CHLOROPHYLL DEGRADATION IN RIPENING CANOLA SEED (*BRASSICA* SP.) AND EFFECTS OF PROCESSING AND STORAGE ON CHLOROPHYLL PIGMENTS IN CANOLA OIL

BY

KERRY A. WARD

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree of

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ABSTRACT

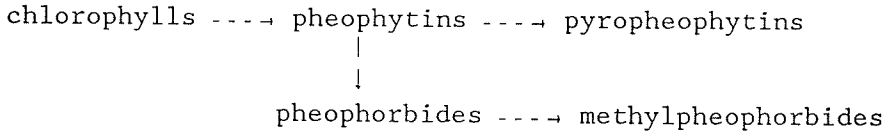
Kerry Ward, Ph.D. The University of Manitoba, August 1994.
Chlorophyll Degradation in Ripening Canola Seed (Brassica sp.) and Effects of Processing and Storage on Chlorophyll Pigments in Canola Oil.
Major Professor: Dr. R. Scarth

High levels of chlorophyll in harvested canola seed result in the downgrading of the crop. These studies have investigated several aspects of chlorophyll breakdown in ripening canola seed and oil. Both the genotype of the plant and the location at which it was grown affected the chlorophyll content of the seed at harvest. Minimal genotype by environment interaction occurred with the four cultivars tested.

Three cultivars of B. napus had significantly different rates of seed chlorophyll breakdown. Lower temperatures resulted in slower chlorophyll breakdown. Moisture and chlorophyll levels were positively correlated in ripening canola seed. All cultivars had the same rate of moisture loss from the seeds and this rate was not affected by environment. The rate of ethylene evolution from siliques containing seed was positively correlated with seed chlorophyll content, but the peak of ethylene production began after the initiation of chlorophyll breakdown and seed moisture loss. This suggests that endogenous ethylene production is not the trigger for these events.

Chlorophyll pigments in ripening canola seed were monitored using high performance liquid chromatography (HPLC). The major pigments detected were chlorophylls A and B and pheophytins A and B, with low levels of pheophorbide A, methylpheophorbide A and pyropheophytin A. Pigment composition was dependent on seed maturity, with green seed containing both chlorophylls and pheophytins, but mainly chlorophylls remaining in

ripe seed. The pheophytins and minor components were breakdown intermediates, formed from the chlorophylls and subsequently degraded. The ratio of chlorophyll A:B increased during seed ripening. The "B" derivatives appeared to degrade faster than the "A" derivatives, suggesting enzymatic reactions. The initial steps in the chlorophyll breakdown pathway appear to be:



Chlorophyll pigments in canola oil were characterized by HPLC immediately after commercial extraction and following oil storage for one month. The main pigments in canola oil were pheophytin A, pyropheophytin A and chlorophylls A and B. The "A" derivatives comprised 81 to 100% of total chlorophyll pigments. During degumming, chlorophylls were converted to pheophytins and pyropheophytins. During oil storage, both exposure to light and high temperatures resulted in the conversion of chlorophylls to pheophytins and pyropheophytins.

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I would like to thank my committee members, Dr. James Daun, Dr. Kevin Vessey and Dr. Bruce McDonald, for reading my thesis and technical papers and providing suggestions for improvements. I also appreciate Dr. Anne Johnson-Flanagan's willingness to act as my external committee member. Finally, I sincerely wish to thank my advisor, Dr. Rachael Scarth, for all of her assistance, encouragement and advice. Thank you for giving me the opportunity to attend numerous conferences, for giving me career advice and for the many hours you spent reading and editing my thesis and papers.

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LIST OF ABBREVIATIONS

PHO = pheophorbide

METHYL = methylpheophorbide

CHL = chlorophyll

PHY = pheophytin

PYRO = pyropheophytin

CHLLIDE = chlorophyllide

GDD = growing degree days

HPLC = high performance liquid chromatography

GxE = genotype by environment

TT = triazine tolerant

RCBD = randomized complete block design

a.i. = active ingredient

GC = gas chromatograph

HB = Harper and Berkenkamp

DPA = days post anthesis

LIST OF PAPERS

The following papers based on material contained in this thesis have been submitted or accepted for publication:

Ward K., Scarth R., Daun J.K. and Thorsteinson C.T. 1994. A comparison of HPLC and spectrophotometry to measure chlorophyll in canola seed and oil. JAOCS 71(9):(in press).

Ward K., Scarth R., Daun J.K. and Thorsteinson C.T. 1994. Effects of processing and storage on the chlorophyll derivatives in commercially extracted canola oil. JAOCS 71(8):(in press).

Ward K., Scarth R., Daun J.K. and Thorsteinson C.T. 1994. Characterization of chlorophyll pigments in ripening canola seed (Brassica napus). JAOCS (in press).

Ward K., Scarth R., Daun J.K. and Vessey J.K. 1994. Chlorophyll degradation in ripening canola seed (Brassica napus). Can. J. Plant Sci. (submitted).

1.0 INTRODUCTION

Canola is the second most important commercial and export crop in Canada, second only to wheat, and Canada is the world's leading exporter of canola seed. In 1992, canola accounted for 77% of total vegetable oil production in Canada (Oilseeds Sector Profile,1994). Canada produces, on average, 3.5 million tonnes of canola annually, and in 1993 new records were set with 4.13 million hectares seeded to canola in western Canada yielding 5.3 million tonnes of seed (Statistics Canada,1993).

High chlorophyll levels in harvested seed result in the crop receiving a lower grade and in lost revenue for producers. This is because seed chlorophyll is extracted with the oil where it causes a number of problems. The greenish or dark coloured oil obtained from high chlorophyll seed is unappealing to the consumer. The colour is removed using bleaching, which acts by adsorption of the pigments to acid activated clays, which are then precipitated and filtered from the oil. This increases the cost of oil processing and the amount of oil loss, as bleaching clay also retains valuable oil (Mag,1990a). Oil which contains chlorophyll is more susceptible to oxidation, leading to a reduced shelf life (Usuki et al., 1983, 1984a; Endo et al.,1984a,b; Kiritsakis and Dugan,1985). Oils extracted from green seed show reduced stability even after the chlorophyll pigments have been removed (Tautorus et al.,1993). Chlorophyll pigments also act as catalyst poisons blocking the active site of the nickel catalyst and impairing hydrogenation (Abraham and deMan,1986).

Approximately 50% of the Canadian canola crop is crushed domestically

while the other half is exported as seed, mainly to Japan (Oilseeds Sector Profile,1994). In Japan, canola oil is sold as a premium product so only top grade seed is imported. Importing countries often lack the bleaching facilities required to remove chlorophyll from lower quality seed. Therefore it is extremely important that top quality seed be produced as there are few markets available for lower grade seed.

The chlorophyll degradation pathway is poorly understood. Canola seed has been found to contain mainly chlorophyll A and chlorophyll B in an approximate 3:1 ratio, while commercially extracted crude canola oil contains primarily pheophytin A and pheophytin B in an approximate 9:1 ratio (Endo et al.,1992; Daun and Thorsteinson,1989). The level of chlorophyll in canola seed at harvest is affected by both the genotype of the plant and by the environment (Ward,1990). In general, the ability to mature in a short growing season correlates well with low chlorophyll levels at harvest, but there are exceptions (Ward,1990).

Ethylene is well established as a ripening hormone (Abeles,1973). It is involved in many aspects of fruit and seed ripening and plant senescence (Kader,1985; Mattoo et al.,1988), including chlorophyll breakdown, with exogenous ethylene often applied commercially to ripen many fruit species (Wilde,1971; Choe and Whang,1986; Abeles,1973). Patterns of ethylene evolution during ripening are, therefore, of interest, as ethylene may have a regulatory role in chlorophyll degradation. Cerone is a trade name for 2-chloroethylphosphonic acid, which spontaneously breaks down to release ethylene over time. Cerone was tested to determine whether it would enhance chlorophyll degradation in physiologically mature but green canola seed.

Due to the economic importance of the Canadian canola crop and the extent to which it is downgraded each year by high seed chlorophyll levels, there is a pressing need for studies which will shed more light on chlorophyll degradation in canola seed. Therefore we conducted five studies which investigated several aspects of chlorophyll breakdown in ripening canola seed. The first study investigated the effects of genotype, environment and genotype by environment (GxE) interactions on the final chlorophyll content of canola seed at harvest. The objectives of the study were to determine:

1) whether variation in seed chlorophyll levels can be attributed to genotype i.e. cultivar differences;
2) whether the chlorophyll content of the seed at harvest is affected by the location at which it is grown;
3) whether there is significant year to year variation in seed chlorophyll levels within cultivars; and
4) whether cultivars perform consistently at all locations and years or whether there is significant GxE interaction.

A field study measured the rates of seed chlorophyll breakdown, moisture loss and ethylene evolution in three cultivars of B. napus during seed ripening. The objectives were to determine:

1) whether there were differences in the rates of seed chlorophyll breakdown among these three cultivars;
2) whether the external environment affected chlorophyll breakdown rates;
3) whether rates of seed moisture loss were affected by genotype or environment; and

4) the relationship between ethylene evolution, chlorophyll breakdown and moisture loss in ripening canola seed.

From this study we hoped to clarify the influence of genotype, environment, seed moisture and endogenous ethylene production on seed chlorophyll degradation.

In a third study, HPLC was used to characterize the chlorophyll pigments that appear in canola seed throughout the ripening period. The purposes of this study were to determine:

1) which chlorophyll derivatives occur in canola seed and in what proportions;
2) whether the pigment composition changes as canola seed ripens;
3) if there are significant differences in the composition of chlorophyll derivatives between seed of different cultivars; and
4) whether the environment affects the composition of chlorophyll derivatives in the seed.

In our fourth study, HPLC was used to examine the effects of processing and storage on the chlorophyll derivatives that occur in commercially extracted canola oil. The objectives were to determine:

1) which chlorophyll derivatives occur in commercially extracted canola oil and in what proportions;
2) whether the types and amounts of these chlorophyll derivatives are affected by the oil extraction and processing conditions; and
3) whether the type or amount of each chlorophyll derivative changes during oil storage under various conditions.

The final study compared the results obtained by two different methods of chlorophyll measurement - HPLC and spectrophotometry.

LITERATURE REVIEW

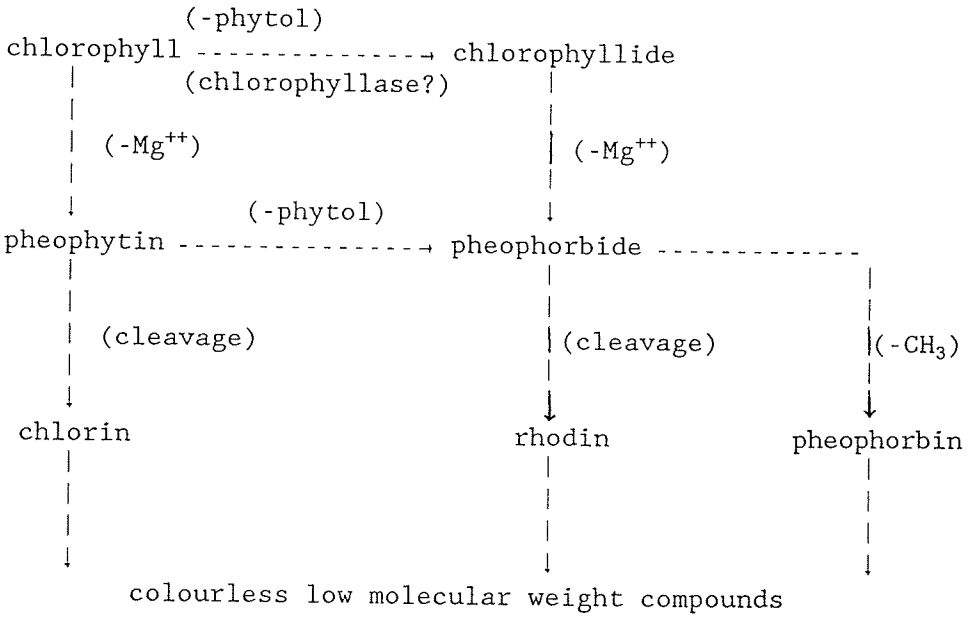
2.1 Chlorophyll Degradation in Higher Plants

2.1.1 The Chlorophyll Breakdown Pathway

One billion tonnes of chlorophyll degrades on earth every year, but the pathway involved and the ultimate fate of the pigment is largely unknown. The chlorophyll biosynthetic pathway has been almost completely elucidated (Castelfranco,1983) but we have only clues about the first steps in the catabolic pathway.

Chlorophyll A (CHL A) and chlorophyll B (CHL B) are the two chlorophyll pigments found in higher plants. The ratio of chlorophyll A:B varies from 2:1 to 4.5:1 depending on the species and growth stage (Hendry et al.,1987). During plant senescence in most species studied, this ratio drops as CHL A degrades more quickly than CHL B (Simpson et al.,1976).

The initial steps in the chlorophyll breakdown pathway are believed to be as follows (Humphrey,1980):



There are a number of reasons why the elucidation of this pathway remains difficult. First, in normal healthy plant tissues, the entire degradative pathway is tightly coupled so intermediates do not accumulate for analysis. Pheophytin A (PHY A) has been identified in ripening fruits, pheophorbide A (PHO A) has been found in dead algae (Hendry et al.,1987) and chlorophyllide A (CHLLIDE A) has been identified in several ripening fruit (Looney and Patterson,1967; Barmore,1975; Shimokawa,1981). Amir-Shapira et al. (1987) detected CHLLIDE A in the peel of ripening citrus fruit and PHY A in senescing parsley leaves. Langmeier et al. (1993) have detected both CHLLIDE and PHO in senescent oilseed rape cotyledons and chloroplasts under conditions which prevented further pigment breakdown. Secondly, care must be taken because artifacts form readily during pigment extraction and analysis, and thirdly, it is necessary to differentiate between degradative and biosynthetic intermediates (Simpson et al.,1976).

The conversion of chlorophylls to chlorophyllides may be catalyzed by chlorophyllase although there is much debate as to whether the in vivo role of this enzyme is biosynthetic, degradative or both. It has been widely implicated in the chlorophyll biosynthetic pathway (Castelfranco,1983). Numerous reports of chlorophyllase degradative activity during fruit ripening have also appeared. A rise in chlorophyllase activity was observed during ethylene induced degreening of citrus fruits (Goldschmidt,1980), and a close relationship was found between chlorophyllase and chlorophyll breakdown in maturing citrus fruit (Hirschfeld and Goldschmidt,1983). Levels of chlorophyllase were reported to increase during leaf senescence and in ripening fruits exposed to

ethylene (Amir-Shapira et al.,1987). Therefore chlorophyllase does seem to be involved in chlorophyll breakdown in certain circumstances.

