 8) to remove all water from the sample. Leaf samples remained in the freeze dryer for 48 h or until completely dry. All extraction procedures took place within the original sample vial. Total leaf lipids were extracted as chloroplast membrane lipids comprise the majority of total leaf lipids (Browse and Somerville 1994) and thus give a sufficiently accurate measurement of chloroplast membrane lipid fatty acid profile. To extract the lipids from the leaf material, 500µl of heptane was added to the dried samples. Using a glass rod, the samples were crushed into very fine pieces in the heptane to facilitate more efficient lipid extraction. The lipid-solvent mixture in capped vials was allowed to stand overnight (8 h) to allow extraction to occur. To this mixture, 500µl of 0.4 N sodium methoxide in methanol was added to extract the more polar lipids and to trans-methylate

the fatty acids. The capped vials were again allowed to stand overnight (8 h) to allow sufficient fatty acid methyl ester (FAME) formation to occur. For each incubation period, the vials were placed in the fridge to ensure minimal loss due to evaporation.

The top layer of the resulting two-layer sample was removed by Pasteur pipette into 200 μl sample vials for gas chromatograph injection and analysis.

3.2.5. Gas chromatograph procedure for leaf fatty acid analysis

Gas chromatography was performed using a Varian (model 3400 GC) chromatograph equipped with a flame ionization detector (280°C). A 15 m (0.25 mm interior diameter, 0.25 μm film thickness) J & W Scientific (model DB225) column was used for all analysis. Helium carrier gas was used. The GC was equipped with an isothermal injector which was set at an initial temperature of 240°C. The initial temperature of the GC column was 160°C, which was held at the start of the program for 2.00 min. The column was then heated at a rate of 5°C min⁻¹ to a temperature of 200°C. The rate of increase then moved to 20°C min⁻¹ to heat to a temperature of 230°C which was held for 2.50 min. The total program lasted 14.00 min., however the integration (Star Chromatography Workstation, version 4.5) from 0.0634 to 4.6587 min. and from 9.7557 to 13.9356 min. was inhibited to eliminate unwanted peaks (e.g. leaf surface waxes) from the analysis. The GC was also equipped with an autosampler (model 8200), which allowed sampling at a 5 μl sec⁻¹ uptake speed and an injection rate of 10 μl sec⁻¹. The injection volume was set at 4.0 μl .

3.2.6. *Arabidopsis* and *B. napus* seed lipid extractions

Extraction methodology for both types of seed was identical. Approximately 25-30 mg of seed was obtained from harvested seed samples. The seed was crushed using a

hammer and the piston and cylinder from a Carver Press and placed in a 10 mL volumetric flask. The extraction was performed using 5 mL of heptane, which was added to the flask and left overnight (8 h). The lipid-solvent mixture was transferred to a clean 10 mL volumetric flask and 500 μ L of 0.4 N sodium methoxide in methanol was added to the solution to form fatty acid methyl esters (FAME). The solution was again left overnight (8 h) to allow for sufficient trans-methylation to occur. From the flask 1 mL aliquots were removed by Pasteur pipette from the top layer of the two-layer solution and placed in the GC sample vial for analysis.

3.2.7. Gas chromatograph procedure for seed fatty acid analysis

Gas chromatography was performed using a Varian (model 3400 GC) chromatograph equipped with a flame ionization detector (280°C). A 15 m (0.25 mm interior diameter, 0.25 μ m film thickness) J & W Scientific (model DB225) column was used for all analysis. Helium carrier gas was used. The GC was equipped with an isothermal injector, which was set at an initial temperature of 240°C. The initial temperature of the GC column was 160°C, which was held at the start of the program for 2.00 min. The column was then heated at a rate of 5°C min⁻¹ to a temperature of 200°C. The rate of increase then moved to 20°C min⁻¹ to a temperature of 230°C, which was held for 2.50 min. The initial column temperature was 190°C, which increased at a rate of 10°C min⁻¹ to a temperature of 220°C. The column temperature was then increased at a rate of 20°C min⁻¹ to a temperature of 240°C and was held for 3.50 min. Thus, the total program time was 7.50 min (Star Chromatography Workstation, version 4.5). The GC was also equipped with an autosampler (model 8200), which allowed sampling at a 5 μ L

sec⁻¹ uptake speed and an injection rate of 10µl sec⁻¹. The injection volume was set at 4.0µl.

3.2.8. Statistical analysis

Analysis of variance (ANOVA) statistical analysis was performed on the fatty acid profile data using Agrobase™ (Agronomix Software Inc. 1999) statistical software. The *Arabidopsis* leaf analysis was performed on the first seven of the ten replicates of each line from each treatment to ensure a balanced data set. The *Arabidopsis* seed was bulked from 5 plants to provide sufficient seed for GC analysis. Thus, two seed samples were obtained for each *Arabidopsis* line from each chamber run. All *B. napus* leaf and seed samples were analyzed individually, without selecting or bulking samples.

For data analysis, fatty acids were grouped into categories of total saturated fatty acids (xx:0), total monounsaturated fatty acids (xx:1), total dienoic fatty acids (xx:2) and total trienoic fatty acids (xx:3). These factors along with a ratio of saturated fatty acids to unsaturated fatty acids (sat./unsat.) and trienoic fatty acids to dienoic fatty acids (xx:3/xx:2) were analyzed. In addition, the number of double bonds found in the average fatty acid (ADB) in the profile was calculated by weighting the profile as follows: $[(\% \text{ xx:0}) \times 0] + [(\% \text{ xx:1}) \times 1] + [(\% \text{ xx:2}) \times 2] + [(\% \text{ xx:3}) \times 3] / 100$. ADB reflects the saturation state of each fatty acid profile; with lower numbers representing a more saturated profile. The primary factors analyzed were temperature level, CO₂ level and entry for the growth room experiment. Interactions between the factors, including entry x temperature, entry x CO₂, temperature x CO₂ and entry x temperature x CO₂, were also analyzed.

3.3 Results and Discussion

3.3.1 *Arabidopsis* mutant lines leaf composition

The analysis of the *Arabidopsis* leaf lipids (Figure 3.1, Appendix B: Table 1) confirmed the fatty acid profiles previously described for the wild type and mutant lines (Appendix A: Table 1). The wild type lines of the Columbia background (N933) and of the Landsberg *erecta* background (NW20) showed high trienoic fatty acid levels (C16:3 & C18:3). N209 and CS3108 in the Columbia and Landsberg *erecta* backgrounds, respectively, are the *fad7* mutation lines. These lines contain a mutated *FAD7* gene (Browse *et al.* 1986) which alters the chloroplast localized ω -3 desaturase creating a deficiency in the trienoic fatty acids and an increased level of dienoic fatty acids (C16:2 & C18:2). Both *fad7* lines showed decreased levels of trienoic fatty acids with increased levels of dienoic fatty acids compared to the wild type lines of their respective backgrounds. The CS8036 line contains mutated *FAD7* and *FAD8* genes which both encode chloroplast localized ω -3 desaturases (Murakami *et al.* 2000). The only functioning ω -3 desaturase is the endoplasmic reticulum localized enzyme encoded by the *FAD4* gene. As a result, the *fad7fad8* double mutant produces lower levels of trienoic fatty acids than does the single *fad7* mutant (Murakami *et al.* 2000). In this fatty acid analysis, CS8036 produced less trienoic fatty acids than N209 and contained significantly higher levels of dienoic fatty acids than did the single *fad7* mutant.

The *fad4* mutation produces a deficient endoplasmic reticulum localized ω -3 desaturase (Browse *et al.* 1985), but because of the two chloroplast ω -3 desaturases, the fatty acid profiles of the leaves of this line appear essentially the same as the wild type. The N205 line produced a very similar profile to the N933 wild type line, illustrating the

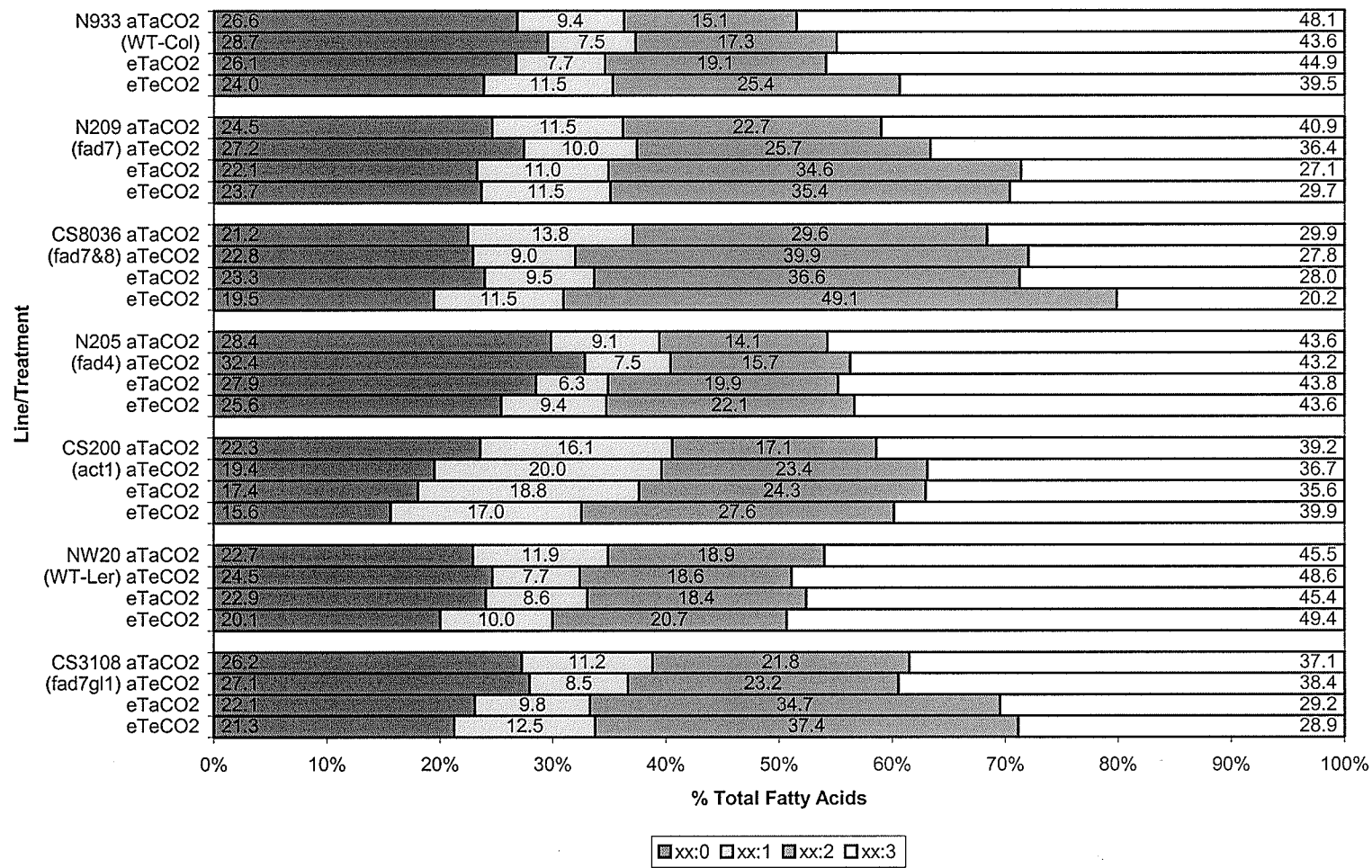


Figure 3.1: Leaf fatty acid profiles of seven *Arabidopsis* lines over four temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

alternate pathways redundancy in the biosynthetic pathway (Figure 3.1). CS200 is an *act1* mutant containing a deficient glycerol-3-phosphate-acyltransferase (Kunst *et al.* 1989), resulting in a large decrease in C16:3, a slight increase in C18:3, a small decrease in C16:0 and an increase in C18:2 (Figure 3.1) compared to the N933 wild type line.

The Columbia (N933) and Landsberg *erecta* (NW20) wild type lines and the *fad4* (N205) and *act1* (CS200) mutant lines had the highest overall ADB values averaged over all treatments at 1.82, 1.93, 1.78 and 1.82, respectively as a result of the high trienoic fatty acid levels and low dienoic fatty acid levels of these lines. The single *fad7* mutants in the Columbia (N209) and Landsberg *erecta* (CS3108) backgrounds as well as the double *fad7fad8* (CS8036) mutant had lower ADB values averaged over all treatments at 1.73, 1.74 and 1.72, respectively. These mutant lines were characterized by elevated dienoic and decreased trienoic fatty acid levels.

The response to the temperature and CO₂ treatments was analyzed using ANOVA of the ADB value for each line x treatment (Table 3.1). Temperature significantly affected mean ADB values of all the lines (P<0.0132). The effect of CO₂ on the mean ADB values over all lines was minimal (P<0.9211) in comparison to the line and temperature effects. Interestingly, the ADB values of the group were affected by the temperature by CO₂ interaction (P<0.0078) despite the non-significance of the CO₂ effect.

The lack of response to changing CO₂ levels in the fatty acid profile of the leaf lipids may be a result of the limiting light level in the growth chamber. The light level in the growth chamber was approximately 625 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is in the normal range for a growth chamber. However, the light level on a sunny day outside may approach

2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In order for plants to fully utilize elevated levels of CO_2 , they must have sufficient light to provide the energy required to maximize the reaction rate of their biosynthetic pathways. Without sufficient light, ATP and NADPH levels will not be high enough to significantly increase CO_2 fixation and reduction (Salisbury and Ross 1992). Conceivably, insufficient energy levels would restrict the enzymes involved in fatty acid biosynthesis, which require electron donation from the energy molecules generated by the light reactions of photosynthesis (Murphy and Piffanelli 1998). As a result, the impact of CO_2 levels on the fatty acid profile may not be evident.

The ANOVA of interactions (Table 3.1) also revealed that the individual lines responded differently to temperature ($P < 0.00005$) and CO_2 effects ($P < 0.0009$), but not to the interaction between temperature and CO_2 ($P < 0.2749$). Therefore, the lines were analyzed individually to determine the relative influence of temperature, CO_2 and their interaction on leaf fatty acid profiles.

Table 3.1: ANOVA analysis summary of number of double bonds in an average fatty acid in *Arabidopsis* leaf fatty acids over temperature and CO_2 treatments.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	391	4.978			
Entry	6	1.772	0.295	40.75	0.0000
Temperature	1	0.045	0.045	6.20	0.0132
CO_2	1	0.000	0.000	0.01	0.9211
Temp x CO_2	1	0.052	0.052	7.16	0.0078
Entry x Temp	6	0.246	0.041	5.66	0.0000
Entry x CO_2	6	0.169	0.028	3.90	0.0009
EntxTempx CO_2	6	0.055	0.009	1.26	0.2749
Residual	364	2.638	0.007		

ANOVA analysis identified the influence of temperature, CO_2 and their interaction in the response of each line to the treatments (Table 3.2). (See Appendix B: Tables 2-8 for ANOVA summary table for each line). Based on probability values

(Table 3.2), temperature affected the ADB value of lines N209, N205, CS200 and NW20 significantly (approximately $P < 0.01$). CO_2 affected the ADB values of N933, CS200 and NW20, but to a lesser extent ($P < 0.05$) than temperature. Also, the temperature by CO_2 interaction was weak among most lines with significant effects in N209 and CS200 lines at the $P < 0.05$ level.

As shown by the ADB value analysis the *fad7* and *fad7fad8* mutant lines were less affected by the treatments than were the other lines. N209 was weakly affected by temperature and the temperature by CO_2 interaction (likely related to the elevated aTaCO_2 ADB value) and CS3108 was not significantly affected by the treatments (Table 3.2, Appendix B: Tables 2-8). The double mutant CS8036 was not significantly affected by the treatments, indicating the stability of the *fad7* and *fad7fad8* mutant lines under different climate conditions.

Table 3.2: Summary of F-values from ANOVA analysis of each *Arabidopsis* line ADB value individually for temperature, CO_2 and temperature x CO_2 effects.

Line	Mutation	Temperature	CO_2	Temperature x CO_2
N933	WT-Col	0.8487 ^{N.S.}	0.0447*	0.6041 ^{N.S.}
N209	<i>fad7</i>	0.0111*	0.0539 ^{N.S.}	0.0341*
CS8036	<i>fad7fad8</i>	0.2222 ^{N.S.}	0.8024 ^{N.S.}	0.6610 ^{N.S.}
N205	<i>fad4</i>	0.0031**	0.3782 ^{N.S.}	0.1319 ^{N.S.}
CS200	<i>act1</i>	0.0000***	0.0105*	0.0107*
NW20	WT-Ler	0.0094**	0.0010**	0.1275 ^{N.S.}
CS3108	<i>fad7gll</i>	0.9464 ^{N.S.}	0.7123 ^{N.S.}	0.6469 ^{N.S.}

* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$

The comparison of ADB values (Figure 3.2) showed the responses of the different lines to the temperature and CO_2 treatments. The lines were grouped according to whether they responded or did not respond to the treatments. The responsive group included the wild type lines of both backgrounds and the *fad4* and *act1* mutant lines. The Columbia wild type (N933) and the *fad4* mutant (N205) lines showed a similar tendency

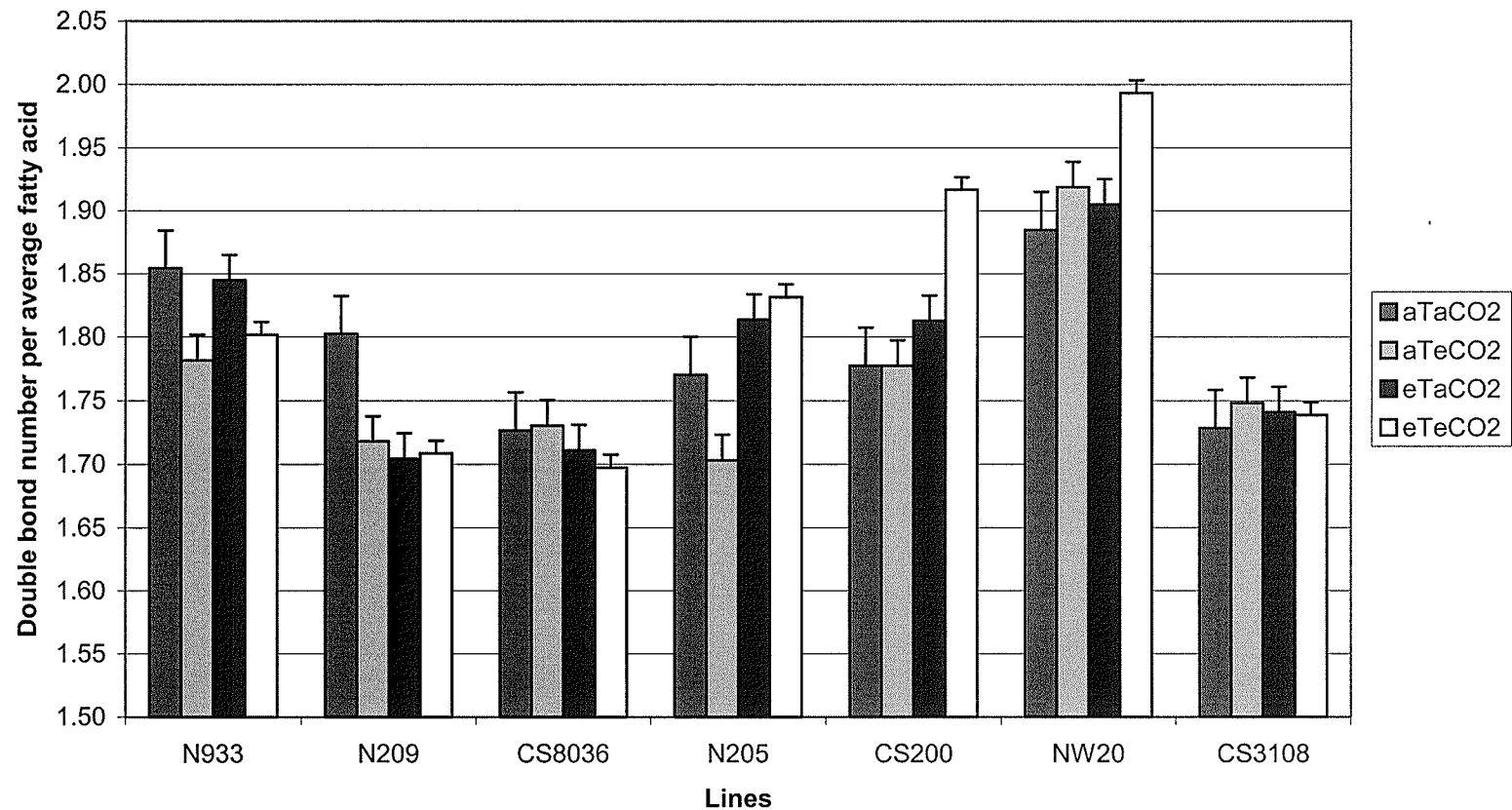


Figure 3.2: The number of double bonds in an average fatty acid in leaf lipid analysis for seven *Arabidopsis* lines over temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

to have no significant difference between the ADB values for the aTaCO₂, eTaCO₂ and eTeCO₂ treatments, but significantly lower ADB values for the aTeCO₂ treatment (Figure 3.2). The *act1* mutant (CS200) and Landsberg *erecta* wild type (NW20) lines responded differently in that their ADB values were similar for aTaCO₂, aTeCO₂ and eTaCO₂ treatments, but were significantly higher for the eTeCO₂ treatment.

With the exception of the aTaCO₂ treatment of the Columbia *fad7* mutant (N209) line, the ADB values remained very stable over treatments for the *fad7* (N209 and CS3108) and *fad7fad8* (CS8036) mutant lines (Figure 3.2).

If a crop plant is not adapted to growth under elevated temperature and CO₂ conditions, the result of the stress treatment would be loss of yield and changes in the quality of the harvestable product which would often be detrimental to its utility (Williams *et al.* 1995, Harwood 1998b). The *fad7* and *fad8* mutations produced elevated leaf lipid saturation levels which maintains normal growth and development under elevated temperatures (Murakami *et al.* 2000). Thus, the *fad7* and *fad8* mutations in leaves may result in the plant having an appropriate relative saturation level of its cellular membranes to tolerate elevated temperatures.

3.3.2 *B. napus* leaf composition

The basis for selecting the *B. napus* lines for the experiment was the seed fatty acid profile altered by mutation treatment to achieve a variation in saturated fatty acids and altered dienoic to trienoic ratio. The doubled haploid mutation lines were chosen to include a wide range of saturated fatty acid levels in the seed oil, with a range from 5% saturates to over 10% saturates (Appendix A: Table 2). The corresponding saturate levels of the leaf lipids was not known prior to this analysis. The pathways of leaf and seed

lipid production include many common steps, thus it was hypothesized that similar mutations may have occurred in leaf lipid biosynthetic pathways.

The fatty acid profiles were converted into ADB values. These values were averaged over all treatments, and ranked from highest ADB value (most unsaturated fatty acid profile) to lowest ADB value (most saturated fatty acid profile) (Table 3.3). The lines were sorted into three clusters based on the pedigree of the line and the class of seed profile of the *B. napus* line (Table 3.3). The first cluster (00DH-1, 00DH-2) had the highest average ADB values (2.20 and 2.18) meaning their lipid profiles were the most unsaturated. This cluster was distinct as the Dynamite by FA18-1 parentage was shared by the two lines; neither parent appeared in any of the other lines' pedigrees (Appendix A: Table 2).

Table 3.3: Number of double bonds per average fatty acid values averaged over temperature treatments and over all treatments for each *B. napus* line presented by cluster. The seed profiles are described as High Saturates (HS), Canola (Canola profile), High Erucic Acid Rapeseed (HEAR), Low Linolenic Acid (LL) and High Oleic Acid (HO).

Group	Line	Type	aT ADBavg	Rank	eT ADBavg	Rank	ADBavg	Rank
1	00DH-1	HS	2.29	1	2.11	5	2.20	1
	00DH-2	Canola	2.23	2	2.13	2	2.18	2
2	DH2-68	HEAR	2.22	3	2.13	1	2.18	3
	DH2-117	HEAR	2.21	6	2.12	4	2.17	4
	DH2-113	HEAR	2.20	10	2.12	3	2.16	5
	DH3-162	HEAR	2.22	5	2.07	7	2.14	6
	DH1-54	HEAR	2.20	7	2.08	6	2.14	7
	DH1-53	HEAR	2.22	4	2.05	10	2.14	8
3	00DH-215	LL, HO	2.19	12	2.06	9	2.12	9
	00DH-70	LL, HO	2.18	15	2.06	8	2.12	10
	00DH-44	HS, LL	2.20	9	2.04	12	2.12	11
	00DH-201	LL, HO	2.19	11	2.04	11	2.12	12
	00DH-293	LL, HO	2.20	8	2.02	19	2.11	13
	00DH-200	LL, HO	2.17	16	2.04	13	2.11	14
	00DH-629	LL, HO	2.18	13	2.02	18	2.10	15
	00DH-124	Canola	2.16	18	2.04	14	2.10	16
	00DH-52	Canola	2.18	14	2.01	21	2.10	17
	00DH-327	LL, HO	2.18	17	2.02	17	2.10	18
	00DH-25	HS, LL	2.16	20	2.02	16	2.09	19
	00DH-627	LL, HO	2.16	19	2.00	24	2.08	20
	00DH-292	LL, HO	2.14	24	2.02	15	2.08	21
	00DH-326	LL, HO	2.15	21	2.01	23	2.08	22
	00DH-294	LL, HO	2.14	23	2.02	20	2.08	23
00DH-27	HS, LL, HO	2.14	22	2.01	22	2.07	24	

The second cluster of lines was comprised of the six HEAR lines, which had intermediate ADB values in the leaf lipids ranging from 2.14 to 2.18 (Table 3.3). These lines all shared Neptune (a HEAR cultivar) as one common parent.

The lowest cluster in the ranking by average ADB value, was composed of a large group of *B. napus* lines, with several common parental lines. The fifteen lines in the group possessed leaf lipid ADB values which ranged from 2.07 to 2.12 (Table 3.3).

The presence of groups in the ADB value ranking of the different lines and pedigrees of *B. napus* indicates that there is a possibility that the mutations affecting the desaturase enzymes involved in seed oil production also affected the enzymes involved in leaf fatty acid production. The Dynamite by FA18-1 (*B. rapa*) cross was made in order to move the low saturated fatty acid trait from the FA18-1 parent (3.4%) into the Dynamite (canola fatty acid profile) background. However, the FA18-1 line also possessed low C18:1 (47.0%) and high C18:2 (35.8%) and C18:3 (12.1%) for a canola fatty acid profile, indicating the C18:1 desaturase had possibly been overexpressed following the mutation treatment. This mutation may also have affected the leaf cell desaturases of the FA18-1 line, producing the leaf profiles of its progeny which are relatively more unsaturated than the other *B. napus* lines.

Data was further analyzed by ANOVA analysis to determine whether the lines within the three clusters were similar to each other and whether the clusters were significantly different from each other. Group averages for each treatment and for each fatty acid group were calculated (Figure 3.3 and in Appendix B: Table 9). The lines all responded to the treatments in a similar fashion with saturated fatty acids higher in eT compared to aT and eCO₂ compared to aCO₂, across all three groups. Both dienoic and trienoic fatty acids were lower in the profile of aT compared to eT treatments and in aCO₂ compared to eCO₂ treatments, across all three groups.

Significant variation ($P < 0.00005$) was found in the ADB values of the lines when the lines from all groups were compared (Table 3.4) and all lines were significantly affected by temperature ($P < 0.00005$), CO₂ ($P < 0.00005$) and the interaction between temperature and CO₂ ($P < 0.0007$). All lines appeared to respond similarly to temperature,

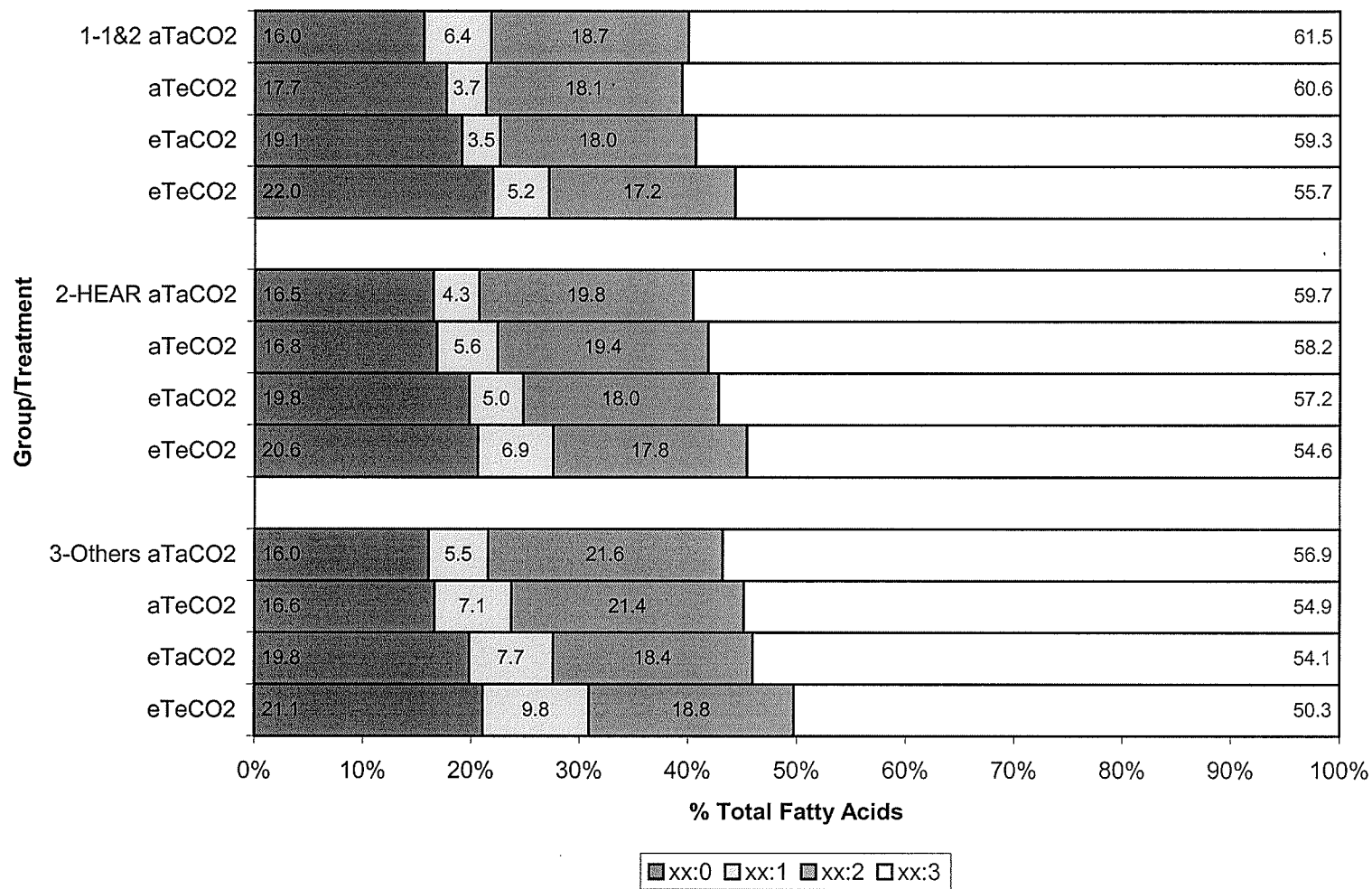


Figure 3.3: Leaf fatty acid profiles of three *B. napus* groups over four temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

CO₂ and the temperature by CO₂ interaction as the entry interactions were non-significant.

Table 3.4: ANOVA analysis summary of number of double bonds in an average fatty acid in *B. napus* leaf fatty acids over temperature and CO₂ treatments.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	383	3.594			
Entry	23	0.474	0.021	8.35	0.0000
Group 1	1	0.002	0.002	0.66	0.4255
Group 2	5	0.022	0.004	2.66	0.0290
Group 3	15	0.063	0.004	1.59	0.0782
1 vs. 2 vs. 3	2	0.386	0.193	74.37	0.0000
Temperature	1	1.784	1.784	723.17	0.0000
CO ₂	1	0.410	0.410	166.31	0.0000
Temp x CO ₂	1	0.029	0.029	11.85	0.0007
Entry x Temp	23	0.081	0.004	1.43	0.0959
Entry x CO ₂	23	0.058	0.003	1.03	0.4333
E x T x CO ₂	23	0.048	0.002	0.84	0.6763
Residual	288	0.710	0.002		

The ADB values of each group were analyzed within groups and between groups by dividing the twenty-three degrees of freedom and the sums of squares between the groups and the group comparison analysis. This analysis revealed that lines within group 1 and 3 were not significantly different from each other ($P < 0.4255$ and $P < 0.0782$, respectively) (Table 3.4), but the HEAR lines (group 2) were significantly different from each other ($P < 0.0290$). From the ANOVA analysis of group 2 (Table 3.5, Appendix B: Tables 10-13) it is evident that there is a significant entry by temperature interaction which is causing the lines of group 2 to be significantly different from each other. While the lines of group 2 do not all respond in the same way to temperature changes, they do all respond with decreases in ADB (a more saturated profile) to higher temperatures (Table 3.3). Thus, the grouping of lines is supported as groups are significantly different from each other and lines within groups are generally similar.

Table 3.5: Summary of F-values from ANOVA analysis of ADB values of each *B. napus* group individually for temperature, CO₂, temperature x CO₂ and entry x temperature effects.

Group	Type	Temperature	CO ₂	Temp x CO ₂	Entry x Temp
1-1 & 2	Dynamite x FA18-1	0.0000***	0.0010***	0.2530 ^{N.S.}	0.0556 ^{N.S.}
2-HEAR	HEAR	0.0000***	0.0000***	0.0198*	0.0185*
3-Others	Canola	0.0000***	0.0000***	0.0158*	0.8338 ^{N.S.}

* - P<0.05; ** - P<0.01; *** - P<0.001

Temperature and CO₂ were significant effects for each group (Table 3.5 and Appendix B: Table 10-13). Interestingly, the effect of the temperature by CO₂ interaction on ADB value was quite small in relation to the individual effects of temperature and CO₂.

The presence of three groups is further supported as the groupings remain intact over treatments (Figure 3.4). For each of the four treatments, average ADB value is highest in group 1 and lowest in group 3. Additionally, all groups respond similarly to the treatments, with average ADB values decreasing from aTaCO₂ to aTeCO₂ to eTaCO₂ to eTeCO₂, indicating a more saturated fatty acid profile in the elevated temperature and CO₂ conditions. The response to elevated temperature with a more saturated leaf fatty acid profile may be required to maintain membrane stability and cell function (Harwood 1998b). Thus, it appears that, while the absolute ADB values of the various groups have been altered by the leaf lipid fatty acid mutations, all lines still maintain the ability to adapt to elevated temperature and CO₂ through leaf membrane fatty acid profile changes. The *FAD7* and *FAD8* genes of the leaf lipid biosynthetic pathways have remained unaffected by the seed mutation treatments of these lines. If the *fad7* or *fad8* mutations had been present in the leaves of the *B. napus* plants, the ADB values would have remained the same over all treatments rather than decreasing with elevated temperature and CO₂ level.

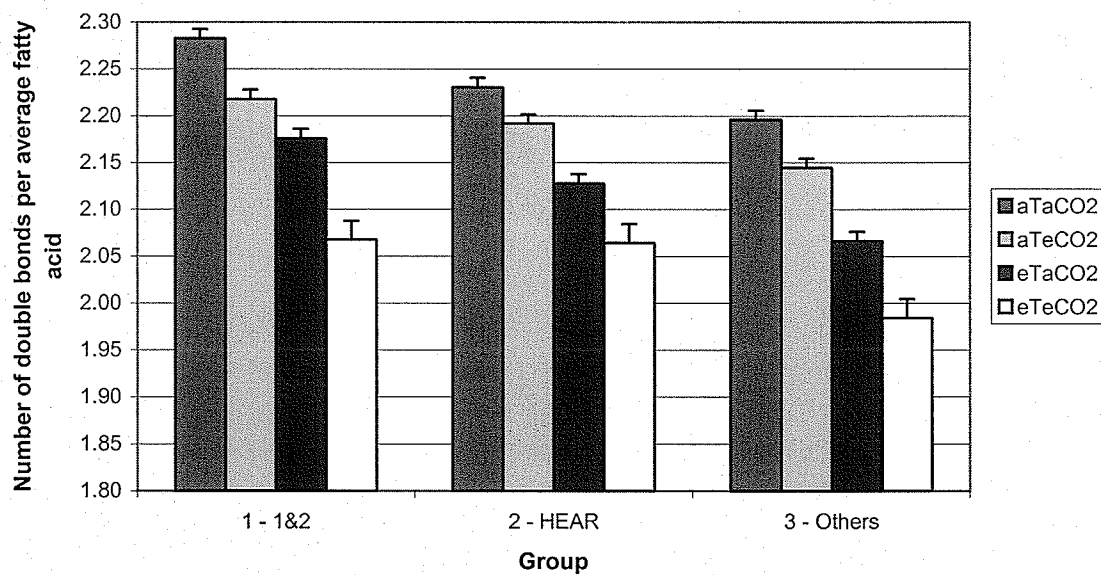


Figure 3.4: The number of double bonds in an average fatty acid in leaf lipid analysis for three groups of *B. napus* lines over temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

3.3.3 *Arabidopsis* seed composition

The treatments of the lines occurred from approximately the 2-leaf to the 6-leaf stage (14-28 DAS), or during the vegetative growth of the plants. Thus, while the vegetative parts of the plant developed in the various treatment environments, the reproductive development occurred in the growth chamber at ambient temperature (20°C) and CO₂ levels (360 ppm). The *Arabidopsis* lines were chosen based on their leaf lipid characteristics and contained no known seed lipid mutations. Any seed fatty acid profile differences between treatments or lines would then be due to a “carry-over” effect of the temperature treatment in the vegetative to reproductive growth stages.

The ADB values for entry are significantly different ($P < 0.0121$) due to the significantly lower ADB values from the CS200 line (Table 3.6). Temperature ($P < 0.0042$), CO₂ ($P < 0.00005$) and the temperature x CO₂ interaction ($P < 0.0002$) are also significant, likely due to the significantly lower ADB values of the aTeCO₂ treatment. All lines responded similarly to the treatments with similar ADB values for the aTaCO₂, eTaCO₂ and eTeCO₂ treatments and lower ADB values for the aTeCO₂ treatment as indicated by the non-significant entry interactions.

Table 3.6: ANOVA summary of the number of double bonds per average fatty acid (ADB) values for *Arabidopsis* seed lipid analysis.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	111	0.161			
Rep	3	0.000	0.000	0.08	0.9682
Entry	6	0.016	0.003	2.94	0.0121
Temperature	1	0.008	0.008	8.69	0.0042
CO ₂	1	0.027	0.027	30.06	0.0000
Temp x CO ₂	1	0.014	0.014	14.95	0.0002
Ent x Temp	6	0.007	0.001	1.29	0.2708
Ent x CO ₂	6	0.007	0.001	1.36	0.2391
E x T x CO ₂	6	0.008	0.001	1.52	0.1835
Residual	81	0.073	0.001		

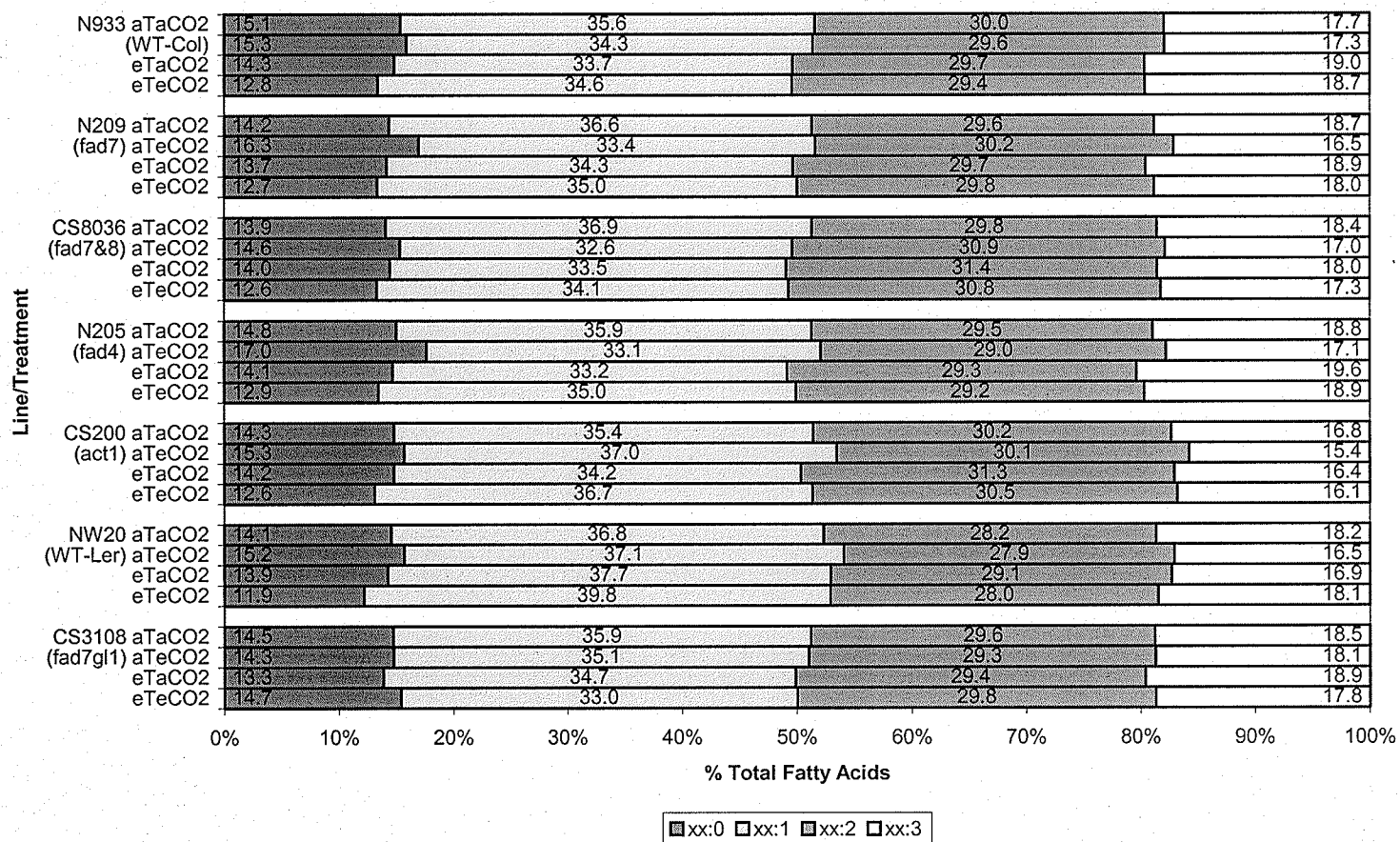


Figure 3.5: *Arabidopsis* seed lipid fatty acid profiles by fatty acid group presented by line over four temperature and CO₂ levels. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

The seed fatty acid profiles of the different lines grown in the 4 temperature and CO₂ treatments are similar (Figure 3.5). However, small differences are evident in the analysis of the lines grown in the aTeCO₂ environment including an increase in saturated fatty acids, lower monounsaturated and trienoic fatty acids. When all the lines are averaged across treatments the changes are small, but significant (Table 3.7). Results by line are presented in Appendix B: Table 14.