The conversion of chlorophylls to pheophytins and pheophorbides is accomplished in the lab by the addition of acid. In vivo, however, the enzymes responsible are not known, although magnesium dechelators producing either pheophytin, pheophorbide or both have been described (Hendry et al.,1987; Langmeier et al.,1993). Neither enzymes nor products are known beyond the point of pheophytin and pheophorbide. The chlorophyll breakdown pathway is not a direct reversal of the biosynthetic pathway. Nor is it believed to parallel the degradation of other tetrapyrroles such as heme because neither ring compounds nor linear tetrapyrroles have been detected beyond pheophytin and pheophorbide. Rapid cleavage to colourless low molecular weight compounds is thought to occur (Hendry et al.,1987).

A number of enzymes have been identified which are not believed to be responsible for the majority of chlorophyll breakdown in vivo, but may play a role in secondary breakdown once chloroplasts begin to degenerate. Chlorophyll normally occurs in chlorophyll-lipoprotein complexes within the thylakoids of the chloroplasts. As senescence proceeds, membranes are destroyed, compartmentalization breaks down and enzymes, acids and so forth are released to contact new substrates.

Lipoxygenase can degrade chlorophyll in the presence of oxygen and unsaturated fatty acids. The chlorophyll is bleached as a secondary substrate during the peroxidation of linoleic and linolenic acids. Bleaching is normally rapid with no intermediates being detected. Lipoxygenase has been detected in the plastids and leaves of several

species but it is not generally believed to be important in chlorophyll breakdown in vivo (Luthy et al.,1984). One exception to this is a report by Minguez-Mosquera et al. (1990) suggesting that lipoxygenase may play a role in chlorophyll breakdown in ripening olive fruits. During storage of harvested olives, CHLS A and B were degraded to PHYS A and B. PHOS were not detected. The time of maximum chlorophyll breakdown coincided with the time of maximum lipoxygenase activity.

A hydrogen peroxide-dependent thylakoid bound peroxidase has also been implicated in chlorophyll bleaching in vivo in the presence of certain phenolics (Huff,1982). It degrades CHL A six to eight times faster than CHL B. The products of this reaction are unknown, but the bleaching is a secondary effect as carotenoids are also bleached. Free radicals are believed to be released during the breakdown of phenolics and these in turn initiate chlorophyll destruction (Kato and Shimizu,1985;1987). Peroxidase levels in cucumber cotyledons have been shown to rise during senescence, but peroxidase synthesis could be inhibited with no effect on chlorophyll breakdown (Abeles and Dunn,1989). This enzyme may have a role in chlorophyll turnover in plants but its levels do not usually correlate with senescence (Hendry et al.,1987).

An oxygen-dependent chlorophyll oxidase has also been reported which requires unsaturated fatty acids and bleaches chlorophyll in a secondary reaction. Chlorophyll oxidase has been reported to be associated with photosystems I and II. ^{13}-hydroxychlorophyll A has been identified as a transient breakdown product which is rapidly destroyed (Schoch et al.,1985). It is uncertain whether this enzyme is active in vivo or not, but it is not believed to catalyze the bulk of chlorophyll breakdown.

In solution, chlorophyll can also be bleached by light. During photobleaching, CHL A and CHL B degrade at the same rate (Jen and Mackinney,1970), so this is clearly not the major in vivo breakdown route. Photobleaching requires light and oxygen and appears to be non-enzymatic. Breakdown products which were more highly oxygenated than chlorophyll and contained the phytol side chain and a N:Mg ratio of 4:1 have been detected in vitro (Jen and Mackinney,1970). Photobleaching is thought to be prevented in vivo by the carotenoids and the intact thylakoids, but it may become important during the later stages of chloroplast senescence when structural integrity is lost (Hendry et al.,1987).

Therefore, the chlorophyll breakdown pathway - its substrates, enzymes and control- are, at present, all poorly understood. As chlorophyll degrades, the phytol side chain and central Mg ion are lost yielding pheophorbide, followed by the rapid breakdown of the tetrapyrrole rings to colourless low molecular weight compounds. Chlorophyllase seems to catalyze the removal of the phytol side chain in certain situations but its activity may not be universal. Magnesium dechelators have been reported and several enzymes (lipoxygenase, peroxidase, chlorophyll oxidase) have been implicated in secondary chlorophyll breakdown. It is not known how any of these enzymes are regulated or how chlorophyll breakdown is controlled.

2.1.2 Ethylene and Chlorophyll Degradation

Ethylene is commonly known as a ripening hormone because it promotes many of the processes associated with fruit ripening, including chlorophyll loss, in a variety of plant species. Two types of fruit can

be distinguished based on their patterns of ethylene evolution during ripening (Bidwell,1979). Climacteric fruit, such as apples and bananas, undergo a burst of respiration as ripening is initiated. This is usually accompanied by a temporary but dramatic increase in ethylene production. Ethylene is believed to affect the permeability of cell membranes, breaking down cellular compartmentalization and bringing enzymes into contact with new substrates, resulting in typical ripening responses such as fruit softening, chlorophyll breakdown and the conversion of starch to sugars. Ethylene may also induce new enzyme synthesis. Non-climacteric fruit, such as oranges and grapefruits, do not normally produce a burst of ethylene as they ripen and do not increase their rate of respiration. However in some of these fruits a climacteric response can be induced by the addition of ethylene, and in many non-climacteric fruit, applied ethylene can induce fruit softening, peel colour changes and other typical ripening responses (Bidwell,1979). All fruit become sensitive to increasingly lower concentrations of ethylene as they mature (Abeles,1973).

Exogenous ethylene is often applied to initiate ripening in fruit such as tomatoes, pineapples, cantaloupes, cherries, blueberries, gooseberries, black currants, apples, figs, peaches, grapes and plums (Wilde,1971; Choe and Whang,1986; Abeles,1973). Ethylene is able to initiate many aspects of fruit ripening irreversibly, including chlorophyll loss. Ethephon is a chemical spray of 2-chloroethylphosphonic acid. It breaks down spontaneously to release ethylene above a pH of 4. Ethephon translocates in the phloem in a source-sink relationship (Weaver et al.,1972). This chemical has a number of trade names including Ethephon, Ethrel, Cerone

and CEPA (Wilde,1971). It is usually applied as a spray approximately two weeks prior to harvest at levels of 250 to 500 ppm (Wilde,1971). Levels of 0 to 5000 mg L^{-1} Ethephon have been used to promote chlorophyll loss and ripen litchi fruit (Sadhu and Chattopadhyay,1989). Five ppm ethylene has been used to degreen squash peel and levels of 0.1 to 10 ppm ethylene have induced chlorophyll loss in cucumber fruits (Kader,1985). Ethylene at levels above 10 μL L^{-1} has been shown to speed ripening of mangoes, having the greatest effects on softening and peel colour changes (Medlicott et al.,1987). Applied to detached apples, ethylene hastened the onset of the respiratory climacteric response and reduced the chlorophyll content of the peel (Knee et. al.,1987). Ethylene and Ethephon are also used to degreen non-climacteric citrus fruits. In citrus fruit, ethylene seems to affect mainly colour changes and volatile production (Abeles,1973; Wilde,1971).

Several authors report an increase in chlorophyllase activity during ethylene induced degreening of citrus fruits. The chlorophyllase enzyme was believed to be present prior to senescence and was activated by ethylene (Amir-Shapira et al.,1987). Aljuburi et al. (1979) showed that Ethephon applied to oranges increased chlorophyllase activity and stimulated chlorophyll loss. During natural degreening and regreening, chlorophyllase levels paralleled chlorophyll content. Knee et al. (1988) treated harvested oranges with 0 to 1250 μL L^{-1} ethylene and found that all levels of ethylene applied accelerated chlorophyll loss. A rise in endogenous ethylene occurred at the same time as the phase of rapid chlorophyll destruction. Therefore endogenous ethylene seems to affect the ripening of citrus fruits, although they are non-climacteric. A rapid

decline in the chlorophyll content of calamondin (Citrus microcarpa) fruit peel occurred when the fruits were exposed to 10 ppm ethylene. Ethylene may stimulate either the synthesis or activation of chlorophyllase (Barmore,1975). In green mandarin fruit (Citrus unshui), ethylene accelerated chlorophyll loss by enhancing chlorophyllase activity more than twenty times (Shimokawa,1981). Apelbaum et al. (1976) showed that when endogenous ethylene levels of oranges were reduced, chlorophyll loss was not impaired, indicating that ethylene may not be the primary inducer of colour change. An exogenous application of ethylene, however, did induce rapid peel degreening. Normally there is a close relationship between the levels of chlorophyllase and chlorophyll breakdown in maturing citrus fruits (Hirschfeld and Goldschmidt,1983).

In addition to affecting chlorophyllase activity, ethylene may also affect chlorophyll breakdown by altering the structural integrity of chloroplasts. Purvis (1980) showed that in degreening calmondin fruit, ethylene disrupted the internal chloroplast membranes and caused a four fold increase in chlorophyllase activity. Levels of chlorophyllase and chlorophyll were inversely correlated. Chlorophyllase levels rose dramatically when the fruits were exposed to ethylene. The chlorophyll loss occurred after the chloroplasts lost their structural integrity, so ethylene may act by disrupting membranes to bring chlorophyll into contact with chlorophyllase. Shimokawa et al. (1978) proved that when harvested mandarins were treated with ethylene the number of chloroplasts decreased, chloroplast size decreased and the grana disintegrated, indicating that ethylene may act by reducing structural integrity and releasing chlorophyll for destruction. Normally both chlorophyll and chlorophyllase

occur in chlorophyll-lipoprotein complexes bound to the inner membranes of the chloroplasts. However, Shimokawa et al. (1978) also showed that ethylene enhanced chlorophyll degradation required de novo synthesis of cytoplasmic enzymes, indicating that a loss of structural integrity is not the only requirement for chlorophyll breakdown. Choe and Whang (1986) incubated isolated chloroplasts with Ethephon. This promoted destruction of the chloroplasts and reduced the chlorophyll content both in the light and dark. Thylakoid activity was lost and photosynthesis stopped prior to chlorophyll loss. Ethylene injected into solution was also able to promote chlorophyll loss, both from isolated chloroplasts and from leaf discs.

Exogenous ethylene application to leaves is widely known to enhance chlorophyll loss. Chlorophyll loss is accompanied by the destruction of the plastids. During dark induced senescence of leaf discs or in dicot leaves senescing on the plant, endogenous ethylene production accompanies chlorophyll loss. In most monocots, on the other hand, ethylene synthesis tends to precede chlorophyll loss (Mattoo and Aharoni,1988). Therefore ethylene is clearly implicated in chlorophyll breakdown, both during fruit ripening and leaf senescence, although the mode of action has not yet been established.

2.2 Chlorophyll Degradation in Canola Seed and Oil

2.2.1 Chlorophyll Measurement in Canola Seed and Oil

To maintain the quality of the Canadian canola crop, all seed delivered to elevators is assigned a grade based on the inclusion of foreign matter, heated seeds, green seeds, overall maturity and soundness.

Price discounts are applied to the lower grades of seed. In order to grade as Canada No. 1, the sample must contain not more than 2% distinctly green seed, assessed by the crush strip method. Canada No. 2 canola may contain up to 6% distinctly green seed and No. 3 up to 20% (Canadian Grain Commission,1991).

The crush strip method is the official method used to determine the percentage of green seeds in a sample. The strips are prepared by transferring 100 seeds to the sticky side of a piece of tape using a special seed counter. The seeds are crushed with a roller and the number of "distinctly green" seeds are counted. A minimum of five crush strips are prepared for each seed sample. If a large number of light green seeds are counted the sample may also be downgraded due to "poor natural colour" (Canadian Grain Commission,1991).

The first problem with this method is that it is subjective in terms of what an inspector considers "distinctly green". The other problem is that the correlation between the actual chlorophyll content of a sample, determined by oil extraction and absorbence using the AOCS Method Cc 13d-55, and the percentage of "distinctly green" seeds counted is less than 0.5 (Daun,1982). Therefore counting the number of green seeds in a sample does not give an accurate measurement of the amount of chlorophyll in the seeds.

Chlorophyll levels can be measured accurately by instrumental analysis. There are several types of analytical methods to measure chlorophyll in canola seed and oil including the use of near infrared reflectance (NIR) spectrophotometers, techniques which extract the oil and measure the absorbence, and methods which utilize high performance liquid

chromatography (HPLC) to separate and quantify each individual chlorophyll pigment in a sample.

The Canadian General Standards Board (1987) allows up to 30 mg kg^{-1} chlorophyll in the top grade of crude canola oil. Seed containing less than 24 mg kg^{-1} chlorophyll yielded oil which met the industry standard of 30 mg kg^{-1} (Daun,1987). Instrument grading methods determine seed chlorophyll levels as mg chlorophyll per kg seed. These methods are more accurate than the crush strip process, but they are also more expensive and time consuming.

Methods which measure the reflectance of the seed sample, using either whole or ground canola seeds, have been developed. These NIR methods are much faster than methods based on solvent extraction, and the whole seed method has the advantage of being non-destructive. The method developed for use with whole seeds measures the reflectance of the seed sample at six wavelengths between 630-754 nm and 1640-2176 nm. The method was developed with seed samples ranging from 1 to 53 mg kg^{-1} chlorophyll. A correlation of 0.939 +/- 4.8 mg kg^{-1} was determined between this method and an older NIR method which utilized ground seed (Tkachuk and Kuzina,1982).

The most recent reflectance method reported which utilizes ground seed involves the use of Dickey-john Instalab 600 NIR spectrophotometers with two of the standard infrared filters replaced with narrow bandpass filters with central wavelengths of 674 and 696 nm. The optimal wavelengths for chlorophyll prediction were 674, 696 and 2100 nm. Seed samples were ground in a coffee grinder for 30 seconds, stirred, placed in a sample dish, levelled and the reflectance measured at the optimal wavelengths. Seed samples containing 2 to 60 mg kg^{-1} chlorophyll were tested. A

correlation of 0.980 +/- 3.1 mg kg^{-1} was observed between this method and a method based on solvent extraction (Tkachuk et al.,1988).