Table 3.7: Averages of seed fatty acid analysis for seven *Arabidopsis* lines presented by fatty acid category, saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid (ADB) value over four temperature and CO₂ level treatments.

Treatment	xx:0	xx:1	xx:2	xx:3	Sat/Unsat	xx:3/xx:2	ADB
aTaCO ₂	14.43b	36.16b	29.56a	18.13b	0.17b	0.61b	1.50b
aTeCO ₂	15.42c	34.64a	29.59a	16.85a	0.19c	0.57a	1.44a
eTaCO ₂	13.93b	34.46a	29.99b	18.24b	0.17b	0.61b	1.49b
eTeCO ₂	12.88a	35.45ab	29.64ab	17.84b	0.16a	0.60b	1.48b

There are significant changes in the ADB values of the seed analysis from the aTeCO₂ treatment compared to the rest of the treatments (Figure 3.6). In all lines the aTeCO₂ treatment resulted in lower ADB values, indicating that the seed fatty acid profiles were more saturated than the other treatments. The most plausible explanation for this result is a temperature regulation problem with one of the growth chambers. No abnormality was noticed in the leaf lipid analysis data from this run, likely since the malfunction occurred late enough in the trial that the leaf lipids had already been developed. Thus, in one of the two runs of the aTeCO₂ treatment the saturated fatty acids are noticeably higher and the monounsaturated fatty acids are lower than the other treatments, creating a lower average ADB value for this treatment. This may have been caused by a temperature spike during early reproductive development.

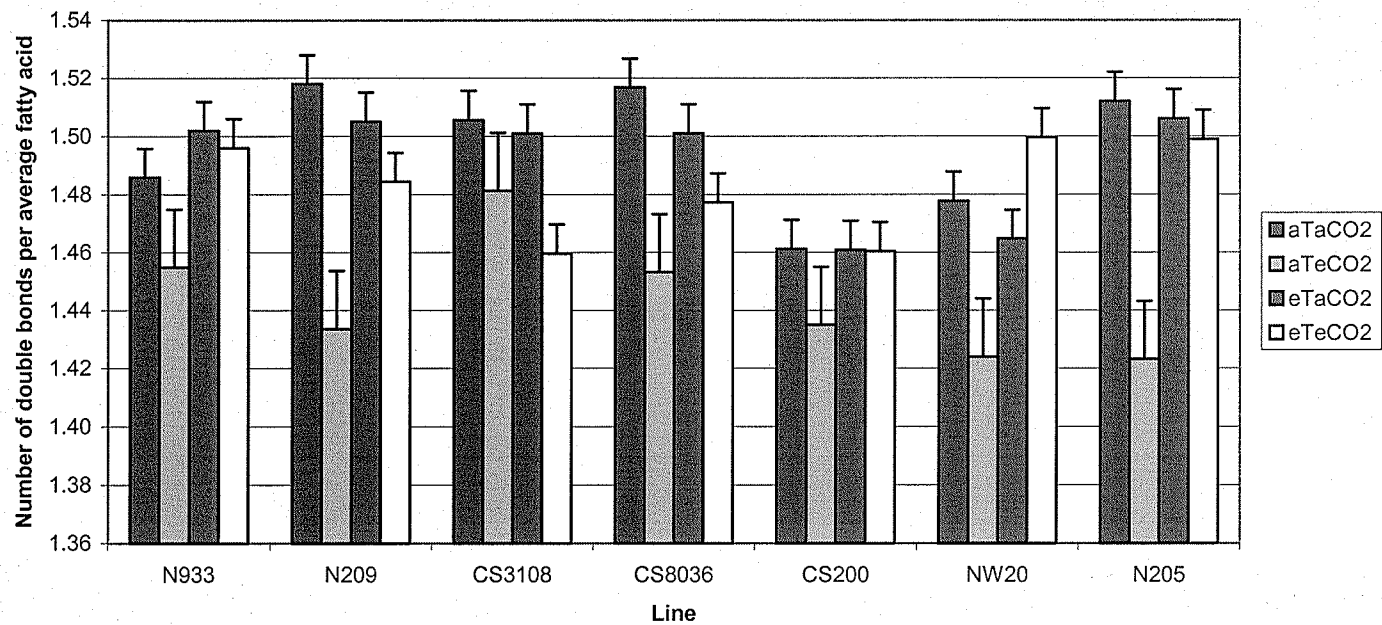


Figure 3.6: *Arabidopsis* seed number of double bonds per average fatty acid (ADB) values presented by line over four temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

The CS200 line (*act1*) has the most saturated seed fatty acid profile (Figure 3.6), as it contained significantly higher monounsaturated and dienoic fatty acids and lower trienoic fatty acids (Figure 3.5) than the wild type (N933). This could indicate a mutation in the lipid biosynthetic pathway of the seed of the CS200, however, if any exists its functional expression is very slight.

3.3.4. *B. napus* seed composition

The *B. napus* lines were chosen to represent a wide range of saturated fatty acid levels and specialty seed fatty acid profile types. Seven groups were developed based on the previous seed fatty acid analysis of the doubled haploid lines: canola profile (7% saturates, 60% C18:1, 20% C18:2, 10% C18:3) (group 1); high erucic acid rapeseed (HEAR)(group 2 – 25% C22:1 and group 3 – 50% C22:1); high saturates (HS)(group 4); high saturates, low C18:3 (LL)(group 5); high saturates, low C18:3, high C18:1 (HS LL HO)(group 6); and low C18:3, high C18:1 (LL HO)(group 7).

As with the *Arabidopsis* experiment, the treatments occurred from the 2-6 leaf stage (14-28 DAS) of the *B. napus* growth. This meant that any changes in seed fatty acid profiles between treatments would be a result of “carry-over” effects from the development of the vegetative material.

ANOVA analysis of the ADB values (Table 3.8) indicated that CO₂ was the main cause of the changes in seed lipid fatty acid profile over treatment. The significant effect of CO₂ (P<0.00005) was the only significant factor in altering the ADB values of the lines, as temperature (P<0.0553) and the temperature x CO₂ (P<0.4884) effects, were non-significant. The entry x temperature and entry x CO₂ effects were also non-significant indicating that all *B. napus* lines responded similarly to the treatments.

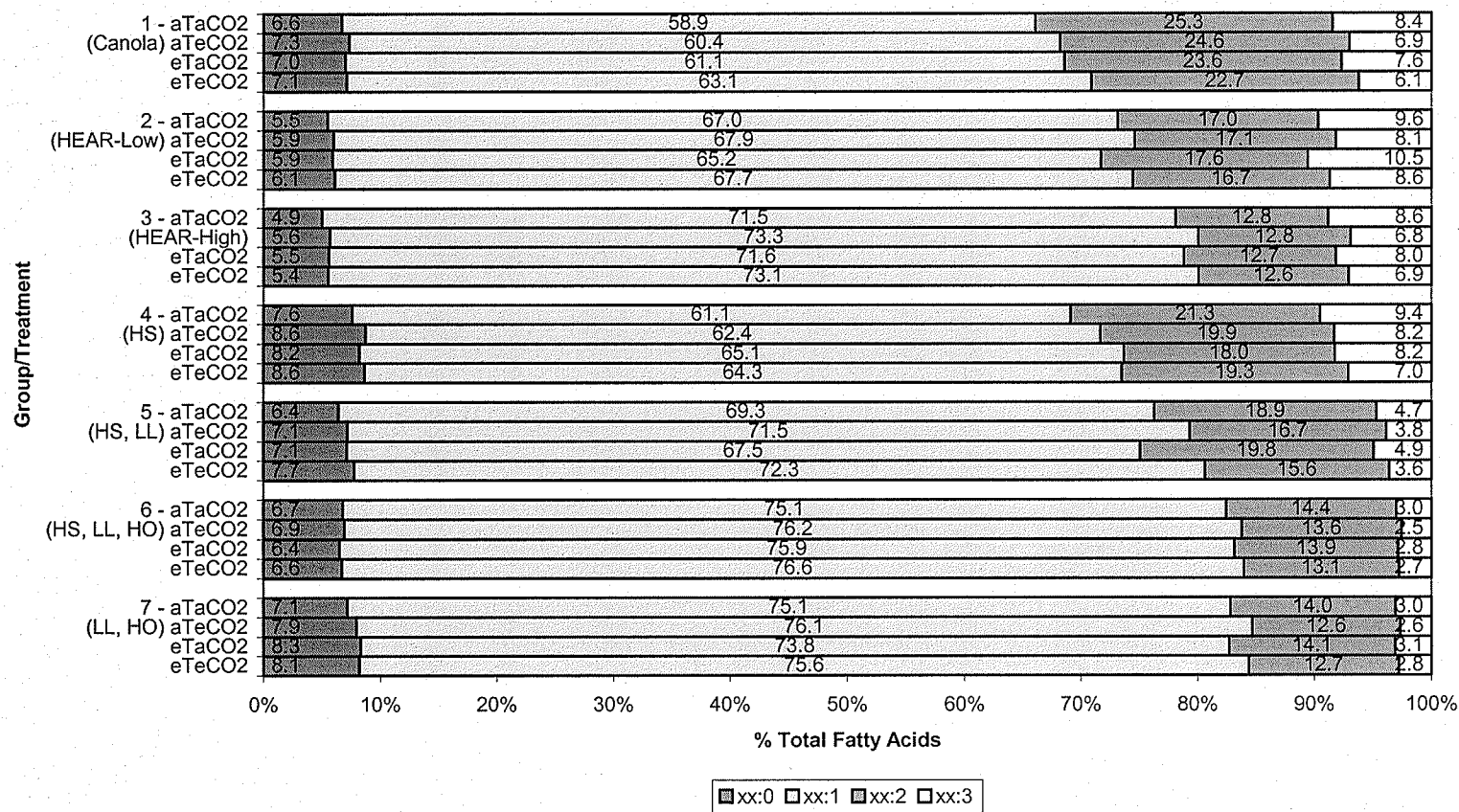


Figure 3.7: *B. napus* seed lipid fatty acid profiles by fatty acid group presented by line over four temperature and CO₂ levels. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

Table 3.8: ANOVA summary of the number of double bonds per average fatty acid (ADB) values for *B. napus* seed lipid analysis.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	383	3.696			
Entry	23	2.746	0.119	47.86	0.0000
Temperature	1	0.009	0.009	3.70	0.0553
CO ₂	1	0.097	0.097	39.06	0.0000
Temp x CO ₂	1	0.001	0.001	0.48	0.4884
Ent x Temp	23	0.035	0.002	0.61	0.9190
Ent x CO ₂	23	0.031	0.001	0.54	0.9625
Residual	311	0.776	0.002		

The groups responded similarly to the various treatments despite the different seed fatty acid profiles of the groups. Changes were small, however, lines from the aTaCO₂ and eTaCO₂ treatments possessed lower monounsaturated fatty acids and higher dienoic and trienoic fatty acid levels, while lines from aTeCO₂ and eTeCO₂ treatments possessed higher monounsaturated fatty acids and lower dienoic and trienoic fatty acid levels (Figure 3.7). This trend was also evident when all lines were averaged over treatment (Table 3.9).

Table 3.9: Averages of seed fatty acid analysis for 24 *B. napus* lines presented by fatty acid category, saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid (ADB) value over four temperature and CO₂ level treatments.

Treatment	xx:0	xx:1	xx:2	xx:3	Sat./Unsat.	xx:3/xx:2	ADB
aTaCO ₂	6.49a	70.75a	16.19b	5.59b	0.07a	0.35b	1.20b
aTeCO ₂	7.18b	72.06b	15.18a	4.65a	0.08b	0.31a	1.16a
eTaCO ₂	7.26b	70.33a	15.97b	5.43b	0.08b	0.35b	1.19b
eTeCO ₂	7.30b	72.27b	14.81a	4.61a	0.08b	0.31a	1.16a

As a result of these changes over treatment, the ADB values for the aTaCO₂ and eTaCO₂ treatments were significantly higher than those for the aTeCO₂ and eTeCO₂ treatments over most of the *B. napus* groups (Table 3.9, Figure 3.8). Results from each line individually are shown in Appendix B: Table 15. This pattern held across groups

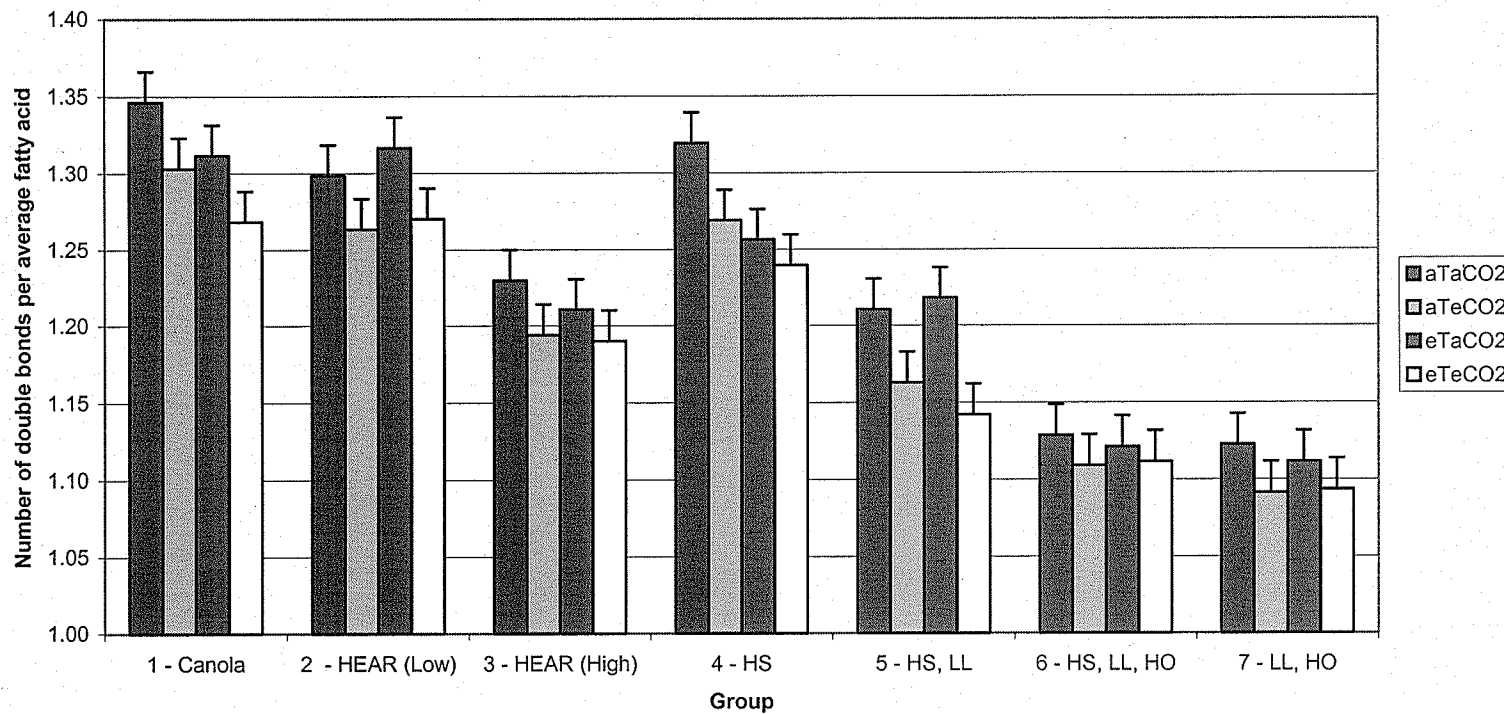


Figure 3.8: *B. napus* seed number of double bonds per average fatty acid (ADB) values presented by line over four temperature and CO₂ treatments. aT: ambient temperature (20/15°C); eT: elevated temperature (30/25°C); aCO₂: ambient CO₂ (360 ppm); eCO₂: elevated CO₂ (720 ppm).

despite the different ADB values and saturation states of the seed lipids of each group, which were lower among the low linolenic acid lines.

3.4. Conclusions

Temperature affected the fatty acid profiles of the leaf lipids in both *B. napus* and *Arabidopsis*. All the *B. napus* lines in the study responded to elevated temperature with higher saturation of the leaf lipid fatty acids. Three clusters appeared in the *B. napus* lines, based on average ADB values, which could be correlated to the pedigree of the different groups. The *Arabidopsis* lines responded differently as the *fad7* and *fad7fad8* mutants maintained a relatively saturated leaf lipid profile despite changes in temperature.

CO₂ played a less significant role in affecting ADB values of the *Arabidopsis* lines. The *B. napus* lines responded to elevated CO₂ with significant decreases in ADB value or more saturated leaf lipid profiles. The limited light level in the growth chamber, compared to full sun conditions, may have been a limitation on the ability of the plants to utilize the elevated level of CO₂ for increased photosynthetic production of energy and carbon skeletons.

There was no significant temperature by CO₂ interaction on ADB values in both *Arabidopsis* and *B. napus* as compared to the temperature effects. In general, it seems that *fad7* and *fad7fad8* mutations limit the ability of the leaf to respond to a wide range of climate conditions by increasing saturation levels. However, the relatively saturated state of their fatty acid profiles provides an adaptation to elevated temperatures. Murakami *et al.* (2000) indicated the *fad* mutations allowed growth under elevated temperature conditions. Incorporation of these *Arabidopsis* mutations into *B. napus* may result in

cultivars that are better adapted to the elevated temperatures associated with climate change conditions.

Elevated temperature and CO₂ were found to impact the seed fatty acid profiles of the *Arabidopsis* lines significantly, even though the treatments occurred during the vegetative growth of the plants. Temperature, CO₂ and the temperature x CO₂ interaction affected the saturation state of the seed with significantly lower ADB values being found in the lines from the aTeCO₂ treatment. This may have been a result of a temperature spike in the treatment chamber during a critical period of seed development, creating a more saturated seed fatty acid profile in one run of one treatment.

Despite the wide-ranging seed ADB values between the *B. napus* lines in the study, all seed classes responded similarly to the treatments. There was very little change in seed fatty acid profile between treatments for all groups of lines, however CO₂ did have a significant effect resulting in higher ADB values for aTaCO₂ and eTaCO₂ treatments and lower ADB values for aTeCO₂ and eTeCO₂ treatments for all lines.

The changes in seed fatty acid profile between treatments for both *Arabidopsis* and *B. napus* indicate that vegetative growth conditions may play a role in the resulting fatty acid profile of the seed. The changes in this study were small, however, even small changes are important for the quality of oil produced by agricultural crops. The more frequent episodic type of extreme weather events predicted to be associated with climate change might significantly affect oilseed quality.

In general, *Arabidopsis* and *B. napus* leaf and seed lipids were more relatively saturated under elevated temperature than under ambient temperature. The effects of CO₂ were more difficult to generalize from the growth chamber study. It also appears

generalizations about species response to climate change conditions will be difficult to make. While the leaf lipids of *Arabidopsis* were significantly affected by temperature, its seed lipids were impacted significantly by temperature and CO₂ levels. In *B. napus*, the leaf lipids were significantly affected by temperature and CO₂, while the seed lipids responded significantly only to CO₂ levels. This is understandable as seed and leaf lipid biosynthetic pathways are related, but quite independent of each other in terms of fatty acid profiles produced and predominant lipid species present in the respective tissues (Somerville *et al.* 2000). More study will be required to determine how individual oilseed species will respond to changing abiotic conditions.

4. THE EFFECT OF ELEVATED TEMPERATURE ON THE LEAF AND SEED FATTY ACID COMPOSITION OF FIELD-GROWN *Brassica napus* FATTY ACID MUTANT LINES.

4.1 Introduction

The rise of atmospheric CO₂ levels as part of climate change is predicted to have an increasingly large impact on the environment. Global mean temperature increases of 0.5°C per decade are also predicted (Environment Canada 2002), with even greater temperature increases forecast for the more northerly latitudes, such as Canada. The combined effects of elevated CO₂ and temperature will have significant impacts on a wide range of human activities (Environment Canada 2002) including agriculture.

Crop plants are susceptible to temperature stress during both vegetative and reproductive growth stages (Ferris *et al.* 1999). Leaf cellular membranes play a significant role in determining the overall health of the plant due to their role in cellular processes (Browse and Somerville 1994). The fatty acid profile of the leaf cellular membrane lipids is particularly important in determining the fluidity of the membrane (Harwood 1998a). Under elevated temperatures, plants must be able to increase the saturation level of its membrane lipid fatty acid profile in order to provide the optimal membrane fluidity for proper cellular function (Harwood 1998b).

Exposure to increased temperatures during reproductive growth and seed development has been associated with increased levels of saturated fatty acids or a relatively more saturated seed oil fatty acid profile. Studies of economically important cereals and oilseeds including wheat (*Triticum aestivum*) (Williams *et al.* 1995),

sunflower (*Helianthus annuus*) (Sarmiento *et al.* 1998), soybean (*Glycine max*) (Cheesebrough 1989) and oilseed rape (*Brassica napus*) (Deng and Scarth 1998) have all shown increased saturation levels in the seed lipids associated with higher temperatures during reproductive development.

The effect of environment on the leaf and seed fatty acid profiles of *Brassica napus* lines was examined in a field study. *B. napus* doubled haploid (DH) lines with a range of seed oil saturation levels were selected for the experiment. Low linolenic acid (C18:3)/high oleic acid (C18:1) canola, high erucic acid (C22:1) rapeseed (HEAR) and conventional canola lines were represented (Appendix C: Table 1). The lines were seeded on two seeding dates at two locations, to obtain distinct environments during vegetative and reproductive development. The study was designed to determine the genotypic response to environment, with the purpose of developing breeding goals for *B. napus* lines with improved adaptation to climate change conditions.

4.2 Methods and Materials

4.2.1. *Brassica napus* lines

Twenty-two *B. napus* lines were chosen for the experiment to represent a wide range of saturated fatty acid levels in the seed oil (Appendix C: Table 1). Six groups of DH and commercial lines were formed based on seed oil fatty acid composition. The groups were comprised of lines representing the canola oil profile (7% C16:0 + C18:0, 60% C18:1, 20% C18:2, 10% C18:3) (group 1); HEAR lines (group 2); high saturate lines (HS) (group 3); high saturate, low C18:3 lines (HS, LL) (group 4); high saturate,

low C18:3, high C18:1 lines (HS, LL, HO) (group 5) and low C18:3, high C18:1 lines (LL, HO) (group 6).

Due to regulatory restrictions, the herbicide resistant HEAR lines which were grown in the controlled environment experiments were replaced with the varieties MilleniUM03, Mercury and Reston for the field study (Appendix C: Table 1).

4.2.2. Growth of *Brassica napus* in field

The field trials were conducted at the University of Manitoba field research station at Carman, Manitoba and at the Point field station at the University of Manitoba campus in Winnipeg, Manitoba. Two trials were planted at each location, for a total of 4 trials. The experimental design was a randomized complete block (RCB) with 4 replicates (rep) per plot. The early (E) seeding dates were May 13 and 14, 2002 and the late (L) seeding dates were June 4, 2002 for both Carman and Winnipeg locations, respectively. The plot row length was 3 m in Winnipeg and 5 m in Carman. The row spacing was 0.4 m and approximately 0.3 g of seed was sown per rep. Counter 5G (Cyanamid) was applied at the time of seeding to provide flea beetle control.

For each line, 2 plants in each rep were selected for leaf and seed samples. When the plants reached the 3-leaf stage, a stake was placed next to the plant and the date was recorded. The emergence dates of the 4th, 5th and 6th leaves were also recorded. Additionally, the dates of flowering and maturity were recorded as well the leaf sample date and harvest date. When the plants reached the stage where the 6th leaf was expanded, the 3rd through 6th leaves were sampled for lipid fatty acid analysis (June 28 and July 12 for the E and L trials at Carman, June 26 and July 10 for the E and L trials at

Winnipeg). The samples were taken using a standard hole punch (approximately 30 mg) and placed on dry ice to halt all cellular processes and prevent breakdown of leaf tissue.

The plants were staked and bagged during flowering in order to obtain selfed seed for fatty acid analysis. At the end of flowering the bags were removed, the selfed racemes were tagged and any remaining flowers on the selfed racemes were removed. When the seed was mature, the racemes containing selfed seed were harvested into paper bags (August 9 and 22 for Carman plots, August 7 and 21 for Winnipeg plots). The samples were then cleaned to provide a seed sample for fatty acid analysis.

Plot maintenance included weed control by manual hoeing and *Sclerotinia* disease control through application of the fungicide Benelate (DuPont Canada Inc.) at approximately the 30% flowering stage and a rate of 0.4 kg acre⁻¹.

Temperature data was recorded via in-canopy measurements by Watchdog temperature loggers (Spectrum Technologies Inc.) in order to determine the temperature regime under which the *B. napus* leaves and seed developed. In-canopy measurements of CO₂ level were taken using a handheld CO₂ monitor (Spectrum Technologies Inc.).

4.2.3. *B. napus* leaf lipid extractions

Frozen leaf samples were placed in a freeze dryer (Labonco Freeze Dryer 8) to remove all water from the sample. Leaf samples remained in the freeze dryer for 48 h or until completely dry. All extraction procedures took place within the original sample vial. Total leaf lipids were extracted as chloroplast membrane lipids comprise the majority of total leaf lipids (Harwood 1998a) and thus give a sufficiently accurate measurement of chloroplast membrane lipid fatty acid profile. 500 µl of heptane was added to the dried samples to extract the lipids from the leaf material. The samples were

crushed into very fine pieces in the heptane with a glass rod to facilitate more efficient lipid extraction. The lipid-solvent mixture in capped vials was allowed to stand overnight (8 h) to allow extraction to occur. To this mixture, 500 μ l of 0.4 N sodium methoxide in methanol was added to extract the more polar lipids and to trans-methylate the fatty acids. The capped vials were again allowed to stand overnight (8 h) to allow sufficient fatty acid methyl ester (FAME) formation to occur. During both overnight periods the vials were placed in the fridge to ensure minimal extraction compound loss due to evaporation.

The top layer of the resulting two-layer sample was removed by Pasteur pipette into 200 μ l sample vials for gas chromatograph injection and analysis.

4.2.4. *B. napus* seed lipid extractions

Approximately 25-30 mg of seed was obtained from harvested seed samples. The seed was crushed using the piston and cylinder of a Carver Press and a hammer and placed in a 10 mL volumetric flask. The extraction was performed using 5 mL of heptane, which was added to the flask and left overnight (8 h). The lipid-solvent mixture was transferred to a clean 10 mL volumetric flask and 500 μ L of 0.4 N sodium methoxide in methanol was added to the solution to form fatty acid methyl esters (FAME). The solution was again left overnight (8 h) to allow for sufficient trans-methylation to occur. From the flask, 1 mL aliquots were removed by Pasteur pipette from the top layer of the two-layer solution and placed in the GC sample vial for analysis.

4.2.5. Gas chromatograph procedure for leaf analysis

Gas chromatography was performed using a Varian (model 3400 GC) chromatograph equipped with a flame ionization detector (280°C). A 15 m (0.25 mm

interior diameter, 0.25 μ m film thickness) J & W Scientific (model DB225) column was used for all analysis. Helium carrier gas was used. The GC was equipped with an isothermal injector which was set at an initial temperature of 240°C. The initial temperature of the GC column was 160°C which was held at the start of the program for 2.00 min. The column was then heated at a rate of 5°C min⁻¹ to a temperature of 200°C. The rate of increase then moved to 20°C min⁻¹ to a temperature of 230°C which was held for 2.50 min. The total program lasted 14.00 min.; however the integration (Star Chromatography Workstation, version 4.5) from 0.0634 to 4.6587 min. and from 9.7557 to 13.9356 min. was inhibited to eliminate unwanted peaks (e.g. leaf surface waxes) from the analysis. The GC was also equipped with an autosampler (model 8200) which allowed sampling at a 5 μ l sec⁻¹ uptake speed and an injection rate of 10 μ l sec⁻¹. The injection volume was set at 4.0 μ l.

4.2.6. Gas chromatograph procedure for seed analysis

The GC used for seed analysis was the same as that used for leaf analysis, equipped with the same FID, column, injector, carrier gas, autosampler and integration software. However, the GC program differed slightly from the one used in leaf analysis. The initial column temperature was 190°C which increased at a rate of 10°C min⁻¹ to a temperature of 220°C. The column temperature was then increased at a rate of 20°C min⁻¹ to a temperature of 240°C and was held for 3.50 min. Thus, the total program time was 7.50 min. One additional change was a 5 μ L min⁻¹ injection rate was used.

4.2.7. Statistical analysis

Analysis of variance (ANOVA) statistical analysis was performed on the fatty acid profile data using Agrobase™ (Agronomix Software Inc. 1999) statistical software.

For both leaf and seed data, fatty acids were grouped into categories of total saturated fatty acids (xx:0), total monounsaturated fatty acids (xx:1), total dienoic fatty acids (xx:2) and total trienoic fatty acids (xx:3). These factors, along with ratios of saturated fatty acids to unsaturated fatty acids (sat./unsat.) and trienoic fatty acids to dienoic fatty acids (xx:3/xx:2), were analyzed. In addition, the number of double bonds found in the average fatty acid (ADB) in the profile was calculated by weighting the profile as follows: $[(\% \text{ xx:0}) \times 0] + [(\% \text{ xx:1}) \times 1] + [(\% \text{ xx:2}) \times 2] + [(\% \text{ xx:3}) \times 3] / 100$. ADB reflects the saturation state of each fatty acid profile; with lower numbers representing a more saturated profile. The primary components analyzed were seeding date (temperature), location and entry. Interactions between the components, including entry x date, entry x location, date x location and entry x date x location, were also analyzed.

4.3 Results and Discussion

4.3.1 Leaf fatty acid composition

Two seeding dates were used to obtain different environments for the growth and development of the *B. napus*. Analysis of the temperature data collected by the Watchdog temperature loggers in the field plots indicates a temperature differential between plots seeded at the two dates (Table 4.1). The difference in average temperatures between the E and L seeding dates at the Winnipeg plots for the 3 to 6 leaf stage (11 d for E and 8 d for L) was 7.6°C and 4.1°C for the 3 leaf stage to the leaf sample date (18 d for E and 15 for L), with L having warmer temperatures in both. For the Carman plots, the difference between average temperatures of the E and L seeding dates for the 3 to 6 leaf stage (12 d for E and 8 d for L) was 5.9°C and 2.6°C for the 3 leaf

stage to the leaf sample date (20 d for E and 15 d for L) with L having warmer temperatures in both.

The use of average temperatures for comparison may not reflect the heat units available to the growing crop. Growing degree days (GDD) measures heat accumulated above a base temperature. For *B. napus* the calculation is the average temperature of the day from which 5 (the minimum temperature in °C required for *B. napus* growth) is subtracted (Morrison *et al.* 1989). The daily GDD values are summed to give a total measure of the heat available to the crop over a given time.

Based on the GDD data for the 3 leaf to sample date and for the purposes of comparison to the controlled environment study, it was decided to designate the E seeding date as the low temperature (lT) and the L seeding date as the high temperature (hT) for Winnipeg. The reverse was true for Carman as the E seeding date was designated hT and the L seeding date was designated lT. It is interesting to note that, for Carman, were the lT and hT labels assigned according to average temperatures of the period, the labels would have been reversed from the GDD method. The seed development temperature data will be discussed in the next section. The CO₂ measurements were 360 ppm throughout the field season.

Table 4.1: Average, minimum and maximum temperatures (°C) and growing degree days (GDD) for two locations and two seeding dates presented by stage of *B. napus* growth and corresponding dates in 2002. Treatment name provided for data comparison of leaf and seed fatty acid analysis over seeding dates.

Location	Seeding Date	Variable	3 - 6 leaf	3 leaf - sample	flower - harvest	seeding - harvest
Winnipeg	Early (May 14)	Date	Jn.9 - Jn.19	Jn.9 - Jn.26	Jn.30 - Au.7	May14 - Au.7
		Average	16.7	18.4	21.3	19.6
		Min./Max	6.5/28.3	6.5/31.2	7.3/34.1	-7.6/38.8
		GDD	128.2	241.6	635.4	1168.4
		Treatment		IT	hT	
	Late (June 4)	Date	Jn.26 - Jl.3	Jn.26 - Jl.10	Jl.16 - Au.21	Jn.4 - Au.21
		Average	24.3	22.5	19.8	20.1
		Min./Max	10.1/38.8	7.3/38.8	7.3/33.2	4.0/38.8
		GDD	154.8	262.1	546.1	1191.7
		Treatment		hT	IT	
Carman	Early (May 13)	Date	Jn.9 - Jn.20	Jn.9 - Jn.28	Jl.3 - Au.9	May13 - Au.9
		Average	16.0	18.7	20.3	19.0
		Min./Max	7.3/27.2	7.3/34.9	6.5/35.8	-4.1/36.6
		GDD	132.1	273.2	581.4	1146.2
		Treatment		hT	hT	
	Late (June 4)	Date	Jn.28 - Jl.5	Jn.28 - Jl.12	Jl.20 - Au.22	Jn.4 - Au.22
		Average	21.9	21.3	18.3	19.3
		Min./Max	8.2/36.6	8.2/36.6	5.3/34.9	5.3/36.6
		GDD	135.5	244.0	449.9	1141.6
		Treatment		IT	IT	

Over all lines, there was a distinct difference in the fatty acid profile of the leaf lipids from Carman-E compared to the other site/seeding dates (Figure 4.1, Table 4.2, Appendix C: Table 2). The Carman-E profiles generally displayed a higher level of saturated fatty acids, thus lower ADB values and lower levels of dienoic and trienoic fatty acids. The plants at Carman-E grew under the warmest environmental conditions of all the seeding dates with a GDD of 273.2 from the 3 leaf to the sample date (Table 4.1).

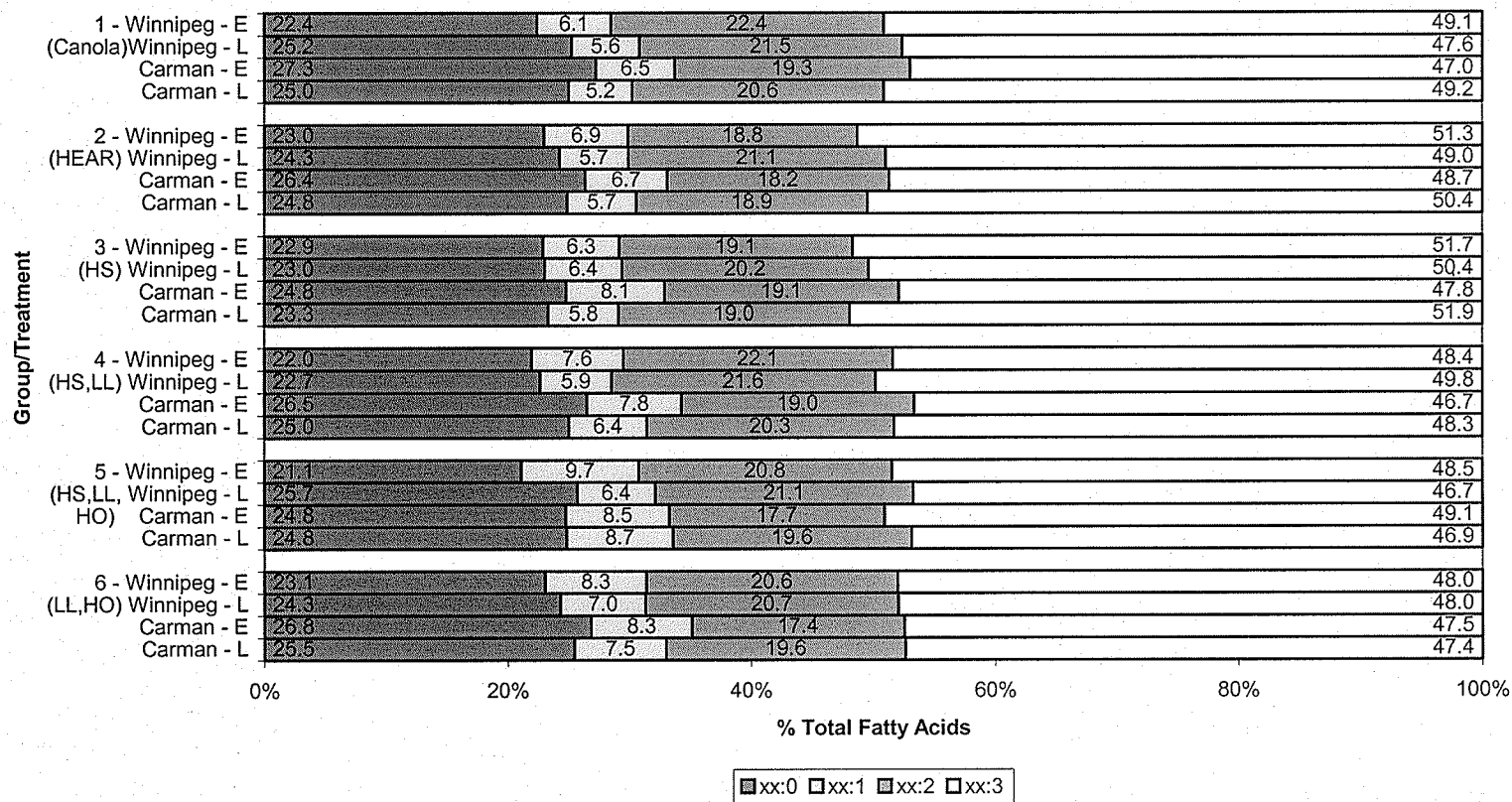


Figure 4.1: Leaf fatty acid profiles presented by fatty acid category of seven groups of *B. napus* lines (based on seed fatty acid analysis) grown in plots at two locations (Winnipeg, Carman) and two seeding dates (E: early date; L: late date).

The more saturated leaf lipid profile of the Carman-E plants would result in decreased cell membrane fluidity under elevated temperatures (Harwood 1998b).

Table 4.2: Leaf lipid fatty acid profile data of seven *B. napus* groups based on seed fatty acid profile grown at two locations and two seeding dates, presented by fatty acid category. Saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid values also presented.