In Sweden, chlorophyll content has been included as a grading factor of canola since 1966, with the chlorophyll level measured by solvent extraction and absorbence (Dahlen,1973). Spectrophotometric methods are based on the fact that chlorophyll pigments have characteristic absorption maxima between 600-700 nm. Chlorophyll pigments also absorb in the 400-500 nm range, but the carotenoids interfere with measurements in this range.

The most commonly used spectrophotometric method is the ISO Method 10519, which was last revised in 1993. Two grams of ground rapeseed are extracted using 30 mL of either 3:1 isooctane:ethanol or 3:1 petroleum ether:ethanol. The sample is shaken with steel ballbearings for one hour, filtered and the spectrophotometric absorbence of the extract is determined at 665 nm to measure the absorption peak for chlorophyll, and at 625 and 705 nm which provide a correction factor. This method measures all chlorophyll pigments that absorb at or near 665 nm, and they are expressed as CHL A. The method has a repeatability of 2 mg kg^{-1} for samples containing 10 to 30 mg kg^{-1} chlorophyll, and 3 mg kg^{-1} for samples containing more than 30 mg kg^{-1} chlorophyll. Reproducibility was 5 and 6 mg kg^{-1} respectively (International Standards Organization,1993).

In North America the AOCS Official Method AK 2-92 is also commonly used. This method was adopted from the ISO method and is based on the same principle. Fifty grams of seed are ground, mixed, and four grams of ground seed are extracted using 30 mL of either 3:1 isooctane:ethanol or 6:1:1 isooctane:propan-2-ol: methanol. Repeatability is 1 mg kg^{-1} (AOCS

Method AK-2-92).

An official spectrophotometric method also exists to measure chlorophyll in vegetable oils. The AOCS Official Method Cc 13d-55 measures the chlorophyll content of vegetable oils based on the spectrophotometric absorbence at 630, 670 and 710 nm. This measures all chlorophyll pigments and expresses them as mg kg^{-1} CHL A. This method is applicable for refined and bleached oils, but is generally applied to canola oil at any stage of processing (AOCS Method Cc 13d-55).

All of these spectrophotometric methods are based on the measurement of the group of chlorophyll pigments with absorption maxima near that of CHL A. Several researchers have noted that these methods tend to underestimate total chlorophyll if the extract contains significant quantities of pheophytins or other pigments which have lower extinction coefficients than CHL A (Tkachuk et al.,1988; Daun and Thorsteinson,1989; Davies et al.,1990; Suzuki and Nishioka,1993).

HPLC methods have been developed which measure each chlorophyll derivative at its absorption maxima and sum them to determine total "chlorophyll". These methods rely on the separation of chlorophyll pigments on a reversed-phase HPLC column, and detection with either a uv/visible or a fluorescence detector. These methods are calibrated with samples of each chlorophyll pigment detected. Suzuki and Nishioka (1993) utilized a series of mobile phases with gradient elution and a fluorescence detector which measured absorbence at 665 and 430 nm. They detected 1.4 times as much total "chlorophyll" by the HPLC method compared to the AOCS Method Cc13d-55. Daun and Thorsteinson (1989) used gradient elution with a photodiode array detector which quantified each pigment at

its adsorption maxima between 408-450 nm. They also found higher levels of "chlorophyll" by HPLC than by the AOCS method. When the AOCS method was recalibrated with PHY A, results were 1.5 times higher and agreed well with results from the HPLC method. There is no standard HPLC method, so the solvents and detectors used vary between laboratories. Davies et al. (1990) have reported a reversed-phase HPLC method using isocratic elution with 70:30 acetone:methanol and a fluorescence detector. Endo et al. (1992) developed a method using isocratic elution with water:methanol:acetone (4:36:60) and a fluorescence detector.

Therefore chlorophyll can be measured in a number of ways. It can be estimated by counting the number of "distinctly green" seeds. The reflectance of either whole or ground seed samples can be measured using a NIR spectrophotometer. The chlorophyll pigments can be extracted and the absorbence measured using a spectrophotometer. Alternatively, each pigment can be separated and quantified separately using a reversed-phase HPLC system with either a photodiode array or fluorescence detector.

2.2.2 Problems Caused By Chlorophyll

Green seed, which contains unacceptably high levels of chlorophyll, receives a lower grade because the chlorophyll from the seed is extracted with the oil where it causes several serious problems. It is well established that chlorophyll pigments promote oxidation of canola oil in the presence of light, thereby reducing its shelf life (Usuki et al.,1983, 1984a; Endo et al.,1984a,b; Kiritsakis and Dugan,1985). Chlorophyll acts as a photosensitizer, accompanying the synthesis of singlet oxygen upon exposure to light that initiates a chain reaction of fatty acid oxidation

(Endo et al.,1984a). During oxidation of the oil, the chlorophyll pigments are degraded. The identity of the chlorophyll decomposition products has not been established but it was determined that they were neither pheophytins nor pheophorbides. The chlorophyll degradation products also had prooxidant activity (Endo et al.,1984b).

Usuki et al. (1984a) studied the effects of chlorophylls and pheophytins on the photooxidation of triglycerides. The pheophytins were stable during oil oxidation while the chlorophylls were degraded. The stability of the pheophytins may account for their stronger prooxidant activity. It was also established that there was no prooxidant synergism between the various degradation products of the chlorophylls. In another study, Usuki et al. (1984b) showed that chlorophyll added to refined soybean oil increased the rate of oxidation when the oil was exposed to light and that the oxidative stability of the oil was dependent on the total chlorophyll content (CHL A + CHL B + PHY A + PHY B).

Even after bleaching to remove chlorophyll, oil extracted from green seed showed reduced stability compared to oil extracted from top quality seed. Evidence suggests that in oil from green seed, some decomposition product, either of chlorophyll or an oxidative breakdown product, remains in the bleached oil and reduces its shelf life. The identity of this prooxidant compound has not yet been established (Tautorus et al.,1993).

A second reason why chlorophyll pigments must be removed from vegetable oil is that they act as physical catalyst poisons by attaching to the hydrogenation catalyst reducing its active surface area. Chlorophyll pigments can also block the pores of the catalyst so triglycerides cannot reach the active sites. Both chlorophylls and

pheophytins act as catalyst poisons (Koseoglu and Lusas,1990a;b). Abraham and deMan (1986) studied the effect of added chlorophyll on the hydrogenation of canola oil. One to ten mg kg^{-1} chlorophyll slowed the hydrogenation rate, increased the solid fat index, increased the synthesis of trans isomers, accelerated the reduction of linolenic acid and increased the production of stearic acid, under both selective and nonselective conditions.

An additional problem is that seed containing high levels of chlorophyll produces a greenish or dark coloured oil that is unappealing to the consumer. This increases the cost of bleaching the oil, which in turn leads to greater oil loss, as bleaching clay can retain 1/3 to 3/4 of its weight in oil (Mag,1990a).

2.2.3 Processing and Its Effect on Chlorophyll Levels

The first step in the processing of canola seed in Canada is cleaning to remove admixtures. Frozen or cold seed is then warmed in order to prevent shattering. Next, the seed is flaked to rupture the oil storing cells and increase the surface area to volume ratio for more efficient oil extraction. The seed is then cooked to denature protein, to cause the oil droplets to coalesce, to decrease oil viscosity for easier extraction and to inactivate myrosinase, the enzyme which hydrolyses glucosinolates to antinutritional compounds (Beach,1983; Unger,1990). Next, the seed is screw pressed, which removes up to 70% of the oil. The oil is filtered and collected and the press cake passes to solvent extraction where hexane is used to dissolve and extract the remaining oil. Distillation removes the hexane and the pressed and solvent extracted oils are mixed to yield

crude oil (Beach,1983; Unger,1990). During degumming, hot water, steam or mild acid is added to the oil, which hydrates the phosphatides, causing them to precipitate from the oil. Alkali refining removes any remaining phosphatides and free fatty acids. Refining makes subsequent bleaching to remove chlorophyll more efficient (Mag,1990b).

After alkali refining, the oil is bleached to remove chlorophyll pigments (chlorophyll and its derivatives), carotenoids, soaps, phosphatides, trace metals and oxidative breakdown products. The oil is heated to 90 to 110°C, 0.5 to 2.5% clay is added depending on the chlorophyll content of the oil and a 5 to 15 minute contact time is allowed. The oil is bleached under vacuum to avoid oxidation, then filtered to remove the chlorophyll pigments to below 0.5 mg kg^{-1} (Teasdale and Mag,1983; Mag,1990b; Unger,1990). If the chlorophyll pigments are not reduced to very low levels prior to hydrogenation, the hydrogenation process converts the chlorophylls to green compounds of unknown composition that are more difficult to remove than the original chlorophyll or pheophytin (Mag,1990b). The final stage in canola oil processing is steam deodorization to remove volatile odour and flavour compounds and improve oxidative stability (Teasdale and Mag,1983; Unger,1990).

The majority of the chlorophyll pigments are removed during bleaching. Natural bleaching earth is available consisting of a bentonite clay with natural adsorptive properties. Generally however, acid activated clays are used as they have a greater capacity for pigment removal. The disadvantage is that they also adsorb more oil, so there is greater oil loss (Swern,1982). Acid activated clays are hydrated aluminum silicates

produced from bentonite or montmorillonite clays that have been chemically altered by acid treatment to improve their adsorptive properties (Richardson,1978). Bleaching earth with a small particle size is the most successful for chlorophyll removal (Segers,1983). Activated carbon can also be used to remove pigments. However it is rarely employed because of its high cost. Small amounts may be mixed with acid activated clays to treat oils that are difficult to bleach.

A number of alternate processes have been tested to remove chlorophyll pigments from canola oil. Levadoux et al. (1987) tested chlorophyll removal using the chlorophyllase enzyme. This enzyme converts oil soluble chlorophyll to water soluble chlorophyllide, that can then be separated from the oil. However, enzymatic hydrolysis of chlorophyll was not possible in crude canola oil unless it was first degummed and desoaped, then approximately 10% of the chlorophyll could be removed. The presence of oil in the reaction mixture impaired hydrolysis so this method has little commercial potential.

Kalmokoff and Pickard (1988) also studied chlorophyllase to remove chlorophylls and pheophytins from canola oil. Chlorophyll which was added to refined, bleached, deodorized oil could be removed using chlorophyllase, but naturally occurring pigments in green oil were not removed.

Ion exchange has been tested to remove chlorophyll from degummed canola oil. A number of different resins were tested but none removed more than half the chlorophyll. Alkali refining followed by ion exchange could remove a maximum of 75%. This method was determined to be impractical as bleaching clay is much cheaper than ion exchange resin, and

the clay is also considerably more effective (Diosady,1991).

A continuous bleaching process to remove chlorophyll from canola oil is being tested by Singh and Chuaqui (1991). Bleaching is generally carried out by a batch mode process which is both expensive and labour intensive. In the contact filtration method, the oil containing the chlorophyll and the adsorbent are fed continuously into an agitated tank where they remain in contact for a certain amount of time before being withdrawn and filtered to remove the clay. Five percent clay with a contact time of 15 minutes reduced the chlorophyll to the target level of below 3 mg kg^{-1}. This method appears promising but requires further evaluation (Singh and Chuaqui,1991).

The final concentration of chlorophyll pigments in the finished oil should be less than 0.1 mg kg^{-1}. Most of the chlorophyll pigments are removed by bleaching, but other steps also have an effect. For instance, phosphoric acid degumming makes the subsequent bleaching step much more effective (Patterson,1989). Twenty-five percent of the chlorophyll pigments in a crude soybean oil with an initial chlorophyll content of 1000 to 1500 $\mu g\ L^{-1}$ were removed by alkali refining, and bleaching subsequently reduced the level to 15 $\mu g\ L^{-1}$ (Swern,1982). Deodorization can remove small quantities of chlorophyll that remain after bleaching. Refined, bleached vegetable oils containing 0.3 to 0.8 mg kg^{-1} chlorophyll had their chlorophyll level reduced below 0.13 mg kg^{-1} by deodorization (Warner et al.,1989). Rapeseed oil that contained 22 mg kg^{-1} pheophytin after degumming, contained 18 mg kg^{-1} following refining, 1 mg kg^{-1} after bleaching and less than 0.02 mg kg^{-1} after deodorization (Thomas,1982). By far the greatest amount of chlorophyll is removed during bleaching.

Canola oil containing 68 mg kg^{-1} chlorophyll was reduced to 60 mg kg^{-1} by degumming and 11 mg kg^{-1} by bleaching (Prior et al.,1991).

2.2.4 Chlorophyll Components in Canola Seed and Oil

According to Mag (1983), the chlorophyll compounds present in canola oil include CHL A, CHL B, PHY A and pheophytin B (PHY B), with the "A" compounds accounting for the greatest proportion. Typical chlorophyll levels in crude canola oil from high quality seed range from 5 to 25 mg kg^{-1} (Koseoglu and Lusas,1990b; Teasdale and Mag,1983). Many authors have reported that lab extracted canola oil contains mainly chlorophylls, while commercially extracted canola oil contains primarily pheophytins (Niewiadomski et al.,1965; Appelqvist and Ohlson,1971; Daun,1982; Johansson and Appelqvist,1984; Daun and Thorsteinson,1989; Davies et al.,1990; Endo et al.,1992). High temperatures and acidic conditions are believed to be responsible for this conversion (Daun,1982). Suzuki and Nishioka (1993) found that heating canola oil above 110°C decreased the ratio of A:B pigments and converted pheophytins to pyropheophytins. Johansson and Appelqvist (1984) observed that seed drying, cooking and the extraction solvent chosen all affected the composition of chlorophyll derivatives in the oil. High temperature drying of moist seeds caused chlorophylls to be converted to pheophytins. Hexane extracted fewer chlorophyll pigments than either acetone or chloroform:methanol, as hexane does not extract the more polar chlorophylls efficiently. Cooking converted these chlorophylls to pheophytins which could then be extracted with hexane. The quality of the seed affected the chlorophyll:pheophytin composition. Good quality, sound canola seed contained mainly

chlorophylls, while mouldy, heated or otherwise damaged seeds contained more pheophytins (Johansson and Appelqvist,1984).

Niewiadomski et al. (1965) determined the composition of the chlorophyll pigments in commercially extracted crude rapeseed oil. They found no CHL A, 0 to 1.79 mg kg^{-1} CHL B, 17.99 to 25.65 mg kg^{-1} PHY A and 0.52 to 6.15 mg kg^{-1} PHY B.