Group/ Type	Location/ Seeding date	xx:0	xx:1	xx:2	xx:3	Sat/ Unsat	xx:3/ xx:2	ADB
1 (Canola)	Winnipeg-E	22.40	6.09	22.37	49.14	0.29	2.25	1.98
	Winnipeg-L	25.22	5.59	21.53	47.57	0.34	2.25	1.91
	Carman-E	27.25	6.48	19.30	46.97	0.38	2.55	1.86
	Carman-L	25.02	5.22	20.61	49.15	0.33	2.42	1.94
2 (HEAR)	Winnipeg-E	22.97	6.92	18.80	51.31	0.30	2.76	1.98
	Winnipeg-L	24.26	5.66	21.07	48.97	0.32	2.36	1.95
	Carman-E	26.38	6.74	18.19	48.69	0.36	2.73	1.89
	Carman-L	24.83	5.69	18.91	50.39	0.33	2.67	1.95
3 (HS)	Winnipeg-E	22.90	6.27	19.13	51.71	0.30	2.76	2.00
	Winnipeg-L	23.03	6.37	20.21	50.41	0.30	2.51	1.98
	Carman-E	24.76	8.06	19.15	47.82	0.33	2.55	1.90
	Carman-L	23.31	5.80	18.97	51.93	0.30	2.74	2.00
4 (HS LL)	Winnipeg-E	21.96	7.55	22.08	48.41	0.28	2.31	1.97
	Winnipeg-L	22.67	5.86	21.64	49.83	0.29	2.37	1.99
	Carman-E	26.52	7.77	19.05	46.67	0.36	2.54	1.86
	Carman-L	25.01	6.41	20.28	48.29	0.33	2.43	1.92
5 (HS LL HO)	Winnipeg-E	21.09	9.66	20.78	48.48	0.27	2.34	1.97
	Winnipeg-L	25.73	6.39	21.14	46.74	0.35	2.22	1.89
	Carman-E	24.75	8.52	17.66	49.07	0.33	2.83	1.91
	Carman-L	24.82	8.75	19.57	46.87	0.33	2.40	1.88
6 (LL HO)	Winnipeg-E	23.06	8.32	20.61	48.02	0.30	2.38	1.94
	Winnipeg-L	24.30	7.02	20.72	47.98	0.32	2.35	1.92
	Carman-E	26.85	8.27	17.42	47.47	0.37	2.92	1.86
	Carman-L	25.45	7.55	19.60	47.39	0.34	2.44	1.89

There are differences in the leaf lipid profiles of the various lines. The separation between entries for the number of double bonds per average fatty acid is highly significant ($P < 0.00005$) (Table 4.3). The HS line (00DH-1) has higher ADB values than the LL HO group (Figure 4.2), for example. However, ranking of the lines by leaf ADB value averaged over all the sites and dates did not provide a clear separation of lines into

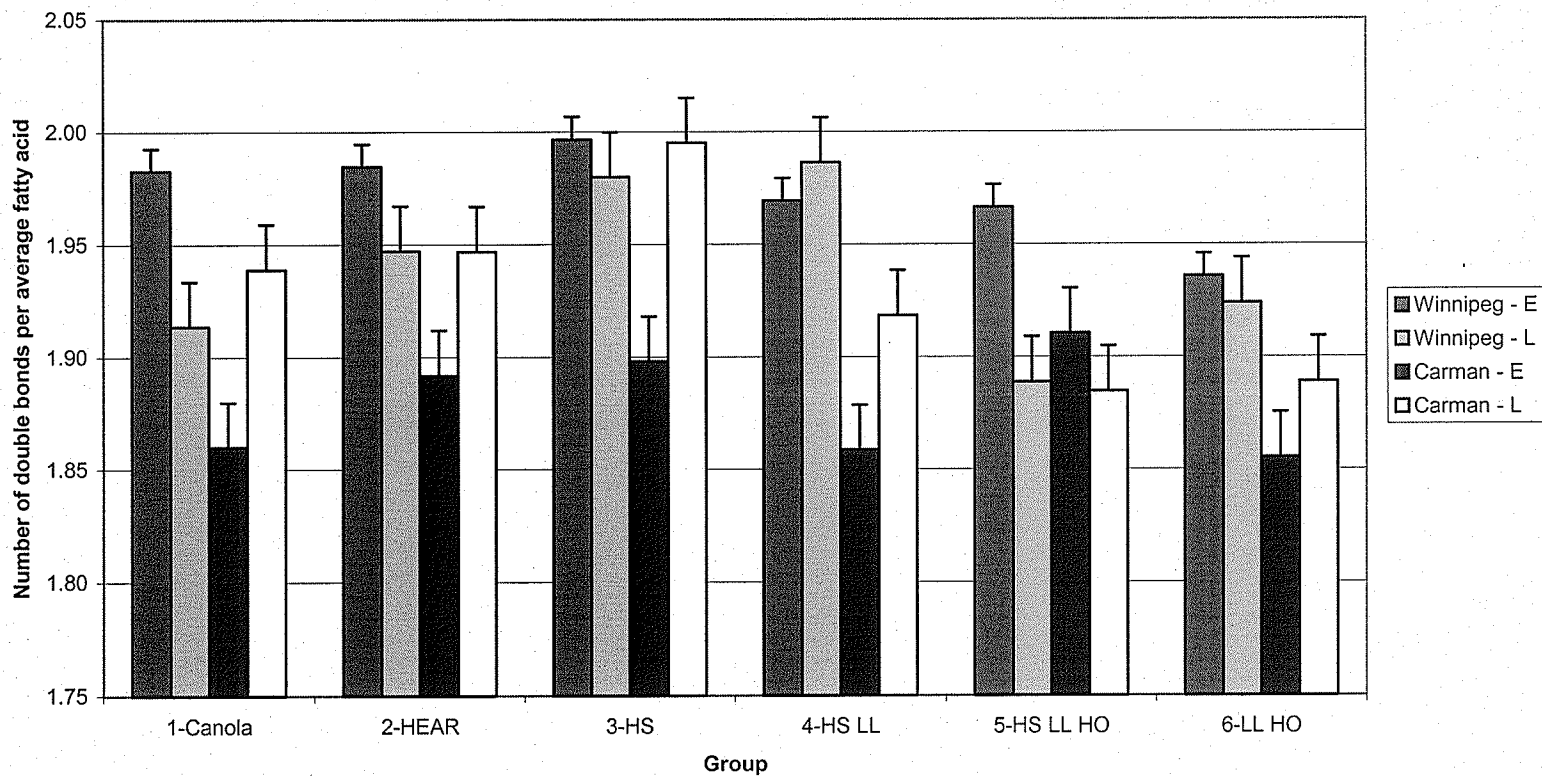


Figure 4.2: Number of double bonds per average fatty acid (ADB) values for leaf lipid fatty acid analysis of seven groups of *B. napus* lines (grouped by seed fatty acid profile). Two locations (Winnipeg, Carman), two seeding dates (E: early date; L: late date).

the groups based on seed fatty acid analysis (data not shown), as occurred in the controlled environment study.

Table 4.3: ANOVA analysis summary table of *B. napus* leaf lipid fatty acid profile number of double bonds in an average fatty acid (ADB) value analyzed by seeding date and plot location.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	351	2.041			
Entry	21	0.313	0.015	3.99	0.0000
Date	1	0.010	0.010	2.62	0.1067
Location	1	0.271	0.271	72.26	0.0000
Date x Loc	1	0.120	0.120	31.99	0.0000
Entry x Date	21	0.135	0.006	1.72	0.0282
Entry x Loc	21	0.121	0.006	1.55	0.0629
E x D x L	21	0.083	0.004	1.05	0.4015
Residual	264	0.988	0.004		

Temperature was not a significant factor in the ANOVA analysis of ADB values ($P < 0.1067$). The overall average of the Winnipeg-E and Carman-E ADB values was 1.91, while the overall average of the Winnipeg-L and Carman-L ADB values was 1.92 (Figure 5.2). Because the overall E ADB value was close to the L value, the effect of temperature was non-significant in the ANOVA analysis despite the significant differences between the different site/seeding dates (Figure 4.2).

Location is a significant factor ($P < 0.00005$) influencing variation in the leaf lipid ADB values (Table 4.3). Across groups the Winnipeg trials had higher ADB values than did the Carman trials (Figure 4.2), as the leaf lipids at Carman were generally more saturated than were those from Winnipeg. The Carman GDD values were higher than at Winnipeg during the vegetative growth stage (for respective IT and hT treatments) (Table 4.1). Therefore, the leaf lipid profiles were responding to increased accumulated heat with increased levels of saturation, as supported by Harwood (1998b).

The date by location interaction was also highly significant ($P < 0.00005$) in the ANOVA analysis of ADB values (Table 4.3). The ADB value at Winnipeg-E is higher than Winnipeg-L and Carman-E is lower than Carman-L. The GDD values are lower for Winnipeg-E than Winnipeg-L and higher for Carman-E than Carman-L (Table 4.1). This relationship between temperature and GDD generally follows the expected increased saturation (lower ADB) under higher temperatures. The temperature difference between seeding dates may only be part of the influence on ADB values and the level of saturation of the leaf tissues, since group 5 lines do not follow the same ADB pattern (Figure 4.2). Other environmental factors such as soil type, fertility and moisture may also influence the leaf lipid profile.

The entry by date interaction was significant ($P < 0.0282$) and examination of the individual lines showed that some lines showed increased saturation (or lower ADB) and others had decreased saturation (or higher ADB) in the lT versus hT environment (data not shown). This may be a result of the individual lines' response to different temperatures or a combination of temperature response with other environmental factors.

All the entries had lower overall ADB values (or more saturated leaf profiles) at Carman compared to the Winnipeg location. The entry by location interaction was insignificant ($P > 0.0629$), indicating all lines responded to the higher GDD values at the Carman site compared to the Winnipeg site during the vegetative stage of growth in the same way (Table 4.1).

The ADB values from both sites follow the relationship of higher GDD value associated with lower ADB values (more saturated) and lower GDD values associated with higher ADB values (less saturated). While some of the influences on lipid

biosynthesis listed above may play a role in determining leaf lipid fatty acid composition, the relationship between temperature and saturation state of the leaf lipid fatty acid profile was confirmed from the controlled environment experiment.

4.3.2 Seed fatty acid composition

Consistent with values during the vegetative development stage, the average temperatures during seed development were higher for the Winnipeg plots than the Carman plots (Table 4.1). At Winnipeg, average temperatures for the flowering to harvest stage for the E seeding date (39 d) were 1.5°C higher than for the L seeding date (36 d). Similarly, the average temperatures recorded at the Carman plots for the flowering to harvest stage were 2°C higher for the E seeding date (37 d) than for the L seeding date (34 d). The flowering to harvest GDD values were higher for the E seeding date (635.4 and 581.4) than the L seeding date (546.1 and 449.9) at Winnipeg and Carman, respectively (Table 4.1).

It was decided to use the L seeding date as the low temperature (lT) treatment and the E seeding date as the high temperature (hT) treatment for the reproductive stage for both locations. This is the reverse of the comparative temperature regimes for the E and L seeding dates during the vegetative growth stages at Winnipeg and the same at Carman.

The HS lines had higher saturation of the seed fatty acids than did the other lines (Figure 4.3 and Table 4.4). The LL HO, HS LL HO and HS LL groups all are noticeably low in trienoic fatty acids (C18:3). The canola, HS and HS LL groups had lower dienoic fatty acids (C18:2) than do the other lines (Figure 4.3 and Table 4.4). All of these values are consistent with the expected profiles for these groups.

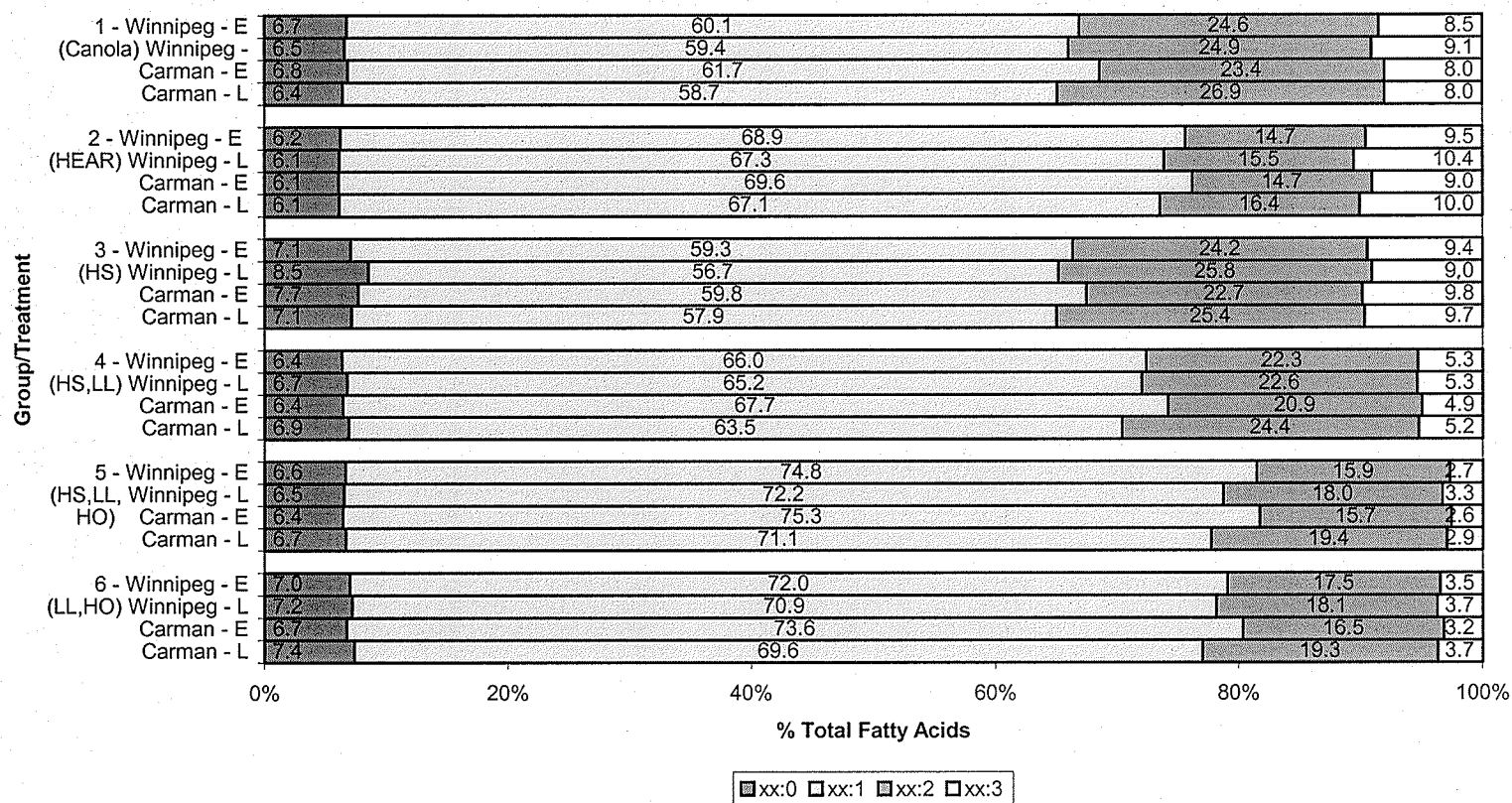


Figure 4.3: Seed fatty acid profiles presented by fatty acid category of seven groups of *B. napus* lines (based on seed fatty acid analysis) grown in plots at two locations (Winnipeg, Carman) and two seeding dates (E: early date; L: late date).

Table 4.4: Seed lipid fatty acid profile data of seven *B. napus* groups based on seed fatty acid profile grown at two locations and two seeding dates, presented by fatty acid category. Saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid values also presented.

Group/ Type	Location/ Seeding date	xx:0	xx:1	xx:2	xx:3	Sat/ Unsat	xx:3/ xx:2	ADB
1 (Canola)	Winnipeg-E	6.73	60.12	24.61	8.49	0.07	0.36	1.35
	Winnipeg-L	6.55	59.42	24.90	9.08	0.07	0.38	1.36
	Carman-E	6.81	61.74	23.42	8.02	0.07	0.36	1.33
	Carman-L	6.38	58.67	26.88	8.02	0.07	0.32	1.36
2 (HEAR)	Winnipeg-E	6.18	68.87	14.72	9.49	0.07	0.65	1.27
	Winnipeg-L	6.10	67.28	15.52	10.44	0.07	0.68	1.30
	Carman-E	6.05	69.64	14.69	8.97	0.06	0.61	1.26
	Carman-L	6.10	67.12	16.36	10.01	0.07	0.63	1.30
3 (HS)	Winnipeg-E	7.06	59.29	24.25	9.42	0.08	0.39	1.36
	Winnipeg-L	8.51	56.68	25.77	9.05	0.09	0.36	1.35
	Carman-E	7.68	59.76	22.69	9.83	0.08	0.44	1.35
	Carman-L	7.13	57.91	25.36	9.65	0.08	0.39	1.38
4 (HS LL)	Winnipeg-E	6.35	66.01	22.31	5.29	0.07	0.24	1.27
	Winnipeg-L	6.75	65.20	22.60	5.35	0.07	0.23	1.26
	Carman-E	6.42	67.72	20.87	4.95	0.07	0.23	1.24
	Carman-L	6.89	63.47	24.39	5.21	0.07	0.21	1.28
5 (HS LL HO)	Winnipeg-E	6.63	74.82	15.86	2.68	0.07	0.17	1.15
	Winnipeg-L	6.49	72.17	18.00	3.29	0.07	0.18	1.18
	Carman-E	6.43	75.28	15.71	2.55	0.07	0.16	1.14
	Carman-L	6.65	71.08	19.36	2.93	0.07	0.15	1.19
6 (LL HO)	Winnipeg-E	7.01	72.04	17.45	3.48	0.08	0.21	1.17
	Winnipeg-L	7.17	70.90	18.12	3.71	0.08	0.21	1.18
	Carman-E	6.74	73.59	16.48	3.17	0.07	0.20	1.16
	Carman-L	7.37	69.64	19.30	3.67	0.08	0.20	1.19

The seed from Carman-E had higher monounsaturated fatty acid levels in all groups than did the other site/seeding dates (Figure 4.3). The Carman-E seed also expressed a lower level of dienoic fatty acids than did the seed harvested from the other site/seeding dates. This was the hT regime for Carman. Higher temperatures increase the rate of plant growth and development. If seed development rate increases, the length of time the developing lipids have to be desaturated by the appropriate desaturases decreases, thus creating a more relatively saturated seed lipid profile. The switch from

dienoic to monounsaturated fatty acids in the seed from Carman-E was a response to the elevated temperatures. This finding agrees with other studies which showed an increase in saturation levels of seed oil under elevated temperatures during seed development (Deng and Scarth 1998).

The number of double bonds in an average fatty acid (ADB) value was significantly different ($P < 0.00005$) between the lines (Table 4.5). The HS LL HO and LL HO groups having significantly lower ADB values (or more saturated) than the other groups (Figure 4.4, Table 4.4 and Appendix C: Table 3), reflecting of the low linolenic acid trait selected in this group of lines.

Table 4.5: ANOVA analysis summary table of *B. napus* leaf lipid fatty acid profile number of double bonds in an average fatty acid (ADB) value analyzed by seeding date and plot location.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	351	2.680			
Entry	21	2.352	0.112	131.70	0.0000
Date	1	0.049	0.049	57.57	0.0000
Location	1	0.001	0.001	1.21	0.2730
Date x Loc	1	0.011	0.011	12.98	0.0004
Entry x Date	21	0.013	0.001	0.72	0.8073
Entry x Loc	21	0.008	0.000	0.46	0.9821
E x D x L	21	0.021	0.001	1.20	0.2499
Residual	264	0.224	0.001		

Temperature (date) was a significant factor ($P < 0.00005$) in the ANOVA analysis of the ADB values (Table 4.5). Winnipeg-E and Carman-E had lower ADB values (more saturated fatty acid profiles) than did Winnipeg-L and Carman-L (Figure 4.4). The seed harvested from Winnipeg-E and Carman-E developed under higher temperatures (higher GDD values) than the seed from Winnipeg-L and Carman-L (Table 4.1). The lower ADB values (increased saturation) in the seed profiles from the E seeding date is a result

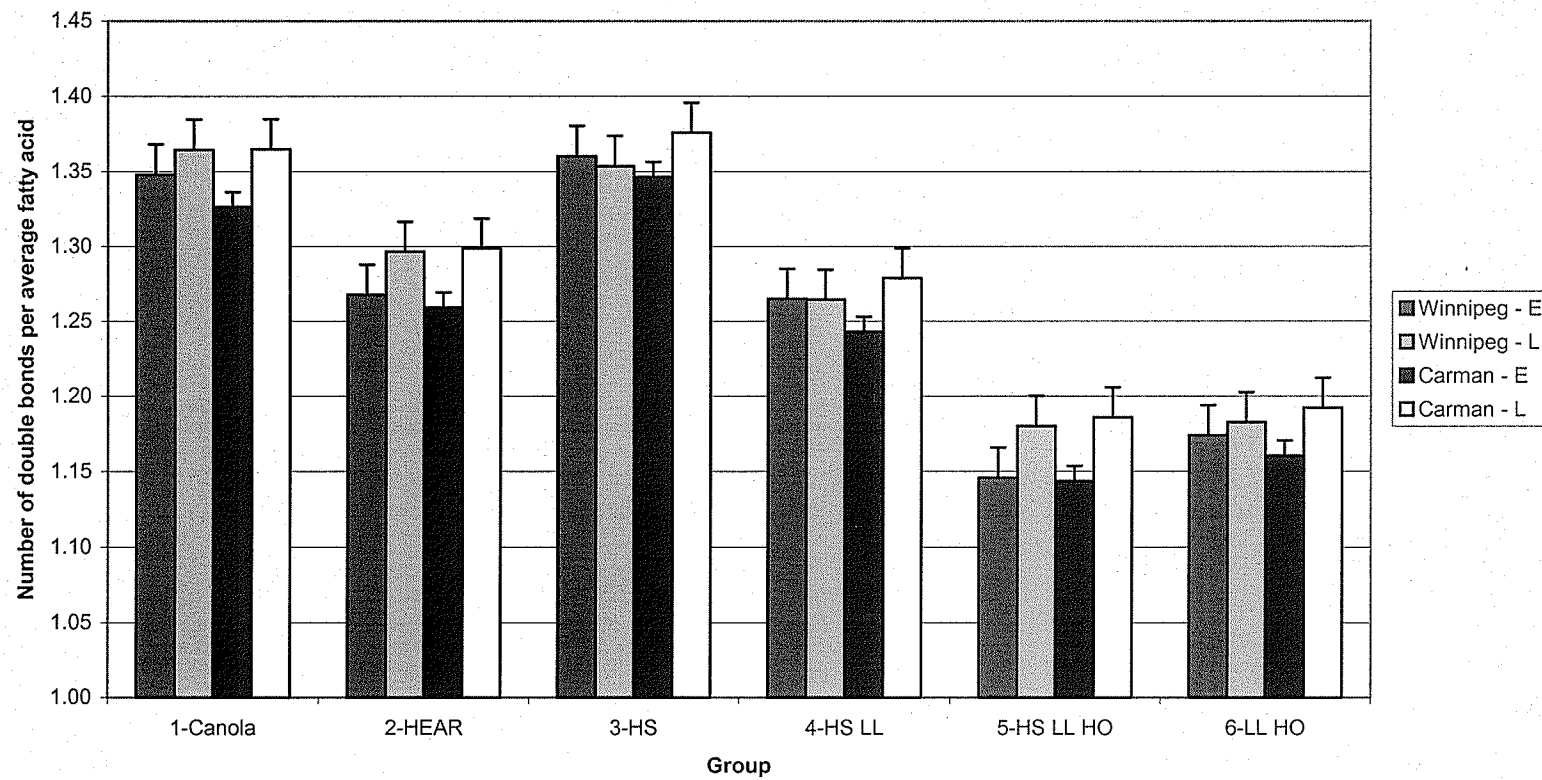


Figure 4.4: Number of double bonds per average fatty acid (ADB) values for seed lipid fatty acid analysis of seven groups of *B. napus* lines (grouped by seed fatty acid profile). Two locations (Winnipeg, Carman), two seeding dates (E: early date; L: late date).

of the shift from dienoic to monounsaturated fatty acids in the E seed profiles. Previous studies showed the effect of elevated temperature increasing the saturation state of seed fatty acid profiles in LL and canola lines (Deng and Scarth 1998). These results confirm the temperature and seed saturation state relationship in LL and canola lines and identify the same response in lines with distinct seed fatty acid profile, of the HEAR, HS and HO type.