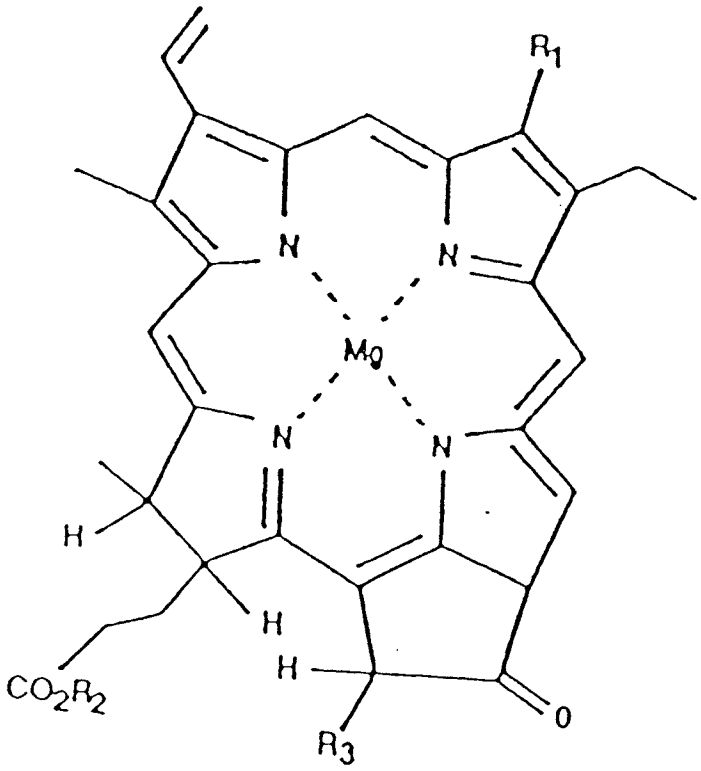
Usuki et al. (1984b) determined the levels of chlorophylls and pheophytins in refined rapeseed oil produced commercially in Japan. Both chlorophylls and pheophytins were present but pheophorbides were absent. Rapeseed oil from several batches was tested and found to contain 0.1 to 15.2 μg kg^{-1} CHL A, 7.2 to 30.6 μg kg^{-1} CHL B, 83.5 to 131.5 μg kg^{-1} PHY A and 27.1 to 49.8 μg kg^{-1} PHY B for a total chlorophyll pigment content of 126.0 to 219.7 μg kg^{-1}. The chlorophyll pigments were approximately 70% of the "A" type and 30% of the "B" type.

Daun and Thorsteinson (1989) also found that commercially extracted crude canola oil contained mainly PHY A and PHY B in an approximate 9:1 ratio, while seed extracts contained mainly CHL A and CHL B in an approximate 3:1 ratio. Therefore the conversion of chlorophyll to pheophytin occurs during commercial oil extraction and the "B" derivatives are preferentially destroyed.

Fraser and Frankl (1985) measured the level of each chlorophyll pigment in soybean oil using high performance liquid chromatography (HPLC). Once again, the predominant pigment was PHY A (40 to 45% of the total) with lesser amounts of PHY A′ (an epimer), pyropheophytin A (PYRO A) and three unidentified pigments with spectral features similar to PHY A. No CHL A or B was detected.

Numerous studies have established that pressed oil tends to contain less chlorophyll than solvent extracted oil. Thomas (1982) determined that pressed rapeseed oil contained 22 to 29 mg kg^{-1} pheophytin, while solvent extracted oil contained 30 to 35 mg kg^{-1}. Niewiadomski et al. (1965) measured the levels of chlorophylls and pheophytins in 15 pressed and 5 solvent extracted rapeseed oils. CHL A was not present in either; CHL B was present at a level of 0.14 to 1.79 mg kg^{-1} in the pressed versus 1.00 to 1.06 mg kg^{-1} in the solvent extracted oil; PHY A was present at 20.60 to 25.13 mg kg^{-1} in the pressed oil versus 17.99 to 25.62 mg kg^{-1} in the solvent extracted oil; and the pressed oil contained 0.52 to 4.52 mg kg^{-1} PHY B, while the solvent extracted oil contained 1.73 to 6.15 mg kg^{-1}. Usuki et al. (1984b) found that pressed rapeseed oil contained a total of 39.9 mg kg^{-1} chlorophyll pigments broken down into 6.3% CHL A, 12.3% CHL B, 76.9% PHY A and 4.4% PHY B. The solvent extracted oil contained 46.1 mg kg^{-1} chlorophyll pigments composed of 5.7% CHL A, 6.3% CHL B, 77.2% PHY A and 10.8% PHY B. Therefore, there is ample evidence that solvent extracted oils do contain more chlorophyll pigments than pressed oils.

Endo et al. (1992) used HPLC to identify and quantify the chlorophyll pigments present in canola seed, meal and oil. In the seed the ratio of A:B pigments was approximately 3:1 but in the oil it was 9:1, which agrees with the earlier results of Daun and Thorsteinson (1989). The HPLC system used was able to resolve PHO A, methylpheophorbide A (METHYL A), CHL B, CHL A, PHY B, PHY A and PYRO A. The structures of these pigments are illustrated in Figure 1.



Pigment	Abbrev.	X	R_1	R_2	R_3
Pheophorbide a	Pho a	H_2	CH_3	H	CO_2CH_3
Methylpheophorbide a	Methyl a	H_2	CH_3	CH_3	CO_2CH_3
Chlorophyll b	Chl b	Mg	CHO	$C_{20}H_{39}$	CO_2CH_3
Chlorophyll a	Chl a	Mg	CH_3	$C_{20}H_{39}$	CO_2CH_3
Pheophytin b	Phy b	H_2	CHO	$C_{20}H_{39}$	CO_2CH_3
Pheophytin a	Phy a	H_2	CH_3	$C_{20}H_{39}$	CO_2CH_3
Pyropheophytin a	Pyro a	H_2	CH_3	$C_{20}H_{39}$	H

Figure 1: Structure of each of the chlorophyll derivatives.

Seeds contained mainly CHL A and CHL B with small amounts of PHY A. Traces of PHO A and METHYL A were also detected in some seed samples. Canola meal contained some PHY A and PYRO A with smaller amounts of PHO A, METHYL A and PHY B, but no CHL A or B. Traces of PHY A′ and B′ were also found. Total levels of chlorophyll pigments in the meal were approximately one tenth of those found in the seed (Endo et al.,1992).

Crude and degummed canola oils contained PHY A and B, PYRO A, PHY A′ and B′ and traces of METHYL A, but no CHL A or B. PHO A was detected in the crude oil but not in the degummed oil. The main pigments in the crude and degummed oils were PYRO A and PHY A and the ratio of PYRO A:PHY A was much higher in the degummed oil than in the crude oil. Total chlorophyll pigments in crude and degummed oils were the same (Endo et al.,1992). Therefore, chlorophylls were converted to pheophytins during oil extraction, and during degumming PHY A was converted to PYRO A.

In a recent study, Suzuki and Nishioka (1993) examined the chlorophyll pigments present in canola oil extracted from Canada No. 1 seed using hexane. The HPLC system used was similar to that used by Endo et al. (1992), but Suzuki and Nishioka were able to resolve PYRO B in addition to the other chlorophyll derivatives. Commercially extracted alkali refined oil contained primarily PHY A, PYRO A, PHY B and PYRO B. Small amounts of CHL A and the epimers of PHY A and PHY B were also detected. This is similar to the results of Endo et al. (1992), but in their study PYRO B could not be resolved from PHY A, thus accounting for its lack of detection.

Suzuki and Nishioka (1993) examined the chlorophyll pigments in green and yellow cotyledons and in the hulls. The green cotyledons contained

the greatest amount of total chlorophyll, of which more than 50% was PHY A and a significant amount was CHL A. Other studies, which used whole seed, found smaller proportions of PHY A. Studies by Endo et al. (1992) and Daun and Thorsteinson (1989) both found seed extracts to contain mainly chlorophylls A and B with low levels of pheophytins. The higher proportion of pheophytins observed by Suzuki and Nishioka may be explained by their use of hexane as the extraction solvent. It has previously been shown that hexane does not extract the polar chlorophylls as well as the less polar pheophytins (Johansson and Appelqvist,1984). Suzuki and Nishioka (1993) found the ratio of the A:B derivatives in alkali refined crude canola oil to be 5:1 and the ratio of (PYROS A + B):(PHYS A + B) to be 1.0. The ratio of the A:B derivatives was similar in both green and yellow cotyledons, but higher in the hulls than in the cotyledons. Suzuki and Nishioka (1993) report a ratio of approximately 7:1 for the cotyledons and 4:1 for the hulls, based on (CHL A + PHY A + PYRO A):(PHY B + PYRO B). These results differ significantly from the results of both Daun and Thorsteinson (1989) and Endo et al. (1993) who, working with whole seed, found the ratio of CHL A:CHL B to be 3:1. A possible reason for this discrepancy is the different extraction solvents used in these three studies. Both Daun and Thorsteinson (1989) and Endo et al. (1992) used ball mill extraction with 3:1 isooctane:ethanol, while Suzuki and Nishioka used Soxhlet extraction with hexane. Pigment extraction with hexane may account for the higher proportion of pheophytins and pyropheophytins observed in the study by Suzuki and Nishioka (1993). Chlorophylls have been shown to be converted to pheophytins and pyropheophytins during commercial oil extraction, where hexane is the solvent employed. A higher

proportion of "A" derivatives was also detected in canola oil commercially extracted with hexane (Daun and Thorsteinson,1989; Endo et al.,1992).

Suzuki and Nishioka (1993) also examined the effect of cooking the seed prior to oil extraction on the composition of chlorophyll derivatives in the oil. As the cooking temperature was raised, the total chlorophyll content of the extracted oil increased. The ratio of the A:B derivatives decreased with either higher cooking temperatures or longer cooking time. The ratio of pyropheophytins:pheophytins increased with increasing heat. Daun and Thorsteinson (1989) and Endo et al. (1992) have both previously found that chlorophylls are converted to pheophytins during oil extraction. Suzuki and Nishioka's results indicate that both the cooking time and temperature are important determinants for pigment conversion, as high temperatures converted pheophytins to pyropheophytins.

Suzuki and Nishioka (1993) compared the pigment composition in solvent extracted and pressed oils. Solvent extracted oils contained more total chlorophyll, which agrees with many earlier studies (Thomas,1982; Niewiadomski et al.,1965; Usuki et al.,1984b), mostly present as PYRO A and B. The ratio of the A:B derivatives was lower in the solvent extracted than in the pressed oil. Endo et al. (1992), on the other hand, found the pigment composition of pressed and solvent extracted oils to be similar. Suzuki and Nishioka found that both alkali refining and degumming reduced total chlorophyll slightly, in agreement with other studies (Swern,1982; Patterson,1989; Jung et al.,1989; Thomas,1982; Prior et al.,1991). CHL A decreased during degumming, but neither degumming nor alkali refining had any effect on the ratio of the A:B derivatives or on the ratio of pyropheophytins:pheophytins. This is in agreement with the

results of Usuki et al. (1984b) who found that each pigment decreased with each successive stage of processing, but the ratio of each pigment remained relatively constant. However it does not agree with the results of Endo et al. (1992) who found the ratio of PYRO A:PHY A to be much higher in the degummed oil than in the crude oil. Suzuki and Nishioka (1993) used water degumming, while Endo et al. (1992) used acid degumming. The use of acid during degumming in the study by Endo et al. (1992) may explain the observed conversion of pheophytins to pyropheophytins.

Suzuki and Nishioka (1993) also studied the behaviour of the various chlorophyll derivatives during bleaching. When activated earth was used, PHY A and PYRO A were adsorbed six times more readily than PHY B and PYRO B. With activated carbon, on the other hand, PHY B was adsorbed slightly more readily than PHY A, and PYRO B was adsorbed twice as well as PYRO A. When activated earth was used, the pheophytins and pyropheophytins were equally well removed, but with activated carbon, PHY A and PYRO A were removed equally well, but PYRO B was adsorbed twice as readily as PHY B. Therefore activated earth removed the "A" derivatives effectively, but activated carbon was more successful for removing the "B" derivatives. Thus the composition of chlorophyll derivatives in canola oil is affected by extraction and processing conditions, and the pigment composition is likely to have an impact on the efficiency of bleaching.

2.2.5 Enzymes Involved in Degreening of Canola Seed

Dr. Anne Johnson-Flanagan and her colleagues have investigated the pathway(s) and enzymes involved in chlorophyll breakdown in ripening canola seed. Johnson-Flanagan and Thiagarajah (1990) studied changes in

chlorophyll pigments during the degreening of B. napus (cv. Westar) seed. The main pigments present in the seed were CHL A and CHL B with low levels of pheophytins, pheophorbides and chlorophyllides. As seed moisture fell from 70 to 50%, chlorophyll breakdown occurred rapidly and CHLLIDE A and PHY A accumulated. The ratio of CHL A:B fell from 2.5 to 2.0. Below 50% seed moisture, the degradation of chlorophylls and their breakdown products was linearly correlated. The presence of CHLLIDE A indicated that chlorophyllase was active during initial seed degreening. However PHY A was also produced, indicating that a non-chlorophyllase system was also present. This study provided the first evidence of CHLLIDE A and PHY A together in the same tissue and suggests that two chlorophyll breakdown pathways operate simultaneously in degreening canola seed. The chlorophyll was bound in chlorophyll-protein complexes and these complexes broke down gradually during seed degreening in a controlled manner (Johnson-Flanagan and Thiagarajah,1990).

There is likely more than one pathway and several enzymes involved in the initial steps of chlorophyll breakdown in canola seed. Johnson-Flanagan and McLachlan (1990a) investigated the role of chlorophyllase in the degreening of canola seeds. Chlorophyllase catalyses the removal of the phytol side chain from chlorophylls or pheophytins yielding chlorophyllides and pheophorbides, respectively. During normal seed degreening it was impossible to correlate chlorophyllase activity with chlorophyll loss because the degradation products did not accumulate for analysis. B. napus seed (cv. Westar) contained chlorophyllase and chlorophyllase activity peaked at 65% seed moisture, when active seed chlorophyll breakdown was occurring, then declined at lower moisture

levels. Below 70% moisture, chlorophyllase activity was positively correlated with the level of chlorophyll in the seed (Johnson-Flanagan and McLachlan,1990a).

Sublethal freezing is known to inhibit seed degreening and result in an accumulation of dephytylated pigments in the seed (Johnson-Flanagan et al.,1990). Therefore, the effect of sublethal freezing on chlorophyllase activity was examined. The substrate specificity of chlorophyllase was not affected by sublethal freezing. Chlorophyllase was more active against the pheophytins than the chlorophylls and the "A" type pigments were hydrolysed more quickly than the "B" derivatives. Sublethal freezing initially had no effect on chlorophyllase activity in the thylakoids, but after a four day recovery period following the freezing temperatures, chlorophyllase activity increased as did de novo synthesis of the enzyme. Therefore sublethal freezing did not stop chlorophyll breakdown by reducing chlorophyllase levels or activity. In frozen seed, chlorophyllides and pheophorbides accumulated, indicating that further breakdown by the next enzyme in the pathway may be inhibited (Johnson-Flanagan and McLachlan,1990a).

The role of peroxidase in canola seed degreening was also investigated by Johnson-Flanagan and McLachlan (1990b). Peroxidase is able to degrade chlorophyll in the presence of H_2O_2 and phenolics in a free radical-mediated reaction. The in vitro activity of this enzyme has been demonstrated by several researchers (Huff,1982; Kato and Shimizu,1985;1987) but its in vivo role is uncertain. Johnson-Flanagan and McLachlan (1990b) have shown that peroxidase is active in thylakoids isolated from degreening B. napus seeds (cv. Westar). Thylakoid bound

chlorophyll pigments were degraded in the presence of H_2O_2 and 2,4-dichlorophenol. As peroxidase is an 79 kDa intrinsic thylakoid protein and H_2O_2 and phenols are both normal components of chloroplasts, this enzyme may be active during normal seed degreening. High levels and activity of peroxidase were associated with the period of rapid seed degreening. Peroxidase activity in the thylakoids increased as the seed moisture content declined, reaching a peak at 65 to 55% seed moisture, then declining. Sublethal freezing reduced peroxidase levels and activity to close to zero, but after a four day recovery period peroxidase levels and activity returned to normal. Therefore, the dephytylated pigments that accumulate in canola seed immediately following sublethal freezing may do so because chlorophyllase is active at this time, but peroxidase, which could catalyze their further degradation, is inactive. The increase in in vitro activity may not have any physiological significance as the seed is desiccating rapidly at this point and may not provide the necessary environment for enzyme activity. Inhibition of peroxidase activity could be responsible for the failure of canola seed to degreen when frozen (Johnson-Flanagan and McLachlan,1990b).

Chlorophyllase and peroxidase have both been shown to function during chlorophyll breakdown in ripening canola seed. Chlorophyllase activity was not inhibited by sublethal freezing, but peroxidase activity was. Several pathways appear to operate during seed chlorophyll breakdown and there are likely many enzymes involved which still await discovery.

2.2.6 Effects of Genotype and Environment on the Chlorophyll Content of Canola Seed

Brassica napus cultivars usually contain higher levels of seed chlorophyll than B. rapa cultivars. This may be a genetic effect of the species or it may be explained by the earlier maturity of B. rapa. Previous work examined canola seed from the Manitoba provincial crop variety trials conducted over two years at multiple sites throughout the province (Ward,1990). It was clearly established that B. rapa cultivars contained less seed chlorophyll at harvest than B. napus cultivars. Significant differences were found between final seed chlorophyll levels of different cultivars of B. napus. The environment also had a significant effect on the seed chlorophyll level at harvest. The length of growing season required was positively correlated with seed chlorophyll content at harvest. Cultivars which required longer growing seasons were more likely to ripen in conditions which are not conducive to chlorophyll breakdown and hence, more chlorophyll remained in the harvested seed (Ward,1990).

Further experiments were carried out to determine the rates of chlorophyll breakdown in different B. napus cultivars, to determine whether there were genetic differences in the rate of seed chlorophyll breakdown (Ward et al.,1992a). Within the same seeding date, all cultivars tested (Westar, Global, Regent and Tribute) had the same rate of seed chlorophyll breakdown. Ripening during cooler temperatures resulted in a slower rate of chlorophyll breakdown for the same cultivar. Also, cultivars which required longer growing seasons initiated chlorophyll breakdown later, increasing the chance that high seed chlorophyll levels

would remain at harvest (Ward et al.,1992a).

McGregor (1991) confirmed that B. rapa cultivars contained less seed chlorophyll at harvest than B. napus cultivars in a study of final seed chlorophyll levels for nine cultivars of B. rapa and sixteen cultivars of B. napus over a five year period. The chlorophyll content of each cultivar varied with the year due to the influence of the external environment. There was a positive correlation (+ 0.69) between chlorophyll content at harvest and days to maturity when both species were considered together, but not when each was considered separately (McGregor,1991).

2.3 Summary

In summary, high levels of chlorophyll in harvested canola seed cause several serious problems in the oil leading to a loss of income for growers. The pathway(s) of chlorophyll degradation and the enzymes involved are, at present, poorly understood. However, several breakdown products of chlorophyll have been identified in canola seed and oil. Seed contains mainly CHL A and CHL B in a ratio of approximately 3:1 while oil contains predominantly PHY A and PYRO A, with the A:B pigments occurring in a 9:1 ratio.

Canola oil processing is designed to remove chlorophyll pigments from the oil. This is accomplished primarily by bleaching with acid activated clays. Oil extraction and processing affects the types of chlorophyll pigments present in the oil. Extraction conditions convert chlorophylls to pheophytins and degumming has been shown to convert PHY A to PYRO A.

Ethylene is well established as a ripening hormone, although its exact

role remains obscure. Ethylene levels may play a role in the regulation of chlorophyll breakdown, either by affecting enzyme synthesis or activity, by altering the structural integrity of chloroplasts, or both. Patterns of ethylene evolution during ripening are, therefore, of considerable interest.

Both the genotype of the plant and the external environment interact to control the final chlorophyll level in canola seed. B. rapa cultivars usually contain less seed chlorophyll at harvest than B. napus cultivars, and B. napus cultivars vary in final seed chlorophyll levels. The length of growing season required generally correlates well with final seed chlorophyll levels at harvest.

MATERIALS AND METHODS

3.1 "Agroman" Trials

3.1.1 Genetic and Environmental Influences on Seed Chlorophyll Content at Harvest

Each year, all registered canola cultivars of both B. napus and B. rapa are grown at a number of locations throughout the province, over seven different climatic zones. The delineation of the zones is based on the average number of frost free days and soil type (Figure A1 in the Appendix). The "Agroman" Trials are conducted to evaluate each cultivar and to recommend where it should be grown. In a previous study (Ward,1990), three B. napus cultivars were identified as having different levels of seed chlorophyll at harvest and these cultivars were selected for further study. Delta generally contained low levels of seed chlorophyll, Stellar had high seed chlorophyll levels and Westar was intermediate. The differences in final seed chlorophyll levels could not be explained by the length of growing season required to reach full maturity (Ward,1990). The B. rapa cultivar Tobin was also included as a comparison for the typically low seed chlorophyll level in this species.

Seed of these four cultivars was obtained from the "Agroman" Trials from 1988 to 1992. In 1988, the following ten locations were available:

Locations

Melita	(zone 1)
Waskada	(zone 2)
Shoal Lake	(zone 2)
Mariapolis	(zone 2A)
Dauphin	(zone 3)
Bagot	(zone 3A)
Beausejour	(zone 4)
Teulon	(zone 4)
Roblin	(zone 5)
Swan River	(zone 5)

In 1989, the following twenty-two cultivars were available:

Locations

Melita	(zone 1)
Shoal Lake	(zone 2)
Mariapolis	(zone 2A)
Dauphin	(zone 3)
Winnipeg	(zone 3)
Bagot	(zone 3A)
Beausejour	(zone 4)
Teulon	(zone 4)
Roblin	(zone 5)
Swan River	(zone 5)
The Pas	(zone 6)

In 1990, seed was obtained from the following five locations:

Locations

Waskada	(zone 1)
Dauphin	(zone 3)
Teulon	(zone 4)
Roblin	(zone 5)
The Pas	(zone 6)

while in 1991, seed was available from the following five locations:

Locations

Waskada	(zone 1)
Pilot Mound	(zone 2)
Portage la Prairie	(zone 3A)
Beausejour	(zone 4)
Roblin	(zone 5)

After harvest, the plants were hung in jute bags to dry prior to threshing. The seed was sieved to remove foreign material and the chlorophyll content of each sample was measured using the NIR method (Tkachuk et al.,1988). A near infrared reflectance spectrophotometer was modified to analyze chlorophyll by replacing the standard infrared filters with filters whose central wavelengths are 674, 696 and 2100 nm. A Dicky-John Instalab 600 machine was used. The canola seed was ground in a coffee grinder for thirty seconds, loaded into a sample cup, levelled, and the reflectance of the sample measured. The reading was then converted to a chlorophyll concentration using a calibration equation. This mehtod is accurate to within 3 mg kg^{-1}.

These data were analyzed to determine whether there were significant differences in chlorophyll levels between different cultivars and to determine whether the environment had a significant effect on seed chlorophyll content. Statistical analysis was carried out on the mainframe computer at the University of Manitoba using the SAS program (Helwig and Council,1979). Appropriate GLMs and means separation tests were performed. GLM results were modified according to Cochran and Cox (1957) in order to combine experiments with heterogeneous error variances. Duncan's mean separation tests were carried out by hand as outlined in Gomez and Gomez (1984) using the appropriate degrees of freedom from the modified GLM analysis and a $p=0.05$ level of significance.

3.1.2 Correlations Between Seed Components

In addition to measuring the chlorophyll content of each seed sample, the NIR method (Panford et al.,1988) was also used to simultaneously

determine oil and protein. The correlations between seed chlorophyll (mg kg^{-1}), oil (%) and protein (%) (seed basis) levels were determined within each trial, within each year of the study, for the entire data set combined over years, and subdivided by cultivar.

3.2 Field Study - Seed Chlorophyll Degradation, Moisture Loss and Ethylene Evolution From Ripening Seed of Brassica napus

3.2.1 1991 Field Study

Based on the preliminary results from the "Agroman" Trials in 1988 and 1989, the cultivars Delta, Stellar and Westar were selected for a field study that was conducted during the summers of 1991 and 1992. In the first year, two seeding dates were used, May 13th and May 27th. Plots were laid out in a randomized complete block design (RCBD) with four replicates of each cultivar and four different spray treatments in each replicate. The four spray treatments were:

A - control (distilled water) (0g a.i. ha^{-1})

B - 1/4 L ha^{-1} Cerone (1/2 X recommended rate) (120g a.i. ha^{-1})

C - 1/2 L ha^{-1} Cerone (1 X recommended rate) (240g a.i. ha^{-1})

D - 1 L ha^{-1} Cerone (2 X recommended rate) (480g a.i. ha^{-1})

The recommended rate of Cerone was the rate recommended to control lodging in canola. Six row plots, three meters long were seeded with 0.3 meter spacings between rows. The recommended seeding rate of 6 kg ha^{-1} was used for Westar with adjustments made for equivalent densities of the other cultivars according to different seed weights. All cultivars were tested prior to planting and found to have at least 95% germination.

Emergence dates (50%) were recorded for each plot and the growth stages of the plants were monitored throughout the growing season. Each plant in the inner four rows of each plot was given a colour coded tag at first flower. When sampling was conducted, plants that flowered within a restricted time period were chosen in order to eliminate any unusually early or late material. For the early seeding date, plants that flowered between June 20 to 26 were chosen for sampling, while for the late seeding date, the plants chosen flowered between July 6 to 11.

All plots were sprayed with the appropriate level of Cerone when at least 50% of the plants in each plot had reached growth stage 5.3 on the Harper and Berkenkamp scale (1975) (Table A1 in Appendix), when the seeds were turning from green to brown. This stage corresponded to 30 to 32 days post anthesis (DPA) if anthesis is considered to occur, on average, at the mid-point of the accepted flowering period. The spray date was July 23 for the early seeding date and August 7 for the late seeding date. Plastic guards were set up on both sides of each plot to prevent spray drift and the appropriate concentration of Cerone or distilled water was applied using a backpack sprayer.

3.2.2 Plot Sampling

Each plot was sampled once a week, beginning the day after the plots were sprayed and continuing until the plants were completely senescent (HB 5.5). In 1991, the first sample from the early seeding date was harvested 33 DPA, while the first sample from the late seeding date was collected 31 DPA, assuming that the average date of anthesis was the middle of the selected flowering period. Each sample involved cutting ten plants, which

flowered within the appropriate time interval, from the inner four rows of each plot, removing the main stems and placing them in a plastic bag. In the lab, the seed was immediately removed from the pods and weighed. The samples were then frozen, freeze-dried and reweighed to determine the moisture content at harvest. The freeze-dried samples were stored in glass vials until analyzed.

3.2.3 1992 Field Study

The design of the second year of the study was similar to the first, except the plot sizes changed slightly and the spray treatments were eliminated. In the second year of the study, the early seeding date was planted on May 12th and the late seeding date on June 2nd. Plots were laid out in a RCB design with six replicates of each cultivar. The first range was eliminated in both the early and late seeding dates, and the remaining five replicates were sampled. Each plot consisted of eight rows, three meters long with 0.3 meter row spacing. Seeding rates were identical to the first year. Plants in the inner six rows were given colour coded tags at first flower. Plants that flowered between June 27 to July 9 were chosen for sampling for the early seeding date and those which flowered between July 17 to 23 were selected in the late seeding date.

3.2.4 Plot Sampling

Sampling began when the majority of the plants in each plot had reached growth stage 5.2 on the Harper and Berkenkamp scale (1975), when the seeds were full size and green. The first sample was taken from the early

seeding date 39 DPA and from the late seeding date 36 DPA. Each plot was sampled twice a week until all plants were senescent (HB 5.5). Sampling and analysis were identical to the first year of the study.

3.2.5 Measurement of Ethylene

In both years of the study, when each sample was taken from the field, the rate of ethylene evolution from the siliques containing seed was also measured. For each sample, one silique was taken from near the base of the main stem of each of the ten plants which were cut. The intact siliques containing seed were placed in a 50 mL erlenmeyer flask, the flask was sealed with a rubber stopper and left on a bench in the light for 2 to 3 hours to allow the siliques to evolve ethylene. A preliminary study had established that the rate of ethylene evolution from the siliques was constant between one and six hours after the flasks were sealed.