Location is non-significant ($P < 0.2730$) (Table 4.5) as the average ADB values from Winnipeg and Carman are 1.24. Since the ADB values are higher for the L seeding date and lower for the E seeding date at both locations, the overall ADB value average from both locations is equal.

In the analysis of ADB values, the date by location interaction was significant ($P < 0.0004$). The seed from Carman-E had a significantly lower ADB value than did the other site/seeding dates (Table 4.5) (Figure 4.4), with higher monounsaturated fatty acid level and the decreased dienoic fatty acid level (Figure 4.3).

Despite the significant date by location interaction, the line by location and line by date interactions indicated the individual lines responded similarly to location and date. The entry by date interaction was non-significant ($P < 0.8073$) (Table 4.5) as all the lines had higher ADB values for the L seeding date than for the E seeding date (Figure 4.4, Appendix C: Table 3). The reduction in saturates corresponds to the lower temperatures during seed development for the L seeding date (Table 4.1). The entry by location interaction was also non-significant ($P < 0.9821$) as the averages of each line by location were similar (Table 4.5) (Appendix C: Table 3).

4.3.3 Conclusions

Leaf lipids were more saturated when grown under elevated temperature conditions (higher GDD) and less saturated when grown under lower temperature conditions (lower GDD). *B. napus* shows an ability to adapt to different temperature conditions with alterations in leaf cell membrane lipid composition to maintain required membrane fluidity. The relationship between leaf lipid fatty acid composition and site/location had a greater influence on the level of saturation than the temperature regime alone. Other agronomic factors like fertility and soil characteristics may play a role in the development of the leaf lipid fatty acid profile characteristics.

The seed fatty acid composition data confirmed the results of previous studies, with additional oil quality types in canola and rapeseed. Elevated temperatures during development of seed lipid fatty acids resulted in more saturated fatty acid profiles in the seed oil. The *B. napus* seed which developed under elevated temperatures showed a significant shift from dienoic to monounsaturated fatty acids producing lower ADB values and a more saturated seed fatty acid profile.

While temperatures (GDD) were higher for the reproductive stage of the E seeding date, they were lower for the vegetative stage of the E seeding date than the L seeding date at Winnipeg and higher for the E seeding date than the L seeding date at Carman. Both leaf and seed fatty acid profiles were more saturated when developing under higher GDD values than under lower GDD values. It appeared the saturation state was more closely linked to GDD values than average temperatures, which could be potentially misleading about the actual number of heat units available for crop growth and development.

The relationship between elevated temperature and elevated seed oil saturation levels (Deng and Scarth 1998) was confirmed in canola and low C18:3 lines. HEAR, HO and HS lines included in the experiment to provide a wide seed oil saturation range also responded to elevated temperatures with increasing seed oil fatty acid saturation levels. This poses a challenge for the canola industry, as temperature increases will increase the saturation levels in canola, including specialty oil lines. This will place an even greater importance on the breeding of low saturate canola varieties in order to maintain canola oil's market share in the vegetable oil industry. Additionally, the ability of the canola industry to deliver specialty oil profiles will depend on the development of knowledge of how individual steps of fatty acid biosynthesis are affected by climate change.

5. GENERAL DISCUSSION AND CONCLUSIONS

The leaf lipids of *Arabidopsis thaliana* and *Brassica napus* lines were significantly affected by elevated temperature in the controlled environment study. Both species had higher levels of saturated fatty acids in their leaf lipids when grown under elevated temperatures. The *B. napus* lines in the study were sorted into three clusters based on the number of double bonds in an average fatty acid (ADB) value for leaf analysis. This was intriguing as the clusters of lines corresponded to the pedigree. Based on this result, there are distinct leaf lipid mutations conferring distinct lipid profiles in *B. napus*. Previous studies of the effect of temperature on leaf lipids in peas (*Pisum sativum* L.) (Chapman *et al.* 1983), corn (*Zea mays*) (Hawke *et al.* 1974), soybean (Alfonso *et al.* 2001) and *B. napus* (Williams *et al.* 1992) also found the direct relationship between elevated temperature and increased saturation in leaf lipids. The *Arabidopsis* fatty acid desaturase (*fad*) mutation results in relatively saturated leaf lipids irrespective of temperature. The altered profile provides increased high temperature tolerance to the *fad7* and *fad7fad8 Arabidopsis* mutant lines (Murakami *et al.* 2000).

Elevated CO₂ level affected leaf lipid composition less significantly than elevated temperature in the controlled environment study. The *Arabidopsis* leaves were relatively unaffected by elevated CO₂ levels, while the *B. napus* leaves were significantly more saturated under elevated CO₂ levels. Little study has been done on leaf lipid response to elevated CO₂, with the exception of winter wheat (*Triticum aestivum*) which was studied for changes in lipid classes and not fatty acid composition. Despite acceptable levels of light in the growth chamber (625 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for indoor trials, levels may not have been high enough to provide full light saturation, comparable to full sun. As a result the plants

may not have responded to the elevated CO₂, as they potentially would under field conditions where light levels are much higher.

Leaf fatty acid analysis of *B. napus* from the field study revealed that the site/location of the trial had a greater influence on saturation level than temperature did. This indicates that other factors, including soil fertility, weather and soil characteristics may play an important role in determining leaf lipid profile. It was also evident that leaf lipids were more saturated when the leaves develop under elevated temperatures (or higher GDD) and less saturated when grown under lower temperatures (lower GDD). This agrees with previous studies (Chapman *et al.* 1983, Hawke *et al.* 1974, Alfonso *et al.* 2001, Williams *et al.* 1992) which indicated a direct relationship between elevated temperature and increased leaf lipid saturation level.

The oil fatty acid profile of seed harvested from the trials grown under elevated temperature at both Carman and Winnipeg was significantly more saturated than seed oil fatty acid profiles from the lower temperature location/dates. The increased saturation levels (lower ADB value) in the seed which developed under elevated temperature conditions was largely a result of a decreased desaturation, with lower C18:2 and higher C18:1 levels, as compared to the seed from the lower temperature treatment. This compared well to the previous study by Deng and Scarth (1998). This study extended the elevated temperature/elevated saturate level relationship to include the distinct oil profiles with high saturate, high C18:1 and high erucic acid rapeseed (HEAR) lines.

For future study, the *B. napus* could be allowed to develop to maturity in the treatment chamber to better simulate climate change conditions. Alternatively, the temperature regime could be altered to create elevated temperature periods to simulate

the sporadic high temperature events predicted to be associated with climate change (Environment Canada 2002). Another potential project would be to run the field trials from this study with equipment in place to create an elevated CO₂ environment [e.g. Free Air Carbon dioxide Enrichment (FACE)] to better take advantage of the light levels in the field. It would also be interesting have data on the effects of climate change on the fatty acid composition of specific lipids. Using High Performance Liquid Chromatography (HPLC), leaf diglycerides and seed triglycerides could be separated prior to gas chromatography analysis to get a more accurate idea of which lipid classes respond most significantly to climate change conditions. This would indicate which enzymes and genes to target for study and manipulation in an attempt to develop *B. napus* lines suited for growth under changing environmental conditions.

Leaf and seed fatty acid profiles are generally unrelated. Mutations rarely affect both profiles despite the common pathway steps in leaf and seed fatty acid biosynthesis. The reason for this is unknown, although one theory suggests cellular compartmentalization is involved (Somerville *et al.* 2000). However, leaf and seed lipid responses to elevated temperature are similar with increases in saturation level or relative saturation level. Canola breeders must decrease the level of saturates in *B. napus* seed oil to avoid increases in saturate levels resulting from increasing temperatures and the resulting loss of market share.

The increase in leaf membrane saturation level found in *fad* mutant lines may be a useful trait to canola breeders for production of canola varieties which will be adapted to climate change conditions. The fatty acid desaturase (*FAD*) genes could be silenced, or otherwise inhibited, in order to produce *B. napus* lines which are better adapted to the

elevated temperatures associated with climate change. In order to maintain the growth, development and oil quality of specialty oil and conventional canola varieties under climate change, the leaf *FAD* genes could be modified in *B. napus* germplasm developed for the low saturate seed oil trait.

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7. APPENDICES

APPENDIX A

Table 1: *Arabidopsis thaliana* lines grown growth chamber experiment

Stock Number	Source	Name	Phenotype	Background
N933	NASC	<i>Col-4</i>	Columbia background	Columbia
CS200	AIMS	<i>act1</i>	chloroplast glycerol-3-phosphate acyltransferase deficient	Columbia
CS8036	AIMS	<i>fad7fad8</i>	chloroplast omega3 fatty acid desaturase deficient/fatty acid desaturase deficient	Columbia
CS3108	AIMS	<i>fad7gl1</i>	chloroplast omega3 fatty acid desaturase deficient/glabra	Landsberg erecta
N209	NASC	<i>fad7</i>	chloroplast omega3 fatty acid desaturase deficient	Columbia
NW20	NASC	<i>Ler-0</i>	Landsberg erecta background	Landsberg erecta
N205	NASC	<i>fad4</i>	chloroplast omega3 fatty acid desaturase deficient	Columbia

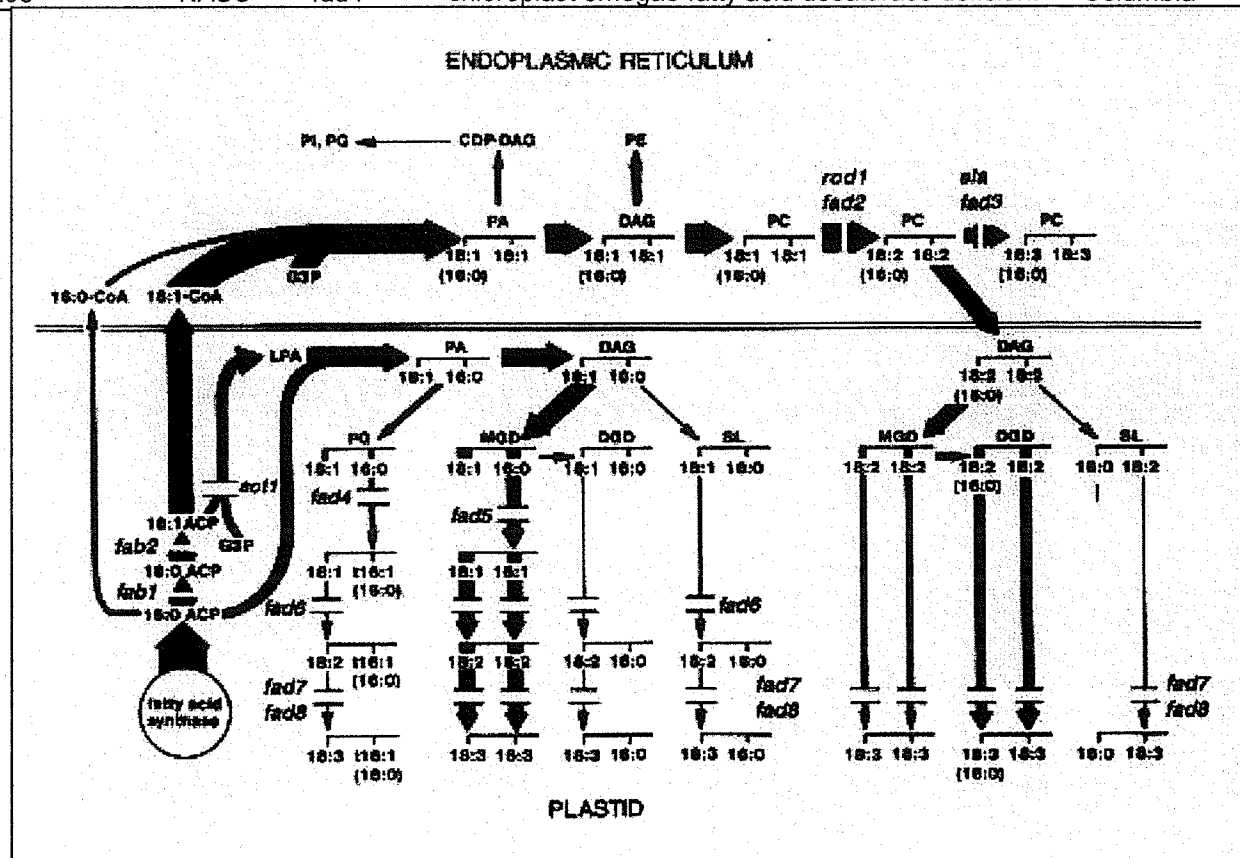


Table 2: Seed fatty acid profiles of doubled haploid *Brassica napus* lines grown in growth chamber experiment

ID Number	Seed Type	Cross	Saturated Cxx:0	Palmitic C16:0	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Arachidic C20:0	Eicosenoic C20:1	Behenic C22:0	Erucic C22:1	24C+
00DH-1	HS	Dynamite x FA18-1	10.1	4.6	3.3	67.2	15.6	4.7	1.4	1.4	0.8	0.0	0.9
00DH-2	Canola	Dynamite x FA18-1	6.5	4.2	1.4	67.3	17.1	6.5	0.6	1.8	0.3	0.0	0.9
00DH-25	HS, LL	970247 x FA16-4	13.4	3.6	6.9	73.0	9.2	2.0	2.1	1.5	0.8	0.0	0.6
00DH-27	HS, LL, HO	970247 x FA16-4	10.1	3.7	4.0	77.0	8.2	1.5	1.6	1.7	0.8	0.1	1.0
00DH-44	HS, LL	97C29 x LG3340	11.5	4.0	5.0	68.0	15.3	2.8	1.7	1.2	0.8	0.0	0.7
00DH-52	Canola	97C29 x LG3340	7.7	4.3	2.0	67.0	18.2	4.7	0.9	1.6	0.5	0.0	0.8
DH1-53	HEAR	(Neptune x W235) x Neptune	4.6	2.6	0.6	12.6	11.9	8.7	0.5	5.0	0.6	53.2	1.4
DH1-54	HEAR	(Neptune x W235) x Neptune	4.8	2.7	0.7	13.8	12.0	7.6	0.6	5.2	0.6	52.6	1.5
DH2-68	HEAR	(Neptune x 235WC) x (Castor x Quantum PB HEAR)	5.3	3.5	1.1	28.8	16.4	9.9	0.5	12.0	0.2	25.0	1.2
00DH-70	LL, HO	970247 x FA16-4	7.7	4.0	2.2	75.9	11.2	2.8	1.0	1.6	0.5	0.0	0.8
DH2-113	HEAR	(Neptune x 235WC) x (Castor x Quantum PB HEAR)	5.6	3.0	1.4	28.1	16.6	9.3	0.6	11.4	0.3	26.2	1.2
DH2-117	HEAR	(Neptune x 235WC) x (Castor x Quantum PB HEAR)	5.3	2.8	0.9	13.7	12.4	9.2	0.7	6.9	0.7	48.6	1.5
00DH-124	Canola	97C29 x LG3340	6.7	4.5	1.3	66.0	21.1	3.4	0.6	1.6	0.3	0.0	0.8
DH3-162	HEAR	HEAR 196 x HBNRes Neptune	4.9	2.9	0.7	10.1	13.3	9.0	0.6	5.3	0.5	53.1	1.5
00DH-200	LL, HO	970428 x FA16-4	8.9	4.0	2.9	78.5	7.9	2.0	1.3	1.4	0.7	0.0	0.6
00DH201	LL, HO	970428 x FA16-4	8.5	3.7	2.9	79.0	8.2	2.0	1.3	1.4	0.6	0.0	0.6
00DH-215	LL, HO	970428 x FA16-4	8.3	3.7	2.8	78.3	9.0	2.1	1.2	1.5	0.6	0.0	0.7
00DH-292	LL, HO	970069 x 97C121	7.6	4.2	2.2	78.0	9.9	1.9	0.8	1.2	0.4	0.1	0.8
00DH-293	LL, HO	970069 x 97C121	7.1	4.1	1.9	75.0	12.5	2.7	0.7	1.3	0.4	0.3	0.8
00DH-294	LL, HO	970069 x 97C121	7.1	3.9	1.9	79.0	9.2	1.3	0.8	1.7	0.5	0.2	0.6
00DH-326	LL, HO	970126 x 97C49	6.7	3.7	1.8	77.5	11.3	1.6	0.7	1.7	0.5	0.1	0.9
00DH-327	LL, HO	970126 x 97C49	6.8	3.6	2.0	76.5	12.4	1.4	0.8	1.5	0.4	0.3	0.9
00DH-627	LL, HO	970001 x 97C49	7.1	3.5	2.0	77.0	11.7	1.3	0.9	1.8	0.7	0.4	0.5
00DH-629	LL, HO	970001 x 97C49	7.3	3.6	2.3	80.8	7.6	1.8	0.9	1.6	0.5	0.0	0.7

APPENDIX B

Table 1: *Arabidopsis* leaf fatty acid analysis grouped by double bond categories, saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and double bonds in an average fatty acid. Different letters indicate significantly different values by LSD test ($P < 0.05$) between treatments in each line.

Line	Treatment	xx:0	xx:1	xx:2	xx:3	Sat./Unsat. xx:3/xx:2	ADB	
N933	aTaCO ₂	26.64ab	9.38b	15.14a	48.11c	0.37a	3.27c	1.85a
	aTeCO ₂	28.69b	7.54a	17.25b	43.64b	0.43b	2.58b	1.78a
	eTaCO ₂	26.15ab	7.70a	19.07c	44.86b	0.37a	2.36b	1.85a
	eTeCO ₂	23.95a	11.49c	25.40d	39.53a	0.32a	1.57a	1.80a
N209	aTaCO ₂	24.53b	11.51a	22.72a	40.86b	0.33a	2.02c	1.80b
	aTeCO ₂	27.20c	9.95a	25.71a	36.36b	0.38b	1.48b	1.72a
	eTaCO ₂	22.08a	11.05a	34.61b	27.15a	0.30a	0.79a	1.70a
	eTeCO ₂	23.72ab	11.49a	35.43b	29.71a	0.31a	0.85a	1.71a
CS8036	aTaCO ₂	21.23b	13.79c	29.57a	29.87b	0.29b	1.21c	1.73a
	aTeCO ₂	22.80bc	9.03a	39.87b	27.82b	0.30b	0.73ab	1.73a
	eTaCO ₂	23.33c	9.46ab	36.63b	27.99b	0.32b	0.77b	1.71a
	eTeCO ₂	19.49a	11.51b	49.05c	20.17a	0.24a	0.41a	1.70a
N205	aTaCO ₂	28.41b	9.11b	14.11a	43.55a	0.43b	3.29c	1.77ab
	aTeCO ₂	32.44c	7.46a	15.65b	43.18a	0.50c	2.78b	1.70a
	eTaCO ₂	27.90ab	6.25a	19.88c	43.83a	0.40b	2.23a	1.81b
	eTeCO ₂	25.59a	9.36b	22.06d	43.61a	0.34a	1.99a	1.83b
CS200	aTaCO ₂	22.29d	16.07a	17.05a	39.17bc	0.31d	2.36b	1.78a
	aTeCO ₂	19.42c	20.01b	23.36b	36.73ab	0.24c	1.61a	1.78a
	eTaCO ₂	17.36b	18.84ab	24.33b	35.62a	0.22b	1.47a	1.81a
	eTeCO ₂	15.64a	16.95a	27.61c	39.90c	0.19a	1.47a	1.92b
NW20	aTaCO ₂	22.67b	11.85c	18.93ab	45.51a	0.30b	2.53a	1.89a
	aTeCO ₂	24.51c	7.70a	18.61a	48.64b	0.33c	2.66a	1.92a
	eTaCO ₂	22.92b	8.57ab	18.44a	45.36a	0.32c	2.50a	1.91a
	eTeCO ₂	20.06a	9.95b	20.74b	49.41b	0.25a	2.43a	1.99b
CS3108	aTaCO ₂	26.24b	11.16b	21.84a	37.10b	0.38b	1.81b	1.73a
	aTeCO ₂	27.14b	8.48a	23.19a	38.36b	0.39b	1.75b	1.75a
	eTaCO ₂	22.11a	9.78a	34.70b	29.18a	0.30a	0.85a	1.74a
	eTeCO ₂	21.25a	12.49c	37.41b	28.87a	0.27a	0.78a	1.74a

Table 2: ANOVA *Arabidopsis* leaf N933 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.627			
Temperature	1	0.000	0.000	0.04	0.8487
CO2	1	0.047	0.047	4.23	0.0447
Temp x CO2	1	0.003	0.003	0.27	0.6041
Residual	52	0.576	0.011		

Table 3: ANOVA *Arabidopsis* leaf N209 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.394			
Temperature	1	0.040	0.040	6.94	0.0111
CO2	1	0.023	0.023	3.89	0.0539
Temp x CO2	1	0.028	0.028	4.73	0.0341
Residual	52	0.303	0.006		

Table 4: ANOVA *Arabidopsis* leaf CS8036 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.289			
Temperature	1	0.008	0.008	1.53	0.2222
CO2	1	0.000	0.000	0.06	0.8024
Temp x CO2	1	0.001	0.001	0.19	0.6610
Residual	52	0.280	0.005		

Table 5: ANOVA *Arabidopsis* leaf N205 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.701			
Temperature	1	0.104	0.104	9.61	0.0031
CO2	1	0.009	0.009	0.79	0.3782
Temp x CO2	1	0.025	0.025	2.34	0.1319
Residual	52	0.563	0.011		

Table 6: ANOVA *Arabidopsis* leaf CS200 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.462			
Temperature	1	0.107	0.107	19.85	0.0000
CO2	1	0.038	0.038	7.04	0.0105
Temp x CO2	1	0.038	0.038	7.01	0.0107
Residual	52	0.279	0.005		

Table 7: ANOVA *Arabidopsis* leaf NW20 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.319			
Temperature	1	0.031	0.031	7.27	0.0094
CO2	1	0.052	0.052	12.05	0.0010
Temp x CO2	1	0.010	0.010	2.40	0.1275
Residual	52	0.225	0.004		

Table 8: ANOVA *Arabidopsis* leaf CS3108 number of double bonds in an average fatty acid.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	55	0.415			
Temperature	1	0.000	0.000	0.00	0.9464
CO2	1	0.001	0.001	0.14	0.7123
Temp x CO2	1	0.002	0.002	0.21	0.6469
Residual	52	0.412	0.008		

Table 9: *B. napus* groups fatty acid analysis by treatment presented in fatty acid categories along with saturated to unsaturated fatty acid ratio, xx:3 to xx:2 fatty acid ratio, number of double bonds per average fatty acid value, and number of double bonds per average fatty acid value averaged for each group. Different letters indicate significantly different values by LSD test ($P < 0.05$) between treatments in each group or between groups for ADB avg.

Group	Treatment	xx:0	xx:1	xx:2	xx:3	Sat./Unsat.	xx:3/xx:2	ADB	ADB avg.
1-1&2	aTaCO ₂	15.97a	6.35b	18.68a	61.53b	0.18a	3.31a	2.28c	
	aTeCO ₂	17.66a	3.67a	18.10a	60.60b	0.22b	3.36a	2.22c	
	eTaCO ₂	19.07b	3.51a	18.04a	59.33b	0.24b	3.32a	2.18b	
	eTeCO ₂	21.95c	5.20ab	17.15a	55.71a	0.28c	3.31a	2.07a	2.19c
2-HEAR	aTaCO ₂	16.49a	4.25a	19.78b	59.73c	0.20a	3.03a	2.23d	
	aTeCO ₂	16.76a	5.63b	19.43b	58.20b	0.20a	3.01a	2.19c	
	eTaCO ₂	19.78b	4.99b	17.99a	57.25b	0.25b	3.21b	2.13b	
	eTeCO ₂	20.60c	6.94c	17.84a	54.63a	0.26c	3.11ab	2.06a	2.15b
3-Others	aTaCO ₂	16.03a	5.55a	21.64b	56.91c	0.19a	2.66a	2.20d	
	aTeCO ₂	16.58b	7.10b	21.40b	54.88b	0.20a	2.60a	2.14c	
	eTaCO ₂	19.81c	7.75c	18.38a	54.05b	0.25b	3.01b	2.07b	
	eTeCO ₂	21.06d	9.80d	18.84a	50.29a	0.27c	2.74a	1.98a	2.10a

Table 10: ANOVA analysis summary table of number of double bond in an average fatty acid value for the leaf fatty acid profiles of group 1 of the *B. napus* lines.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	31	0.303			
Entry	1	0.002	0.002	0.66	0.4255
Temperature	1	0.150	0.150	43.25	0.0000
CO2	1	0.049	0.049	14.09	0.0010
Temp x CO2	1	0.005	0.005	1.37	0.2530
Ent x Temp	1	0.014	0.014	4.05	0.0556
Ent x CO2	1	0.000	0.000	0.07	0.7893
E x T x CO2	1	0.000	0.000	0.07	0.7893
Residual	24	0.083	0.003		

Table 11: ANOVA analysis summary table of number of double bond in an average fatty acid value for the leaf fatty acid profiles of group 2 of the *B. napus* lines.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	95	0.589			
Entry	5	0.022	0.004	2.66	0.0290
Temperature	1	0.311	0.311	183.80	0.0000
CO2	1	0.083	0.083	49.03	0.0000
Temp x CO2	1	0.010	0.010	5.68	0.0198
Ent x Temp	5	0.025	0.005	2.93	0.0185
Ent x CO2	5	0.003	0.001	0.32	0.8988
E x T x CO2	5	0.015	0.003	1.75	0.1338
Residual	72	0.122	0.002		

Table 12: ANOVA analysis summary table of number of double bond in an average fatty acid value for the leaf fatty acid profiles of group 3 of the *B. napus* lines.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	255	2.315			
Entry	15	0.063	0.004	1.59	0.0782
Temperature	1	1.340	1.340	508.94	0.0000
CO2	1	0.281	0.281	106.70	0.0000
Temp x CO2	1	0.016	0.016	5.94	0.0158
Ent x Temp	15	0.026	0.002	0.65	0.8338
Ent x CO2	15	0.053	0.004	1.34	0.1836
E x T x CO2	15	0.032	0.002	0.81	0.6660
Residual	192	0.505	0.003		

Table 13: ANOVA analysis summary table of the comparison of the number of double bond in an average fatty acid value for the leaf fatty acid profiles of the three groups of *B. napus* lines.

Source	Degrees of freedom	Sums of squares	Mean square	F-value	Pr > F
Total	383	3.594			
Group	2	0.386	0.193	74.37	0.0000
Temperature	1	1.784	1.784	687.52	0.0000
CO2	1	0.410	0.410	158.11	0.0000
Temp x CO2	1	0.029	0.029	11.27	0.0009
Group x Temp	2	0.017	0.008	3.22	0.0410
Ent x CO2	2	0.002	0.001	0.46	0.6297
E x T x CO2	2	0.001	0.000	0.15	0.8650
Residual	372	0.965	0.003		

Table 14: Averages of seed fatty acid analysis for seven *Arabidopsis* lines presented by fatty acid category, saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid (ADB) value over four temperature and CO₂ level treatments.

Line	Treatment	xx:0	xx:1	xx:2	xx:3	Sat./Unsat.	xx:3/xx:2	ADB
N933	aTaCO ₂	15.12b	35.61a	30.01a	17.66a	0.18b	0.59a	1.49ab
	aTeCO ₂	15.31b	34.29a	29.64a	17.31a	0.19b	0.58a	1.45a
	eTaCO ₂	14.33b	33.68a	29.74a	19.01b	0.18ab	0.64b	1.50b
	eTeCO ₂	12.75a	34.58a	29.42a	18.73b	0.16a	0.64b	1.50ab
N209	aTaCO ₂	14.23ab	36.62a	29.59a	18.67b	0.17ab	0.63b	1.52b
	aTeCO ₂	16.35b	33.41a	30.19a	16.53a	0.21b	0.55a	1.43a
	eTaCO ₂	13.66a	34.31a	29.74a	18.91b	0.17a	0.64b	1.51b
	eTeCO ₂	12.73a	35.03a	29.76a	17.97ab	0.16a	0.60ab	1.48ab
CS8036	aTaCO ₂	13.92ab	36.87b	29.80a	18.41b	0.17a	0.62b	1.52b
	aTeCO ₂	14.55b	32.56a	30.94a	16.97a	0.18a	0.55a	1.45ab
	eTaCO ₂	13.97ab	33.52a	31.36a	17.96ab	0.17a	0.57ab	1.50ab
	eTeCO ₂	12.62a	34.15a	30.83a	17.31ab	0.16a	0.56ab	1.48a
N205	aTaCO ₂	14.84b	35.94a	29.48a	18.77b	0.18a	0.64b	1.51b
	aTeCO ₂	16.95c	33.09a	28.98a	17.09a	0.22b	0.59a	1.42a
	eTaCO ₂	14.11a	33.16a	29.34a	19.60b	0.17a	0.67b	1.51b
	eTeCO ₂	12.91a	34.97a	29.16a	18.88b	0.16a	0.65b	1.50b
CS200	aTaCO ₂	14.29b	35.39a	30.22a	16.77b	0.17b	0.56b	1.46a
	aTeCO ₂	15.35c	36.95a	30.12a	15.44a	0.19c	0.51a	1.44a
	eTaCO ₂	14.20b	34.20a	31.31b	16.42ab	0.17b	0.52ab	1.46a
	eTeCO ₂	12.57a	36.66a	30.53ab	16.11ab	0.15a	0.53ab	1.46a
NW20	aTaCO ₂	14.14bc	36.76a	28.25a	18.18b	0.17b	0.64bc	1.48b
	aTeCO ₂	15.18c	37.08a	27.92a	16.50a	0.19b	0.59ab	1.42a
	eTaCO ₂	13.91b	37.71a	29.06a	16.88a	0.17b	0.58a	1.46b
	eTeCO ₂	11.90a	39.81a	27.99a	18.06b	0.14a	0.65c	1.50b
CS3108	aTaCO ₂	14.49a	35.93b	29.59a	18.49a	0.17a	0.63a	1.51b
	aTeCO ₂	14.29a	35.10b	29.32a	18.13a	0.18a	0.62a	1.48ab
	eTaCO ₂	13.34a	34.67ab	29.41a	18.88a	0.16a	0.65a	1.50ab
	eTeCO ₂	14.65a	32.97a	29.80a	17.80a	0.18a	0.60a	1.46a

Table 15: Averages of seed fatty acid analysis for seven *B. napus* lines presented by fatty acid category, saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid (ADB) value over four temperature and CO₂ level treatments.

Line	Treatment	xx:0	xx:1	xx:2	xx:3	Sat/Unsatxx:3/xx:2	ADB	
00DH-1	aTaCO ₂	7.56	61.09	21.27	9.44	0.08	0.45	1.32
	aTeCO ₂	8.65	62.42	19.91	8.23	0.10	0.40	1.27
	eTaCO ₂	8.15	65.09	17.98	8.20	0.09	0.45	1.26
	eTeCO ₂	8.58	64.33	19.27	7.04	0.09	0.36	1.24
00DH-2	aTaCO ₂	7.25	63.37	20.06	8.44	0.08	0.42	1.29
	aTeCO ₂	7.73	64.85	19.13	7.43	0.09	0.37	1.25
	eTaCO ₂	7.03	66.44	18.55	7.44	0.08	0.40	1.26
	eTeCO ₂	7.22	66.27	18.58	6.75	0.08	0.36	1.24
00DH-25	aTaCO ₂	6.42	73.05	15.59	4.18	0.07	0.27	1.17
	aTeCO ₂	7.03	72.96	15.50	3.49	0.08	0.22	1.14
	eTaCO ₂	6.71	72.54	16.06	3.93	0.07	0.25	1.16
	eTeCO ₂	8.55	74.17	13.09	3.33	0.10	0.25	1.10
00DH-27	aTaCO ₂	6.73	75.09	14.45	2.97	0.07	0.21	1.13
	aTeCO ₂	6.87	76.24	13.56	2.52	0.07	0.19	1.11
	eTaCO ₂	6.42	75.94	13.89	2.80	0.07	0.20	1.12
	eTeCO ₂	6.64	76.63	13.15	2.74	0.07	0.21	1.11
00DH-44	aTaCO ₂	6.33	65.57	22.14	5.18	0.07	0.23	1.25
	aTeCO ₂	7.19	69.98	17.84	4.17	0.08	0.22	1.18
	eTaCO ₂	7.42	62.38	23.61	5.88	0.08	0.25	1.27
	eTeCO ₂	6.81	70.35	18.14	3.82	0.07	0.21	1.18
00DH-52	aTaCO ₂	6.69	55.57	26.75	10.44	0.07	0.39	1.40
	aTeCO ₂	7.37	56.72	26.87	8.11	0.08	0.31	1.35
	eTaCO ₂	7.07	54.57	27.59	10.04	0.08	0.37	1.40
	eTeCO ₂	7.05	60.05	24.73	7.19	0.08	0.29	1.31
DH1-53	aTaCO ₂	4.56	72.26	12.22	8.69	0.05	0.71	1.23
	aTeCO ₂	5.48	72.61	12.99	7.17	0.06	0.56	1.20
	eTaCO ₂	5.36	72.35	12.39	7.56	0.06	0.61	1.20
	eTeCO ₂	5.45	73.14	12.48	6.80	0.06	0.54	1.18
DH1-54	aTaCO ₂	4.79	73.11	12.33	7.63	0.05	0.62	1.21
	aTeCO ₂	5.50	74.93	11.94	6.15	0.06	0.51	1.17
	eTaCO ₂	5.81	71.86	12.47	7.48	0.06	0.60	1.19
	eTeCO ₂	5.42	74.31	12.23	6.12	0.06	0.50	1.17
DH2-68	aTaCO ₂	5.34	67.12	16.86	9.89	0.06	0.59	1.30
	aTeCO ₂	5.98	68.27	16.85	7.96	0.06	0.48	1.26
	eTaCO ₂	5.93	65.68	17.28	10.28	0.06	0.60	1.31
	eTeCO ₂	6.20	68.74	16.01	8.14	0.07	0.50	1.25

00DH-70	aTaCO ₂	6.57	70.04	17.80	4.73	0.07	0.27	1.20
	aTeCO ₂	6.49	71.46	16.67	4.21	0.07	0.25	1.17
	eTaCO ₂	6.77	69.75	17.98	4.66	0.07	0.26	1.20
	eTeCO ₂	7.03	71.77	16.02	4.25	0.08	0.27	1.17
DH2-113	aTaCO ₂	5.57	66.98	17.18	9.29	0.06	0.55	1.29
	aTeCO ₂	5.91	67.60	17.29	8.23	0.06	0.47	1.27
	eTaCO ₂	5.79	64.67	17.83	10.66	0.06	0.60	1.32
	eTeCO ₂	5.90	66.73	17.45	9.09	0.06	0.52	1.29
DH2-117	aTaCO ₂	5.30	70.73	12.87	9.19	0.06	0.71	1.24
	aTeCO ₂	5.68	73.59	12.37	7.23	0.06	0.58	1.20
	eTaCO ₂	5.53	71.61	12.40	8.44	0.06	0.68	1.22
	eTeCO ₂	5.55	72.84	12.15	7.72	0.06	0.63	1.20
00DH-124	aTaCO ₂	6.00	57.89	29.04	6.26	0.06	0.22	1.35
	aTeCO ₂	6.75	59.55	27.81	5.23	0.07	0.19	1.31
	eTaCO ₂	6.75	62.43	24.67	5.34	0.07	0.22	1.28
	eTeCO ₂	6.91	63.05	24.77	4.44	0.08	0.18	1.26
DH3-162	aTaCO ₂	4.93	70.00	13.74	9.03	0.05	0.66	1.25
	aTeCO ₂	5.75	72.22	14.09	6.67	0.06	0.47	1.20
	eTaCO ₂	5.16	70.72	13.69	8.50	0.06	0.62	1.24
	eTeCO ₂	5.21	72.03	13.43	7.12	0.06	0.53	1.20
00DH-200	aTaCO ₂	7.32	77.33	11.63	3.18	0.08	0.27	1.10
	aTeCO ₂	9.50	77.08	10.02	2.58	0.11	0.26	1.05
	eTaCO ₂	8.64	75.99	11.60	2.98	0.10	0.26	1.08
	eTeCO ₂	9.23	78.54	9.25	2.37	0.10	0.26	1.04
00DH-201	aTaCO ₂	7.36	78.84	10.18	2.84	0.08	0.28	1.08
	aTeCO ₂	9.58	77.00	9.93	2.68	0.11	0.27	1.05
	eTaCO ₂	8.91	77.00	10.61	2.80	0.10	0.26	1.07
	eTeCO ₂	9.72	77.06	9.65	2.65	0.11	0.27	1.04
00DH-215	aTaCO ₂	8.22	75.89	12.09	3.13	0.09	0.26	1.09
	aTeCO ₂	9.48	77.34	10.02	2.62	0.11	0.27	1.05
	eTaCO ₂	8.80	73.95	13.24	3.30	0.10	0.25	1.10
	eTeCO ₂	9.72	75.70	10.98	2.97	0.11	0.27	1.07
00DH-292	aTaCO ₂	7.13	76.06	12.90	3.32	0.08	0.26	1.12
	aTeCO ₂	8.00	77.61	10.99	2.74	0.09	0.25	1.08
	eTaCO ₂	8.73	75.12	12.35	3.11	0.10	0.25	1.09
	eTeCO ₂	8.48	77.06	11.06	2.71	0.09	0.24	1.07
00DH-293	aTaCO ₂	7.31	73.20	14.69	4.10	0.08	0.28	1.15
	aTeCO ₂	7.76	75.85	12.37	3.30	0.08	0.27	1.11
	eTaCO ₂	8.44	73.76	13.33	3.61	0.09	0.26	1.11
	eTeCO ₂	8.14	75.39	12.46	3.43	0.09	0.27	1.11
00DH-294	aTaCO ₂	7.40	78.40	11.43	2.21	0.08	0.20	1.08
	aTeCO ₂	7.27	79.87	10.29	1.94	0.08	0.19	1.06
	eTaCO ₂	8.42	76.38	12.13	2.31	0.09	0.19	1.08
	eTeCO ₂	7.87	77.99	11.12	2.29	0.09	0.21	1.07

00DH-326	aTaCO ₂	7.02	71.98	17.21	2.73	0.08	0.16	1.15
	aTeCO ₂	6.45	74.62	15.62	2.36	0.07	0.15	1.13
	eTaCO ₂	8.60	73.75	14.27	2.56	0.10	0.19	1.10
	eTeCO ₂	6.36	75.59	14.98	2.32	0.07	0.16	1.13
00DH-327	aTaCO ₂	7.10	76.76	13.32	2.29	0.08	0.17	1.10
	aTeCO ₂	7.44	77.34	12.72	1.83	0.08	0.14	1.08
	eTaCO ₂	6.92	72.95	16.49	2.72	0.08	0.17	1.14
	eTeCO ₂	6.90	76.85	13.29	2.30	0.08	0.18	1.10
00DH-627	aTaCO ₂	6.63	71.26	19.07	2.16	0.07	0.11	1.16
	aTeCO ₂	7.46	71.74	17.48	2.17	0.08	0.13	1.13
	eTaCO ₂	8.63	69.33	18.54	2.73	0.10	0.15	1.15
	eTeCO ₂	9.45	68.85	18.28	2.56	0.11	0.15	1.13
00DH-629	aTaCO ₂	6.18	76.54	13.84	2.78	0.07	0.20	1.13
	aTeCO ₂	7.09	77.58	12.11	2.54	0.08	0.21	1.09
	eTaCO ₂	8.17	73.69	14.40	3.00	0.09	0.21	1.12
	eTeCO ₂	6.75	77.16	12.90	2.59	0.07	0.20	1.11

Table 1: Seed fatty acid profiles of doubled haploid *Brassica napus* lines utilized in field experiment.