After 2 to 3 hours, the level of ethylene present in each flask was measured using a Carle Analytical Gas Chromatograph (GC), Model 311, equipped with a 1.8 m x 1/16" Poropak T 50/80 column. The operating temperature was 80°C, the carrier gas was helium at a flow rate of 20 mL $min.^{-1}$ and a flame ionization detector was used. One mL samples were taken from each flask after the 2 to 3 hour incubation period, injected onto the GC column and the ethylene level of the sample measured. Results were quantified by comparing the peak height or area to that of a 10 $\mu L\ L^{-1}$ ethylene standard. The signal response of the GC was linear within the range of analysis. In the first year of the study, the peak heights were measured, using a ruler, while in the second year the peak area was

measured using a microcomputer and an integration package (Inject from IMB-Bioanalytical Systems Co., State College, PA, 16801). In the first year of the study, one measurement was taken for each sample, while in the second year two readings were taken and the results averaged. The volume of each flask containing siliques was calculated, the fresh weight of the siliques was measured and the rate of ethylene evolution was calculated as μL ethylene/g fresh wt./hour.

3.2.6 Chlorophyll Analysis

Each seed sample taken from the field was analyzed for chlorophyll by the AOCS Official Method AK 2-92 (1992) using a Varian DMS 200 UV-Visible spectrophotometer, that provides measurements accurate to within 1 mg kg^{-1}. One gram samples of freeze-dried seed were weighed out and placed in stainless steel extraction tubes with three ball bearings and 30 mL of 3:1 isooctane:ethanol. Samples were shaken for one hour, then filtered. Dilutions were prepared if required and the absorbance readings were measured. Three wavelengths were used - 625.5, 665.5 and 705.5 nm - to measure the absorption peak for chlorophyll with corrections on either side. Two extractions and measurements were made on each sample and the results averaged. The absorbance readings were converted to chlorophyll levels using the following formula which was developed using standards of known chlorophyll concentration:

$$C.O.D. = Abs.(665.5\ nm) - [Abs.(625.5\ nm) + Abs.(705.5\ nm)] \div 2$$

$$Chl.\ (mg\ kg^{-1}) = (13.0 \times C.O.D. \times 30.0)$$

3.3 Chlorophyll Derivatives in Ripening Canola Seed

3.3.1 Seed Sample Selection and Preparation

The objective of this study was to characterize the chlorophyll pigments that appear in ripening canola seed. The seed samples of the cultivars Delta, Stellar and Westar described in the previous section, were also used in this study. Of the seed samples taken in 1991, only the control treatment which had not been sprayed with Cerone was used in this study. All four replicates of the 1991 material were used. In 1992, a much larger number of samples were available, so in order to reduce this to a more manageable number, only replicates 2, 4 and 6 were included in this study. Samples which had been harvested approximately every seven days were selected for analysis. All the seed for this study was freeze-dried at the time the samples were taken from the field. Due to time constraints, these freeze-dried samples were stored in sealed glass vials at room temperature for some time prior to analysis. The samples harvested in 1991 were analyzed after 18 to 21 months of storage, while the samples harvested in 1992 were analyzed within 3 to 8 months.

To extract and prepare the samples for HPLC analysis, two gram samples of freeze-dried seed were weighed out and placed in stainless steel extraction tubes with three ball bearings and 30 mL of 3:1 isooctane:ethanol. Samples were shaken for one hour, filtered and the oil extract containing the chlorophyll was collected. Dilutions were prepared for the high chlorophyll samples if required, to prevent peaks from overlapping, and results were adjusted accordingly. Samples were evaporated to dryness under nitrogen at room temperature and resuspended in acetone. Each set of samples was then loaded onto the HPLC for

analysis along with extracts from two check seed samples of known chlorophyll content (determined by spectrophotometry), included as internal standards, and standards of CHL A, CHL B, PHY A and PHY B, which were included at the beginning and end of each run.

3.3.2 HPLC

HPLC analysis was carried out according to the method of Endo et al. (1992), except the fluorescence detector was replaced with a photodiode array detector. The HPLC system consisted of two Waters model 510 pumps, a Waters model 715 Ultra Wisp sample processor, and a Waters model 994 programmable photodiode array detector. The column was stainless steel (220 mm x 4.6 mm) packed with ODS 5μm (Pierce Chemical Co.). The mobile phase was water:methanol:acetone (4:36:60) at a flow rate of 1 mL $min.^{-1}$. An injection volume of between 10 and 50 microlitres was used, depending on the concentration of chlorophyll pigments in the sample. Check samples were run using 50 μL injections and the standards were injected at 10 μL. A run time of thirty minutes was used for all samples, sufficient to allow all of the chlorophyll derivatives to elute. The photodiode array detector was used to scan peaks to identify the chlorophyll pigments by their characteristic absorption maxima. The wavelengths used for quantification were 642, 655, 662 and 667 nm. Chlorophyll pigments were identified by their absorption spectra and retention times by comparison with standards of known composition (Endo et al.,1992).

3.3.3 Preparation of Standards

CHL A was purchased from Fluka Chemical Co. and CHL B was purchased

from Sigma Chemical Co. PHY A and B were prepared from CHL A and B respectively by reaction with HCl (Hyanninen and Ellfolk,1973). Calibration curves were prepared using standard solutions of CHL A, CHL B, PHY A and PHY B as described in Endo et al. (1992). All curves were linear with R^2 values ranging from 0.95-0.98. Standards of PHO A, METHYL A and PYRO A were not prepared so these were quantified using the PHY A standard and multiplying the result by the ratio of the extinction coefficients (Table 1) (ie. 1.24 for PHO A, 1.33 for METHYL A and 1.10 for PYRO A).

Table 1: Absorption Characteristics of Chlorophylls and Related Pigments

E = Absorbtivity (molar extinction coefficient)[a]

Pigment	Max. λ	E	Max. λ	E	Ref.[b]
Pheophorbide A	409	119200	667	55200	3
Methylpheophorbide A	408.5	122500	667	59200	1
Chlorophyll B	455	131000	645	47100	2
Chlorophyll A	430	94700	663	75000	2
Pheophytin B	434.5	145000	654	27800	3
Pheophytin A	409	101800	666	44500	4
Pyropheophytin A	409	102400	667	49000	1

[a] In. acetone (except METHYL A in ether)

[b] 1. Pennington et al.,1964
2. Mackinney,1940
3. Wasielewski and Svec,1980
4. Wilson et al.,1962

3.4 Chlorophyll Derivatives in Commercially Extracted Canola Oil

3.4.1 Oil Sample Collection and Storage

A study was conducted to determine which chlorophyll derivatives occur in commercially extracted canola oil at different stages in the extraction process, and whether the composition of these chlorophyll derivatives changes during oil storage. Freshly extracted canola oil samples were obtained from a western Canadian oilseed crushing plant. Samples were taken of pressed, solvent extracted, crude and acid degummed oils. Sampling was repeated three times resulting in three separate batches of oil containing, by spectrophotometric analysis, 28, 54 and 79 mg kg^{-1} total chlorophyll respectively. For the second two batches of oil, crude oil could not be obtained directly from the processor and was prepared in the lab using a 50:50 mixture of pressed and solvent extracted oils. The fresh oil samples were placed in plastic bottles in a cooler and taken to the lab for immediate analysis. For the first batch of oil, the samples were refrigerated overnight prior to analysis. The second and third batches of oil were analyzed on the same day that they were collected from the crushing plant.

A subsample of each of the fresh oil samples was analyzed by HPLC to identify and quantify the chlorophyll pigments present immediately after processing. The remaining oil was split into four treatments. For each treatment, twenty millilitres each of the pressed, solvent extracted, crude and degummed oils were placed in glass test tubes with screw caps. The oils were stored for one month under four sets of conditions - in a freezer below -20°C in the dark, in a refrigerator at +10°C in the dark, on a bench at room temperature (~22°C) in the dark, or on a bench at room

temperature in the light. Dark storage was achieved by wrapping each test tube with aluminum foil to completely exclude light.

The oil from each test tube was sampled three times - after 8, 15 and 25 days of storage for the first batch of oil, and after 7, 14 and 28 days for the second and third batches of oil. Each sample was analyzed by HPLC to identify and quantify the chlorophyll derivatives present, in order to examine changes in these pigments during oil storage.

3.4.2 HPLC

The oil was dissolved in acetone prior to analysis to give a solution of 25% oil. Analysis was carried out by HPLC according to the method of Endo et al. (1992) as described in the previous study. Each batch of oil samples (fresh and stored) was run on the HPLC using a 50 μL injection volume and a run time of thirty minutes, which was sufficient to allow all of the chlorophyll derivatives to elute. Chlorophyll derivatives were identified by their absorption spectra and retention times compared to standards. For the first batch of oil samples, quantification was carried out at 410, 430, 450 and 490 nm. However this resulted in a large carotenoid peak appearing in all chromatograms at a retention time of two to five minutes, which obscured PHO A and METHYL A. For the second and third batches of oil, quantification was carried out at 642, 655, 662 and 667 nm which allowed PHO A, METHYL A, CHL B, CHL A, PHY B, PHY A and PYRO A all to be detected.

3.4.3 Preparation of Standards

Standards of CHL B, CHL A, PHY B, PHY A and PYRO A were available.

The first four were prepared as described in the previous section. PYRO A was prepared according to the method described by Pennington et al. (1964). PHY A was dissolved in pyridine, placed in a sealed glass tube and heated at 160°C for two hours. The pyridine was allowed to evaporate and the residue was dissolved in acetone, placed in a sealed glass vial and stored in the freezer until required. Standards were not available for PHO A or METHYL A, so both were quantified using the PHY A standard and multiplying by the ratio of the extinction coefficients (ie. 1.24 for PHO A and 1.33 for METHYL A).

3.4.4 Spectrophotometry

In addition to the HPLC analysis, the total chlorophyll content of each oil sample was also measured spectrophotometrically using the AOCS Official Method 13d-55 (1989). For the first batch of oil, total chlorophyll was determined only once for each sample, after 25 days of storage. Oil samples were dissolved in methylene chloride to give a solution of 10% oil, filtered, and the absorbance was measured at 627, 667 and 707 nm using a 1 cm cuvette and a Varian DMS 200 UV-Visible spectrophotometer. Two measurements were made on each oil sample and the results averaged. For the second and third batches of oil, total chlorophyll was measured spectrophotometrically for the fresh oil samples and after 7, 14 and 28 days of storage, to determine whether or not total chlorophyll levels changed during oil storage. The AOCS Official Method 13d-55 (1989) was used, recalibrated with isooctane:ethanol substituted for methylene chloride. Oil samples were dissolved in a 3:1 mixture of isooctane:ethanol to give a solution of 10% oil. Samples were filtered

and the absorbance measured at 625, 665 and 705 nm using a 1 cm cuvette. Two measurements were made on each sample and the results averaged.

RESULTS AND DISCUSSION

4.1 "Agroman" Trials

4.1.1 Genetic and Environmental Influences on Seed Chlorophyll Content at Harvest

This study examined the effects of genotype, environment and GxE interactions on the final chlorophyll content of canola seed at harvest in three cultivars of *B. napus* (Stellar, Delta and Westar) and one cultivar of *B. rapa* (Tobin).

A GLM analysis was initially carried out for each location in each year of the study (Table 2). At all locations but one, the effect of cultivar on seed chlorophyll level was significant at the p=.05 level. Duncan's means separation tests were carried out for each location, but the grouping of the cultivars varied with the location. At all locations, the cultivar Stellar had the highest seed chlorophyll level at harvest, followed by Westar. Delta and Tobin consistently produced low seed chlorophyll levels.

Table 2: Duncan's Means Separation Test Results for Four Canola Cultivars (Stellar, Westar, Delta and Tobin) Obtained from the Provincial Crop Variety Trials from 1988 to 1991(*)

Year	Location	Stellar	Westar	Delta	Tobin
1988	Bagot	66.5 a	43.9 ab	7.6 bc	14.6 c
1988	Beausejour	35.5 a	20.9 ab	3.3 b	14.5 b
1988	Dauphin	30.1 a	21.7 ab	11.9 b	0.0 c
1988	Mariapolis	42.7 a	28.9 a	12.9 b	4.9 b
1988	Melita	40.5 a	33.3 ab	18.3 b	16.8 b
1988	Roblin	39.4 a	25.3 b	12.3 c	0.2 d
1988	Shoal Lake	68.6 a	45.9 a	45.3 a	13.3 b
1988	Swan River	35.1 a	23.9 b	14.1 c	12.7 c
1988	Teulon	37.7 a	22.4 b	14.8 c	13.4 c
1988	Waskada	66.2 a	28.0 b	26.2 b	4.9 c
1989	Beausejour	41.8 a	22.2 b	18.3 b	15.7 b
1989	Dauphin	17.0 a	9.8 b	4.8 c	2.4 d
1989	Mariapolis	45.2 a	21.8 b	14.7 c	3.2 d
1989	Melita	43.8 a	33.9 ab	20.2 bc	13.6 c
1989	The Pas	72.1 a	52.0 b	29.4 c	6.3 d
1989	Roblin	22.6 a	10.8 b	7.1 b	1.6 c
1989	Shoal Lake	38.8 a	30.5 b	15.4 c	14.1 c
1989	Swan River	18.6 a	10.4 b	6.4 c	2.7 d
1989	Teulon	41.2 a	35.2 a	23.4 b	14.8 c
1989	Winnipeg	29.5 a	16.5 b	9.9 b	7.1 b

(*) within the same location, values followed by the same letter were not significantly different at the p=0.05 level

Table 2 (cont.): Duncan's Means Separation Test Results for Four Canola Cultivars (Stellar, Westar, Delta and Tobin) Obtained from the Provincial Crop Variety Trials from 1988 to 1991(*)

Year	Location	Stellar	Westar	Delta	Tobin
1990	Dauphin	26.1 a	10.9 b	10.4 b	3.7 c
1990	The Pas	27.9 a	10.9 b	12.3 b	2.7 c
1990	Roblin	33.3 a	23.6 b	10.4 c	13.2 c
1990	Teulon	30.8 a	27.5 a	14.4 b	8.8 b
1990	Waskada	61.4 a	43.5 b	36.1 b	14.7 c
1991	Beausejour	32.2 a	25.7 a	16.2 b	8.3 b
1991	Pilot Mound	25.2 a	16.3 b	13.3 b	4.7 c
1991	Portage	9.6 a	5.8 a	4.5 a	2.2 a
1991	Roblin	12.5 a	9.6 ab	5.7 bc	2.6 c
1991	Waskada	19.0 a	11.9 b	11.5 b	1.4 c

(*) within the same location, values followed by the same letter were not significantly different at the p=0.05 level

Bartlett's test was carried out for each year's data to determine whether or not the error variances obtained at different locations were homogeneous. In 1988, Bartlett's test yielded a chi square value of 45.13 with 9 degrees of freedom, significant at the 1% level; in 1989 the chi square value was 56.30 with 9 degrees of freedom, significant at the 1% level; in 1990 a chi square value of 14.90 with 4 degrees of freedom was obtained, significant at the 1% level; and in 1991 the chi square value was 7.12 with 4 degrees of freedom, which was non-significant at the 5% level. Therefore, the first three years of data could not be validly combined over locations, due to error variance heterogeneity, unless the analysis was modified.

A GLM analysis was run on each year's data combined over locations and the results were then modified according to Cochran and Cox (1957). Some components of the location by cultivar interaction may be heterogeneous due to environmental variability between locations. If the interaction mean square is heterogeneous, the F-test for locations must be modified. According to Cochran and Cox (1957), the most conservative test possible assumes that the tabular F-value is distributed approximately with 1 and p-1 degrees of freedom, where p is the number of locations. This takes into account the maximum possible distortion in F which could occur.

Heterogeneity among the experimental error variances, indicated by the results of Bartlett's test, invalidates the F-test of the interaction mean square against the pooled error mean square. The most conservative test possible assumes that the F-value of the location by cultivar interaction is distributed approximately as the tabular F-value, but the number of degrees of freedom is reduced to t-1 and n′, where t is the number of cultivars being tested and n′ is the number of error degrees of freedom associated with the location having the greatest error variance (Cochran and Cox,1957).

Finally, heterogeneity of the experimental error variances also affects the F-test for cultivars. Cultivars should be tested against the interaction (location*cultivar) mean square, instead of against the pooled error term, since this F-value will be less disturbed by the heterogeneity among the error variances (Cochran and Cox,1957).

Modified GLM results for each year's data combined over locations are presented in Tables 3 to 6. The data was analyzed as a split-plot design with locations as the main effect and cultivars as the sub-plots. The

1991 data were not modified as the error variances were shown by Bartlett's test to be homogeneous.

Table 3: Modified GLM Results for Seed Chlorophyll Levels in Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over Locations in 1988. (loc=location, cult=cultivar)

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Reps	3	30.08	0.25	-	-	ns
Locations	9	1101.52	9.23	-	(1,9)	**(1)
Error a (rep*loc)	26	119.34	1.07			
Cultivars	3	9870.10	88.57	36.98	-	**
Loc*Cult	27	266.93	2.40	-	(3,9)	ns
Error b	85	111.44				

(1) ns = non-significant at p=.05
** = significant at p=.01
* = significant at p=.05

Table 4: Modified GLM Results for Seed Chlorophyll Levels in Four Canola Cultivars (Stellar, Delta, Westar and Tobin Combined Over Locations in 1989

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Reps	3	72.61	1.81	-	-	ns
Locations	9	1645.09	40.96	-	(1,9)	**(1)
Error a (rep*loc)	27	40.17	1.60			
Cultivars	3	6173.34	245.60	30.08	-	**
Loc*Cult	27	205.24	8.17	-	(3,8)	**
Error b	88	25.14				

(1) ns = non-significant at p=.05
** = significant at p=.01

Table 5: Modified GLM Results for Seed Chlorophyll Levels in Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over Locations in 1990

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Reps	3	44.42	0.93	-	-	ns
Locations	4	1786.97	37.56	-	(1,4)	**(1)
Error a (rep*loc)	12	47.58	2.80			
Cultivars	3	2661.60	156.73	20.79	-	**
Loc*Cult	12	128.05	7.54	-	(3,9)	**
Error b	45	16.98				

(1) ns = non-significant at p=.05
** = significant at p=.01

Table 6: GLM Results (not modified) for Seed Chlorophyll Levels in Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over Locations in 1991

Source	DF	MS	F-Value	Sig.
Reps	3	20.05	0.63	ns
Locations	4	541.13	16.93	**(1)
Error a (rep*loc)	12	31.97	2.07	
Cultivars	3	747.96	48.45	**
Loc*Cult	12	39.26	2.54	*
Error b	40	15.44		

(1) ns = non-significant at p=.05
** = significant at p=.01
* = significant at p=.05

In every year of the study, both cultivars and locations were highly significantly different (p=0.01), which indicates that both the genotype of the plant and the location at which it is grown contribute to the final chlorophyll content of the seed at harvest. A significant (p=0.05)

genotype by environment (cult*loc) interaction also occurred in most years.

A GLM analysis was then performed on the data set combined over years. Different locations were involved in each year of the study, so in order to combine the data over years, each location in each year was considered as a separate trial. This resulted in a split-plot design with years as the main effect, trials nested within years, and cultivars as the sub-plots. Years were tested against trials within years as the error term, trials within years were tested against reps within trials, and both cultivars and the cultivar by trial interaction were tested against error b. Due to the heterogeneous error variances among locations in the first three years of the study, the GLM analysis was also modified according to Cochran and Cox (1957) as described previously. The modified GLM analysis is presented in Table 7.

Table 7: Modified GLM Results for Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 30 Trials Conducted Over 4 Years

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Years	3	3185.32	2.46	-	(1,3)	ns
Trials(years)	26	1293.63	20.58	-	(1,29)	**(1)
Reps(trials)	89	62.87	1.24	-	-	ns
Cultivars	3	17977.16	354.98	84.05	-	**
Trial*Cult	87	213.88	4.22	-	(3,8)	ns
Error b	258	50.64				

(1) ns = non-significant at p=.05
** = significant at p=.01

Both cultivars and trials within years were highly significant

(p=0.01), confirming that final chlorophyll levels in harvested canola seed were affected by both the genotype of the plant and the environment in which it was grown. Years were not significant at the p=.05 level, indicating that these four cultivars performed similarly in all four years of the study. Using the modified degrees of freedom, the trial by cultivar interaction was not significant at the p=.05 level. This conflicts with the analysis of the individual years, where a significant G x E interaction (p=0.05) occurred in three of the four years of the study. However the non-significance of the trial by cultivar interaction in the analysis combined over years does not prove conclusively that there is no G x E interaction. The modified degrees of freedom provide the most conservative test possible. The real distribution of the trial by cultivar interaction lies somewhere between the original degrees of freedom (87,258) where the interaction was highly significant, and the modified degrees of freedom (3,8) where the interaction was non-significant at p=.05. Therefore, it is not possible to draw a conclusion with certainty with regard to the significance of the G x E interaction. A previous study, that involved fourteen cultivars and ten locations in the first year and twenty-two cultivars and ten locations in the second year, indicated that B. napus cultivars contained more seed chlorophyll at harvest than B. rapa cultivars, that the final chlorophyll content of B. napus seed was affected by the cultivar, location of the trial and year of the trial, and that there was significant (p=0.05) GxE interaction (Ward,1990).

4.1.1.1 Genotypic Effect

In each year of the study, cultivar effects were significant at the p=.01 level. To investigate these cultivar differences, Duncan's means separation tests were performed on each year's data combined over replicates and locations (Tables 8 to 11). These were carried out by hand as outlined in Gomez and Gomez (1984) using the appropriate degrees of freedom from the modified GLM analysis.

Table 8: Duncan's Means Separation Test Results (p=0.05) for Average Seed Chlorophyll Content of Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 4 Replicates and 10 Locations in 1988. (chl=total chlorophyll content)

Cultivar	Mean Chl.(mg kg^{-1})	Group
Stellar	46.5	A
Westar	29.5	B
Delta	16.5	C
Tobin	9.7	C

Table 9: Duncan's Means Separation Test Results (p=0.05) for Average Seed Chlorophyll Content of Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 4 Replicates and 10 Locations in 1989

Cultivar	Mean Chl.(mg kg^{-1})	Group
Stellar	37.1	A
Westar	24.1	B
Delta	14.1	C
Tobin	9.0	C

Table 10: Duncan's Means Separation Test Results (p=0.05) for Average Seed Chlorophyll Content of Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 4 Replicates and 5 Locations in 1990

Cultivar	Mean Chl.(mg kg^{-1})	Group
Stellar	35.9	A
Westar	23.3	B
Delta	16.7	B C
Tobin	8.6	C

Table 11: Duncan's Means Separation Test Results (p=0.05) for Average Seed Chlorophyll Content of Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 4 Replicates and 5 Locations in 1991. (not modified)

Cultivar	Mean Chl.(mg kg^{-1})	Group
Stellar	19.0	A
Westar	13.5	B
Delta	10.5	C
Tobin	3.7	D

The ranking of these four cultivars was the same in every year of the study. The cultivar Stellar always contained the highest level of seed chlorophyll at harvest, followed by Westar, Delta and Tobin, respectively. Compared to Westar, which requires, on average, 100 days to reach full maturity, both Stellar and Delta require an extra four days to reach full maturity, and Tobin requires twelve days less (Western Canadian Co-operative Trials 1988-1992). The shorter growing season required for the cultivar Tobin provides an explanation for the low seed chlorophyll at

harvest. Tobin is able to reach full maturity in the length of growing season available in Manitoba. However differences in the reported maturation times required do not adequately explain the differences observed between the cultivars Stellar, Westar and Delta. Delta consistently produced low seed chlorophyll at harvest, somewhat higher than Tobin, but not significantly higher at the p=.05 level in three of the four years of the study. Due to the modified analysis, the Duncan's means separation test used was the most conservative test possible. Therefore, it is not possible to say with certainty that Delta was not significantly different from Tobin, but we can conclude that it consistently produced the lowest seed chlorophyll of the B. napus cultivars tested. According to data from the Western Canadian Co-operative Trials, the cultivars Stellar and Delta require the same length of growing season to reach full maturity. However our data indicates that Stellar is more likely to be harvested prematurely, while seed chlorophyll levels remain high.

These three B. napus cultivars have distinct genetic backgrounds. Stellar is a low linolenic canola cultivar developed at the University of Manitoba in 1987 from a mutation line backcrossed to Regent. Delta is a Swedish canola cultivar developed at the Weibullsholm Plant Breeding Institute in 1989, and Westar is a canola cultivar developed from a backcross to Tower, released by the Saskatoon Agriculture Canada research station in 1982.

A Duncan's means separation test was also carried out on the data set combined over relicates, locations and years (Table 12).

Table 12: Duncan's Means Separation Test Results (p=0.05) for Average Seed Chlorophyll Content of Four Canola Cultivars (Stellar, Delta, Westar and Tobin) Combined Over 4 Replicates, 30 Locations and 4 Years (1988-1991)

Cultivar	Mean Chl.(mg kg^{-1})	Group
Stellar	37.1	A
Westar	24.0	B
Delta	14.7	C
Tobin	8.4	D

These results confirm those obtained when each year of the study was considered separately. Seed chlorophyll levels were consistently higher for Stellar > Westar > Delta > Tobin. When the data was combined over years all four cultivars were clearly separated, indicating that the B. napus cultivars contained more seed chlorophyll at harvest than the B. rapa cultivar, and the B. napus cultivars produced a range of seed chlorophyll levels due to genotypic differences.

It should be noted that under favourable growing conditions all cultivars would eventually reach extremely low seed chlorophyll levels. High chlorophyll levels in the harvested seed indicate that seed maturation has been terminated prematurely. This can result from a number of causes such as frost, drought or extreme lodging. Therefore Stellar is more likely to be harvested prematurely than Westar, Delta or Tobin, respectively. Cultivars which tend to contain high levels of chlorophyll in harvested seed might have slower rates of seed chlorophyll breakdown than other cultivars. Differences in the visual appearance of the plants or seeds might also explain why certain cultivars are more likely to be

harvested prematurely. If the plants appear brown and ripe they are likely to be harvested although green seeds may remain. Also there is a poor correlation between percent green seed and the actual chlorophyll content of the seed (Daun,1982) which might allow certain cultivars to appear ripe, while still containing significant amounts of seed chlorophyll. Finally, the extent of branching and indeterminate growth of a cultivar will affect its seed chlorophyll level at harvest. The greater the degree of branching the more likely immature seed will remain at harvest.

4.1.1.2 Environmental Effects

The GLM analysis indicated significant differences in seed chlorophyll content among locations in all years of the study ($p=0.01$). Duncan's means separation tests were performed on the data averaged over replicates and cultivars to rank the various locations in each year of the study (Tables 13 to 16).

Table 13: Duncan's Means Separation Test Results (p=0.05) Based on Average Seed Chlorophyll Content Comparing Ten Locations in 1988. (Values were averaged over 4 replicates and 4 cultivars)

Location	Mean Chl.(mg kg^{-1})	Group
Shoal Lake	43.3	A
Bagot	33.1	B
Waskada	31.3	B
Melita	27.8	B C
Mariapolis	22.3	C D
Teulon	22.1	C D
Swan River	21.5	C D
Roblin	19.8	C D
Beausejour	18.5	D
Dauphin	15.9	D

Table 14: Duncan's Means Separation Test Results (p=0.05) Based on Average Seed Chlorophyll Content Comparing Ten Locations in 1989. (Values were averaged over 4 replicates and 4 cultivars)

Location	Mean Chl.(mg kg^{-1})	Group
The Pas	38.9	A
Teulon	28.6	B
Melita	27.5	B
Shoal Lake	24.7	B C
Beausejour	24.6	B C
Mariapolis	21.2	C
Winnipeg	15.7	D
Roblin	10.5	E
Swan River	9.5	E
Dauphin	8.5	E

Table 15: Duncan's Means Separation Test Results (p=0.05) Based on Average Seed Chlorophyll Levels Comparing Five Locations in 1990. (Values were averaged over 4 replicates and 4 cultivars)

Location	Mean Chl.(mg kg^{-1})	Group
Waskada	38.9	A
Teulon	20.4	B
Roblin	20.1	B
The Pas	13.4	C
Dauphin	12.7	C

Table 16: Duncan's Means Separation Test Results (p=0.05) Based on Average Seed Chlorophyll Levels Comparing Five Locations in 1991. (Values were averaged over 4 replicates and 4 cultivars)

Location	Mean Chl.(mg kg^{-1})	Group
Beausejour	20.6	A
Pilot Mound	14.9	B
Waskada	10.9	B C
Roblin	8.3	C D
Portage la Prairie	5.5	D

The ranking of locations based on seed chlorophyll levels at harvest changed from year to year. Only one location was carried through the full four years of the study, with new locations being added and others dropped each year. Therefore, it is difficult to make any conclusions about specific locations. Material grown at Dauphin consistently yielded the lowest levels of seed chlorophyll of any location tested, in the three years that this location was included in the study. Although located quite far north, the Riding Mountains create a microclimate which may

account for the low chlorophyll levels obtained at the Dauphin location. The Roblin location also ranked low for seed chlorophyll in all four years of the study. The Roblin location is quite far north and has a shorter growing season than locations in the south of the province. However adequate rainfall may explain the low seed chlorophyll levels, as drought may halt seed maturation prematurely, resulting in high seed chlorophyll at harvest (Harris,1988; Ward et al.,1992a).