ID Number	Seed Type	Cross	Saturated Palmitic Stearic Oleic Linoleic Linolenic Arachidic Eicosenoic Behenic Erucic										
			Cxx:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	24C+
00DH-1	HS	Dynamite x FA18-1	10.1	4.6	3.3	67.2	15.6	4.7	1.4	1.4	0.8	0.0	0.9
00DH-2	Canola	Dynamite x FA18-1	6.5	4.2	1.4	67.3	17.1	6.5	0.6	1.8	0.3	0.0	0.9
00DH-25	HS, LL	970247 x FA16-4	13.4	3.6	6.9	73.0	9.2	2.0	2.1	1.5	0.8	0.0	0.6
00DH-27	HS, LL, HO	970247 x FA16-4	10.1	3.7	4.0	77.0	8.2	1.5	1.6	1.7	0.8	0.1	1.0
00DH-44	HS, LL	97C29 x LG3340	11.5	4.0	5.0	68.0	15.3	2.8	1.7	1.2	0.8	0.0	0.7
00DH-52	Canola	97C29 x LG3340	7.7	4.3	2.0	67.0	18.2	4.7	0.9	1.6	0.5	0.0	0.8
00DH-70	LL, HO	970247 x FA16-4	7.7	4.0	2.2	75.9	11.2	2.8	1.0	1.6	0.5	0.0	0.8
00DH-124	Canola	97C29 x LG3340	6.7	4.5	1.3	66.0	21.1	3.4	0.6	1.6	0.3	0.0	0.8
00DH-200	LL, HO	970428 x FA16-4	8.9	4.0	2.9	78.5	7.9	2.0	1.3	1.4	0.7	0.0	0.6
00DH-201	LL, HO	970428 x FA16-4	8.5	3.7	2.9	79.0	8.2	2.0	1.3	1.4	0.6	0.0	0.6
00DH-215	LL, HO	970428 x FA16-4	8.3	3.7	2.8	78.3	9.0	2.1	1.2	1.5	0.6	0.0	0.7
00DH-292	LL, HO	970069 x 97C121	7.6	4.2	2.2	78.0	9.9	1.9	0.8	1.2	0.4	0.1	0.8
00DH-293	LL, HO	970069 x 97C121	7.1	4.1	1.9	75.0	12.5	2.7	0.7	1.3	0.4	0.3	0.8
00DH-294	LL, HO	970069 x 97C121	7.1	3.9	1.9	79.0	9.2	1.3	0.8	1.7	0.5	0.2	0.6
00DH-326	LL, HO	970126 x 97C49	6.7	3.7	1.8	77.5	11.3	1.6	0.7	1.7	0.5	0.1	0.9
00DH-327	LL, HO	970126 x 97C49	6.8	3.6	2.0	76.5	12.4	1.4	0.8	1.5	0.4	0.3	0.9
00DH-629	LL, HO	970001 x 97C49	7.3	3.6	2.3	80.8	7.6	1.8	0.9	1.6	0.5	0.0	0.7
Apollo	LL		7.4	4.2	2.4	65.3	22.6	1.5	0.8	1.2	0.0	0.4	0.3
Mercury	HEAR		5.5	2.8	0.9	12.9	11.6	9.3	0.8	7.1	0.8	50.8	1.4
MilleniUM03	HEAR		5.8	2.9	1.0	14.9	12.0	6.8	0.9	8.2	0.8	49.1	1.3
Q2	Canola		7.1	4.0	1.8	62.5	18.5	9.6	0.7	1.3	0.4	0.0	0.3
Reston	HEAR		5.9	3.1	1.2	23.9	13.6	6.7	0.8	12.6	0.6	34.4	1.0

Table 2: Leaf lipid fatty acid profile data of *B. napus* lines based on seed fatty acid profile grown at two locations and two seeding dates, presented by fatty acid category. Saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid values also presented.

Line/ Type	Location/ Seeding date		xx:0	xx:1	xx:2	xx:3	Sat/ Unsat	xx:3/ xx:2	ADB
00DH-1 (HS)	Winnipeg-E		22.90	6.27	19.13	51.71	0.30	2.76	2.00
	Winnipeg-L		23.03	6.37	20.21	50.41	0.30	2.51	1.98
	Carman-E		24.76	8.06	19.15	47.82	0.33	2.55	1.90
	Carman-L		23.31	5.80	18.97	51.93	0.30	2.74	2.00
00DH-2 (Canola)	Winnipeg-E		22.81	6.54	20.31	50.34	0.30	2.55	1.98
	Winnipeg-L		26.01	5.44	19.49	48.89	0.35	2.51	1.91
	Carman-E		28.23	6.61	16.93	48.23	0.39	2.87	1.85
	Carman-L		26.17	5.83	18.60	49.39	0.36	2.67	1.91
00DH-25 (HS LL)	Winnipeg-E		22.60	8.41	18.32	50.68	0.29	2.82	1.97
	Winnipeg-L		22.66	6.60	19.01	51.73	0.29	2.77	2.00
	Carman-E		26.13	8.87	16.08	48.92	0.35	3.05	1.88
	Carman-L		25.41	7.17	17.79	49.64	0.34	2.80	1.92
00DH-27 (HS LL HO)	Winnipeg-E		21.09	9.66	20.78	48.48	0.27	2.34	1.97
	Winnipeg-L		25.73	6.39	21.14	46.74	0.35	2.22	1.89
	Carman-E		24.75	8.52	17.66	49.07	0.33	2.83	1.91
	Carman-L		24.82	8.75	19.57	46.87	0.33	2.40	1.88
00DH-44 (HS LL)	Winnipeg-E		21.32	6.70	25.84	46.15	0.27	1.79	1.97
	Winnipeg-L		22.68	5.13	24.27	47.93	0.29	1.98	1.97
	Carman-E		26.90	6.68	22.02	44.41	0.37	2.04	1.84
	Carman-L		24.62	5.66	22.78	46.94	0.33	2.06	1.92
00DH-52 (Canola)	Winnipeg-E		22.61	5.94	24.05	47.41	0.29	1.98	1.96
	Winnipeg-L		27.15	5.68	22.24	44.77	0.37	2.04	1.84
	Carman-E		27.46	7.47	20.41	44.67	0.38	2.27	1.82
	Carman-L		25.11	4.15	22.48	48.28	0.34	2.17	1.94
00DH-70 (LL HO)	Winnipeg-E		23.10	9.02	19.26	48.62	0.30	2.52	1.93
	Winnipeg-L		23.18	6.74	19.66	50.42	0.30	2.61	1.97
	Carman-E		24.86	8.81	17.12	49.21	0.33	2.89	1.91
	Carman-L		24.22	7.91	17.89	49.99	0.32	2.80	1.94
00DH-124 (Canola)	Winnipeg-E		20.72	5.78	25.60	47.91	0.26	1.88	2.01
	Winnipeg-L		23.28	5.29	24.38	47.05	0.30	1.93	1.95
	Carman-E		25.27	4.47	20.71	49.55	0.34	2.65	1.95
	Carman-L		22.71	5.32	22.43	49.54	0.29	2.22	1.99
00DH-200 (LL HO)	Winnipeg-E		24.00	6.88	18.30	50.83	0.32	2.80	1.96
	Winnipeg-L		26.30	6.74	20.84	46.13	0.36	2.23	1.87
	Carman-E		26.47	7.90	17.12	48.52	0.36	2.90	1.88
	Carman-L		28.07	7.96	18.93	45.08	0.39	2.39	1.81

00DH-201 (LL HO)	Winnipeg-E	23.49	7.68	19.95	48.88	0.31	2.45	1.94
	Winnipeg-L	24.76	6.17	18.49	50.58	0.33	2.74	1.95
	Carman-E	26.87	7.72	16.40	49.02	0.37	3.01	1.88
	Carman-L	27.48	8.08	18.40	46.05	0.38	2.51	1.83
00DH-215 (LL HO)	Winnipeg-E	23.94	9.09	20.69	46.30	0.32	2.24	1.89
	Winnipeg-L	23.66	6.53	20.01	49.82	0.31	2.51	1.96
	Carman-E	28.59	9.99	19.20	42.23	0.40	2.23	1.75
	Carman-L	27.15	7.62	18.44	46.63	0.37	2.55	1.84
00DH-292 (LL HO)	Winnipeg-E	22.15	8.85	19.62	49.38	0.28	2.52	1.96
	Winnipeg-L	22.67	9.35	20.55	47.43	0.29	2.34	1.93
	Carman-E	27.61	8.50	14.54	49.36	0.38	3.60	1.86
	Carman-L	24.60	7.96	19.35	48.10	0.33	2.50	1.91
00DH-293 (LL HO)	Winnipeg-E	23.42	10.44	22.86	43.30	0.31	1.90	1.86
	Winnipeg-L	23.43	7.82	21.34	47.41	0.31	2.25	1.93
	Carman-E	27.25	10.32	17.76	44.68	0.38	2.52	1.80
	Carman-L	24.68	7.91	20.12	47.29	0.33	2.35	1.90
00DH-294 (LL HO)	Winnipeg-E	21.61	8.92	25.89	43.59	0.28	1.69	1.91
	Winnipeg-L	25.78	7.18	23.60	43.45	0.35	1.87	1.85
	Carman-E	24.47	8.85	21.54	45.27	0.32	2.11	1.88
	Carman-L	23.60	7.56	22.55	46.30	0.31	2.05	1.92
00DH-326 (LL HO)	Winnipeg-E	22.65	8.03	18.99	50.33	0.29	2.71	1.97
	Winnipeg-L	25.42	7.96	21.36	45.45	0.34	2.13	1.87
	Carman-E	26.01	8.28	17.88	47.83	0.35	2.68	1.88
	Carman-L	25.79	7.34	19.78	47.10	0.35	2.39	1.88
00DH-327 (LL HO)	Winnipeg-E	24.25	8.96	18.89	47.90	0.32	2.57	1.90
	Winnipeg-L	24.80	5.85	19.18	50.18	0.33	2.63	1.95
	Carman-E	28.27	6.78	14.84	50.13	0.40	3.93	1.87
	Carman-L	25.76	7.85	17.57	48.81	0.35	2.79	1.89
00DH-629 (LL HO)	Winnipeg-E	21.53	7.65	19.42	51.42	0.27	2.67	2.01
	Winnipeg-L	22.48	6.69	20.15	50.68	0.29	2.52	1.99
	Carman-E	24.36	10.72	20.03	44.90	0.32	2.24	1.85
	Carman-L	23.87	8.00	20.21	47.92	0.31	2.37	1.92
Apollo (LL)	Winnipeg-E	23.52	5.98	22.86	47.64	0.31	2.09	1.95
	Winnipeg-L	24.80	6.18	22.79	46.23	0.33	2.05	1.90
	Carman-E	30.58	3.16	15.19	51.07	0.45	4.04	1.87
	Carman-L	24.78	4.82	22.42	47.99	0.33	2.14	1.94
Mercury (HEAR)	Winnipeg-E	23.19	6.36	18.28	52.18	0.30	2.90	1.99
	Winnipeg-L	25.02	4.83	19.11	51.04	0.33	2.68	1.96
	Carman-E	26.14	6.51	17.14	50.22	0.36	3.04	1.91
	Carman-L	24.17	6.42	19.12	50.10	0.32	2.62	1.95
MilleneUM03 (HEAR)	Winnipeg-E	21.73	7.27	20.31	50.70	0.28	2.50	2.00
	Winnipeg-L	24.35	6.28	22.33	46.92	0.32	2.11	1.92
	Carman-E	25.02	6.27	18.55	50.15	0.33	2.73	1.94
	Carman-L	24.16	4.67	19.42	51.75	0.32	2.67	1.99

Q2 (Canola)	Winnipeg-E	23.47	6.09	19.53	50.92	0.31	2.61	1.98
	Winnipeg-L	24.44	5.98	20.01	49.59	0.32	2.50	1.95
	Carman-E	28.05	7.36	19.16	45.43	0.39	2.40	1.82
	Carman-L	26.09	5.59	18.93	49.40	0.35	2.62	1.92
Reston (HEAR)	Winnipeg-E	23.99	7.14	17.81	51.07	0.32	2.89	1.96
	Winnipeg-L	23.40	5.88	21.76	48.96	0.31	2.29	1.96
	Carman-E	27.97	7.44	18.87	45.71	0.39	2.43	1.82
	Carman-L	26.16	5.98	18.21	49.33	0.36	2.71	1.90

Table 3: Seed lipid fatty acid profile data of *B. napus* lines based on seed fatty acid profile grown at two locations and two seeding dates, presented by fatty acid category. Saturated to unsaturated fatty acid ratio, trienoic to dienoic fatty acid ratio and number of double bonds per average fatty acid values also presented.

Line/ Type	Location/ Seeding date	xx:0	xx:1	xx:2	xx:3	Sat/ Unsat		ADB
						xx:3/ xx:2		
00DH-1 (HS)	Winnipeg-E	7.06	59.29	24.25	9.42	0.08	0.39	1.36
	Winnipeg-L	8.51	56.68	25.77	9.05	0.09	0.36	1.35
	Carman-E	7.68	59.76	22.69	9.83	0.08	0.44	1.35
	Carman-L	7.13	57.91	25.36	9.65	0.08	0.39	1.38
00DH-2 (Canola)	Winnipeg-E	6.76	61.29	21.73	10.23	0.07	0.47	1.35
	Winnipeg-L	6.68	60.30	22.99	10.07	0.07	0.44	1.37
	Carman-E	7.31	64.74	19.17	8.77	0.08	0.46	1.29
	Carman-L	4.98	59.82	28.44	6.78	0.05	0.27	1.37
00DH-25 (HS LL)	Winnipeg-E	6.03	73.54	16.55	3.81	0.06	0.23	1.18
	Winnipeg-L	6.14	70.87	18.85	3.99	0.07	0.21	1.21
	Carman-E	6.28	72.88	17.05	3.72	0.07	0.22	1.18
	Carman-L	6.98	69.94	19.26	3.84	0.08	0.20	1.20
00DH-27 (HS LL HO)	Winnipeg-E	6.63	74.82	15.86	2.68	0.07	0.17	1.15
	Winnipeg-L	6.49	72.17	18.00	3.29	0.07	0.18	1.18
	Carman-E	6.43	75.28	15.71	2.55	0.07	0.16	1.14
	Carman-L	6.65	71.08	19.36	2.93	0.07	0.15	1.19
00DH-44 (HS LL)	Winnipeg-E	6.68	58.48	28.08	6.77	0.07	0.24	1.35
	Winnipeg-L	7.36	59.54	26.36	6.71	0.08	0.25	1.32
	Carman-E	6.56	62.57	24.69	6.18	0.07	0.25	1.30
	Carman-L	6.80	57.00	29.52	6.59	0.07	0.22	1.36
00DH-52 (Canola)	Winnipeg-E	6.95	57.35	27.17	8.54	0.07	0.31	1.37
	Winnipeg-L	6.32	56.10	27.05	10.49	0.07	0.39	1.42
	Carman-E	6.87	57.23	26.84	9.07	0.07	0.34	1.38
	Carman-L	7.13	55.17	27.76	9.86	0.08	0.36	1.40
00DH-70 (LL HO)	Winnipeg-E	7.01	65.69	21.62	5.61	0.08	0.26	1.26
	Winnipeg-L	6.30	66.26	21.88	5.42	0.07	0.25	1.26
	Carman-E	6.75	66.69	21.65	4.93	0.07	0.23	1.25
	Carman-L	6.17	65.75	22.78	5.32	0.07	0.24	1.27

00DH-124 (Canola)	Winnipeg-E	6.84	58.61	28.97	5.54	0.07	0.19	1.33
	Winnipeg-L	6.76	57.93	29.32	5.92	0.07	0.20	1.34
	Carman-E	6.56	59.80	28.11	5.48	0.07	0.20	1.32
	Carman-L	6.84	57.45	29.75	5.96	0.07	0.20	1.35
00DH-200 (LL HO)	Winnipeg-E	7.34	75.37	13.56	3.73	0.08	0.27	1.14
	Winnipeg-L	7.30	73.57	14.81	4.26	0.08	0.29	1.16
	Carman-E	6.55	75.88	13.86	3.72	0.07	0.27	1.15
	Carman-L	6.72	74.72	14.72	3.85	0.07	0.26	1.16
00DH-201 (LL HO)	Winnipeg-E	6.82	74.47	14.57	4.14	0.07	0.28	1.16
	Winnipeg-L	7.01	74.48	14.40	4.06	0.08	0.28	1.15
	Carman-E	6.58	74.93	14.74	3.75	0.07	0.25	1.16
	Carman-L	7.89	69.67	17.98	4.47	0.09	0.25	1.19
00DH-215 (LL HO)	Winnipeg-E	6.85	72.58	16.14	4.41	0.07	0.27	1.18
	Winnipeg-L	8.31	67.27	19.23	5.19	0.09	0.27	1.21
	Carman-E	6.36	74.70	15.11	3.94	0.07	0.26	1.17
	Carman-L	9.48	65.47	20.16	4.90	0.10	0.24	1.20
00DH-292 (LL HO)	Winnipeg-E	6.92	75.67	13.83	3.59	0.07	0.26	1.14
	Winnipeg-L	7.18	74.33	15.22	3.21	0.08	0.21	1.14
	Carman-E	6.95	77.20	12.70	3.07	0.07	0.24	1.12
	Carman-L	7.09	73.70	15.57	3.54	0.08	0.23	1.15
00DH-293 (LL HO)	Winnipeg-E	7.29	69.34	19.21	4.18	0.08	0.22	1.20
	Winnipeg-L	7.14	69.83	17.90	4.95	0.08	0.28	1.20
	Carman-E	7.06	70.88	17.91	4.16	0.08	0.23	1.19
	Carman-L	9.11	67.74	18.87	4.00	0.10	0.21	1.17
00DH-294 (LL HO)	Winnipeg-E	7.51	72.17	17.32	3.01	0.08	0.17	1.16
	Winnipeg-L	7.75	71.77	17.29	3.15	0.08	0.18	1.16
	Carman-E	7.64	74.42	15.62	2.32	0.08	0.15	1.13
	Carman-L	5.52	71.67	21.45	1.36	0.06	0.07	1.19
00DH-326 (LL HO)	Winnipeg-E	5.93	74.27	17.24	2.55	0.06	0.15	1.16
	Winnipeg-L	6.35	71.52	18.71	3.29	0.07	0.17	1.19
	Carman-E	6.06	74.76	16.68	2.45	0.06	0.15	1.15
	Carman-L	6.18	72.41	18.81	2.63	0.07	0.15	1.18
00DH-327 (LL HO)	Winnipeg-E	6.71	73.71	17.27	2.33	0.07	0.14	1.15
	Winnipeg-L	6.19	75.80	15.36	2.54	0.07	0.17	1.14
	Carman-E	6.28	77.57	13.81	2.24	0.07	0.16	1.12
	Carman-L	6.33	74.61	16.55	2.46	0.07	0.15	1.15
00DH-629 (LL HO)	Winnipeg-E	7.54	73.60	15.79	3.03	0.08	0.19	1.14
	Winnipeg-L	7.93	72.31	16.77	2.75	0.09	0.16	1.14
	Carman-E	7.41	76.07	14.05	2.42	0.08	0.17	1.11
	Carman-L	8.86	67.70	18.79	4.65	0.10	0.24	1.19
Apollo (LL)	Winnipeg-E	7.20	65.59	25.41	1.76	0.08	0.07	1.22
	Winnipeg-L	7.39	62.74	27.80	1.96	0.08	0.07	1.24
	Carman-E	6.58	66.41	25.15	1.87	0.07	0.07	1.22
	Carman-L	7.68	62.59	26.59	3.15	0.08	0.12	1.25

Mercury (HEAR)	Winnipeg-E	6.39	68.55	13.88	10.07	0.07	0.72	1.27
	Winnipeg-L	6.03	66.94	14.89	11.59	0.06	0.78	1.31
	Carman-E	6.20	68.52	14.57	10.01	0.07	0.69	1.28
	Carman-L	6.13	66.91	15.94	10.48	0.07	0.67	1.30
MilleneUM03 (HEAR)	Winnipeg-E	6.17	70.42	13.66	8.98	0.07	0.66	1.25
	Winnipeg-L	5.88	68.33	14.44	10.29	0.06	0.71	1.28
	Carman-E	5.93	70.40	14.41	8.28	0.06	0.58	1.24
	Carman-L	5.90	68.72	14.54	10.13	0.06	0.70	1.28
Q2 (Canola)	Winnipeg-E	6.35	63.23	20.58	9.66	0.07	0.47	1.33
	Winnipeg-L	6.43	63.35	20.25	9.83	0.07	0.49	1.33
	Carman-E	6.49	65.19	19.56	8.78	0.07	0.45	1.31
	Carman-L	6.58	62.24	21.58	9.49	0.07	0.44	1.34
Reston (HEAR)	Winnipeg-E	5.97	67.66	16.64	9.42	0.06	0.57	1.29
	Winnipeg-L	6.40	66.56	17.24	9.46	0.07	0.56	1.29
	Carman-E	6.03	70.01	15.08	8.61	0.06	0.57	1.26
	Carman-L	6.27	65.72	18.59	9.43	0.07	0.51	1.31