From the data available, it is impossible to tell exactly which environmental variables affect seed chlorophyll levels. Some of the location effect may be due to differences in agronomic practices between sites. The data come from the provincial crop variety trials, so each site was seeded and harvested on different dates and management may not have been uniform. Each site differs in a wide range of environmental variables including temperature, length of growing season, soil type and amount of precipitation. The microclimate of the plots may also be more important than general weather conditions at each location.

When the data were combined over years, it was possible to examine the effect of both years and trials. Specific locations could not be combined over years because different locations were used in each year of the study. Therefore, each location in each year was considered as a separate trial in the combined analysis over years. The GLM analysis indicated that there were no significant differences between years at the $p=.05$ level, but the effect of trials within years was highly significant (at the $p=.01$ level).

Duncan's means separation tests were carried out by hand according to Gomez and Gomez (1984) for the data from the modified GLM analysis, in

order to rank the trials conducted over the four years of the study when the results were averaged over replicates and cultivars (Table 17). (Values followed by the same letter were not significantly different at the p=0.05 level).

Table 17: Means Separation Test Combined Over Years and Locations

Trial	Year	Location	Mean Chl.	Group
7	1988	Shoal Lake	43.3	A
20	1989	The Pas	39.9	A
22	1990	Waskada	38.9	A
1	1988	Bagot	33.1	B
4	1988	Waskada	31.3	B
11	1989	Teulon	28.6	B C
6	1988	Melita	27.8	B C D
16	1989	Melita	27.5	B C D
12	1989	Shoal Lake	24.7	C D E
13	1989	Beausejour	24.6	C D E
5	1988	Mariapolis	22.3	C D E F
3	1988	Teulon	22.1	D E F
10	1988	Swan River	21.5	D E F
15	1989	Mariapolis	21.2	D E F G
26	1991	Beausejour	20.6	E F G
23	1990	Teulon	20.4	E F G
21	1990	Roblin	20.1	E F G
9	1988	Roblin	19.8	E F G
2	1988	Beausejour	18.5	E F G H
8	1988	Dauphin	15.9	F G H I
14	1989	Winnipeg	15.7	F G H I
27	1991	Pilot Mound	14.9	G H I J
25	1990	The Pas	13.4	H I J K
24	1990	Dauphin	12.7	H I J K
30	1991	Waskada	10.9	I J K
19	1989	Roblin	10.5	I J K L
17	1989	Swan River	9.5	I J K L
18	1989	Dauphin	8.5	J K L
29	1991	Roblin	8.3	K L
28	1991	Portage	5.5	L

Clearly there was a great deal of variation in cultivar performance over the different trials. In general, seed chlorophyll levels at harvest were lower in 1990 and 1991 than in 1988 and 1989, but there were exceptions to this. The Dauphin and Roblin sites fall within the lower one third of the trials in all years of the study when they are ranked from highest to lowest on the basis of seed chlorophyll content. Once again, it is impossible to determine which environmental variables are affecting seed chlorophyll, but the existence of a significant environmental effect is clear.

Duncan's means separation tests were performed to rank the seed chlorophyll levels obtained in different years of the study by combining results over replicates, cultivars and locations (Table 18).

Table 18: Duncan's Means Separation Test Results (p=0.05) Based on Average Seed Chlorophyll Levels for the Four Years of the Study (1988-1991) Combined Over Replicates, Locations and Cultivars

Year	Mean Chl.(mg kg^{-1})	Group
1988	25.7	A
1990	21.1	A
1989	21.0	A
1991	11.9	B

There were no significant differences detected (p=0.05) between the different years of the study, except in 1991 which recorded lower average seed chlorophyll levels than the previous three years. This may be due to either climatic conditions in 1991 or due to the choice of locations harvested that year.

4.1.1.3 Genotype By Environment Interaction

A significant G x E interaction was observed in some years of the study but not in others. In 1988, the location by cultivar interaction was not significant at the p=.05 level. In 1989 and 1990, the interaction was significant at the p=.01 level, and in 1991 the G x E interaction was significant at the p=.05 level, but not at the p=.01 level. When the data were combined over years, the modified GLM analysis indicated that the trial by cultivar interaction was not significant at the p=.05 level, however the unmodified GLM indicated significance at the p=.0001 level. Therefore the true level of significance lies somewhere in between. One cannot conclude that there is a significant G x E interaction, but neither can it be concluded with certainty that there is not one. Therefore, cultivar evaluation trials should be conducted in such a way that a G x E effect, if present, will be taken into account. In other words, trials should be conducted at a number of representative locations over several years to adequately measure a cultivar's performance and stability with regard to final seed chlorophyll levels.

A graph of the average chlorophyll level in each cultivar at each trial site reveals that over the four years of the study, for the four cultivars tested, the lines representing each cultivar rarely intersect, with the exception of the two cultivars Delta and Tobin. This indicates that the relative performance of each cultivar with regard to final seed chlorophyll levels follows the same pattern at all trial sites. Stellar consistently produced seed containing more chlorophyll at harvest than Westar, which in turn had higher seed chlorophyll levels than Delta or Tobin. This consistent cultivar performance indicates that G x E

interaction was minimal in this study, with regard to final seed chlorophyll levels (Figure 2). However, it should be noted that these four cultivars were selected from the preliminary study based on their stable performance in producing high, low, or intermediate levels of seed chlorophyll at harvest. Therefore, a minimal G x E effect in this study does not mean that this finding would necessarily apply if other cultivars were tested. The preliminary study tested a much wider range of cultivars and found significant G x E interaction (Ward,1990).

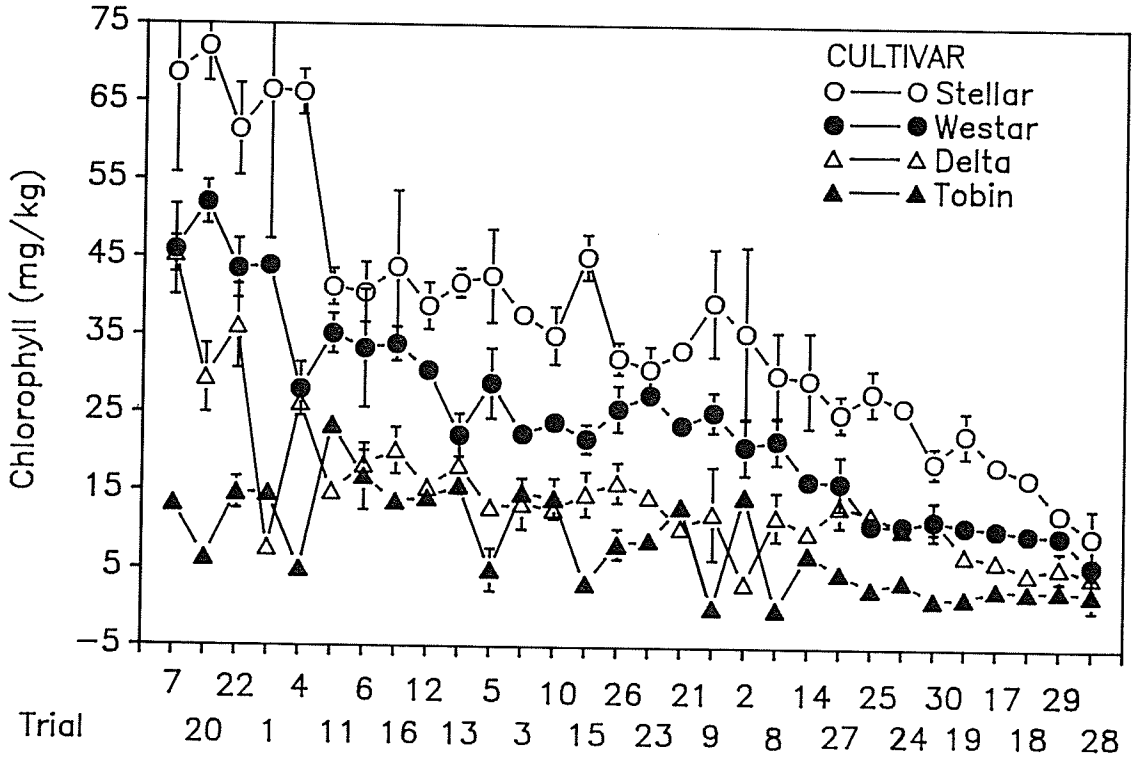


Figure 2: Average Chlorophyll Levels (mg kg^{-1}) in Selected B. napus Cultivars from the "Agroman" Trials Conducted from 1988 to 1991. (Error bars represent standard errors. Each value was averaged over 4 replicates.)

4.1.1.4 Summary

Both the genotype of the plant and the location at which it was grown contributed significantly to the chlorophyll level of the seed at harvest. The cultivar Stellar consistently produced the highest seed chlorophyll levels, followed by Westar, Delta and Tobin, respectively. The cultivars containing the highest levels of seed chlorophyll at harvest are the most likely to be harvested prematurely. In this study, the location of the trial had a greater effect on seed chlorophyll levels than the year in which the trial was conducted. Minimal GxE interaction was observed in this study, but this did not apply when a larger number of cultivars were tested (Ward,1990).

4.1.2 Correlations Between Seed Components

Levels of chlorophyll (mg kg^{-1}), oil (%) and protein (%) (seed basis) were determined for every seed sample taken in each year of the "Agroman" Trial study. The correlations between these seed components are summarized in Table 19.

Table 19: Correlations Between Chlorophyll (mg kg^{-1}), Oil (%) and Protein (%) Levels in 4 Cultivars of Canola Seed (Stellar, Westar, Delta and Tobin) Harvested at 30 Locations in 1988 to 1991

Year	N	Oil x Protein (sig.)	Oil x Chl. (sig.)	Protein x Chl. (sig.)
1988	154	-0.87 (.0001)	+0.089 (.2710)	+0.096 (.2371)
1989	158	-0.80 (.0001)	-0.050 (.5355)	+0.28 (.0003)
1990	80	-0.92 (.0001)	-0.60 (.0001)	+0.63 (.0001)
1991	75	-0.87 (.0001)	+0.58 (.0001)	-0.31 (.0070)
88-91	467	-0.86 (.0001)	-0.081 (.0814)	+0.26 (.0001)

In every year of the study, large significant (p=0.0001) negative correlations were found between the oil and protein contents of the seed. The correlations between oil and chlorophyll were highly variable. No consistent correlations were found regardless of whether the data was analyzed within trials, subdivided by cultivar, or in a combined analysis. In three of the four years of the study the seed protein and chlorophyll contents were significantly correlated (p=0.01). The correlation between protein and chlorophyll was positive in two years, negative in one year and non-significant (p=0.05) in the remaining year of the study. No consistent correlation between protein and chlorophyll was observed when the data was analyzed by cultivar.

Increased oil and protein contents are both objectives of canola breeding programs. The strong negative correlation between these two seed components, expressed as a percentage of seed, confirms that the only way to achieve higher levels of both will be to select for total oil plus protein (Grami and Stefansson,1977a,b; Grami et al.,1977). If only a single component were selected, gains in that component would result in a

loss of the other. The lack of correlation between oil and chlorophyll and the variable correlations between protein and chlorophyll can be explained by the fact that the majority of oil and protein is laid down prior to the initiation of seed chlorophyll breakdown (Norton and Harris,1975; Horodyski and Tabola,1983). There does not appear to be any direct connection between the oil or protein content of canola seed and its chlorophyll content at harvest. Therefore, it should be possible to select for both high oil content and low seed chlorophyll simultaneously.

4.2 Field Study - Seed Chlorophyll Degradation, Moisture Loss and Ethylene Evolution From Ripening Seed of Brassica napus

4.2.1 Seed Chlorophyll Degradation Rates in Three Cultivars of Brassica napus

Three cultivars of B. napus, Stellar, Delta and Westar, sown on two different dates were compared for rates of seed chlorophyll breakdown in the field in 1991 and 1992. In the first year of the study, some plots were sprayed with Cerone to determine whether this would enhance seed chlorophyll loss. Only the control plots, which were sprayed with distilled water, were used in this study. Seed samples harvested once or twice a week throughout the ripening period were analyzed for seed chlorophyll. Results were averaged over replicates and in order to linearize the data so that chlorophyll breakdown rates in each cultivar could be compared, the logarithms of the chlorophyll levels for each cultivar were plotted against the number of days from the start of sampling. A regression analysis was performed on the raw data for each seeding date (Table 20) and the best straight line fitted to each cultivar (Figure 3).

The slope of each line represents the rate of seed chlorophyll breakdown in that cultivar. Paired T-tests were performed to test for homogeneity of the regression coefficients to determine whether the rates of seed chlorophyll breakdown were significantly different among the cultivars tested (Table 21).

Table 20: Regression Analysis Results for Three Cultivars of B. napus (Stellar, Delta and Westar) Based on Log Chlorophyll (mg kg^{-1}) vs. Days After First Sample.

Year	Cultivar	Seeding Date	Slope	MS Error	df	Adj. r^2	Sig.
1991	Delta	Early	-0.1085	0.1178	22	0.92	0.0001
1991	Stellar	Early	-0.08414	0.06449	22	0.93	0.0001
1991	Westar	Early	-0.07894	0.09025	22	0.91	0.0001
1991	Delta	Late	-0.1139	0.02899	14	0.96	0.0001
1991	Stellar	Late	-0.1020	0.03101	14	0.95	0.0001
1991	Westar	Late	-0.1113	0.08004	11	0.91	0.0001
1992	Delta	Early	-0.07852	0.05353	48	0.94	0.0001
1992	Stellar	Early	-0.06073	0.04846	48	0.91	0.0001
1992	Westar	Early	-0.07490	0.03634	48	0.95	0.0001
1992	Delta	Late	-0.05288	0.05372	43	0.91	0.0001
1992	Stellar	Late	-0.04064	0.04222	43	0.88	0.0001
1992	Westar	Late	-0.05035	0.03291	43	0.94	0.0001

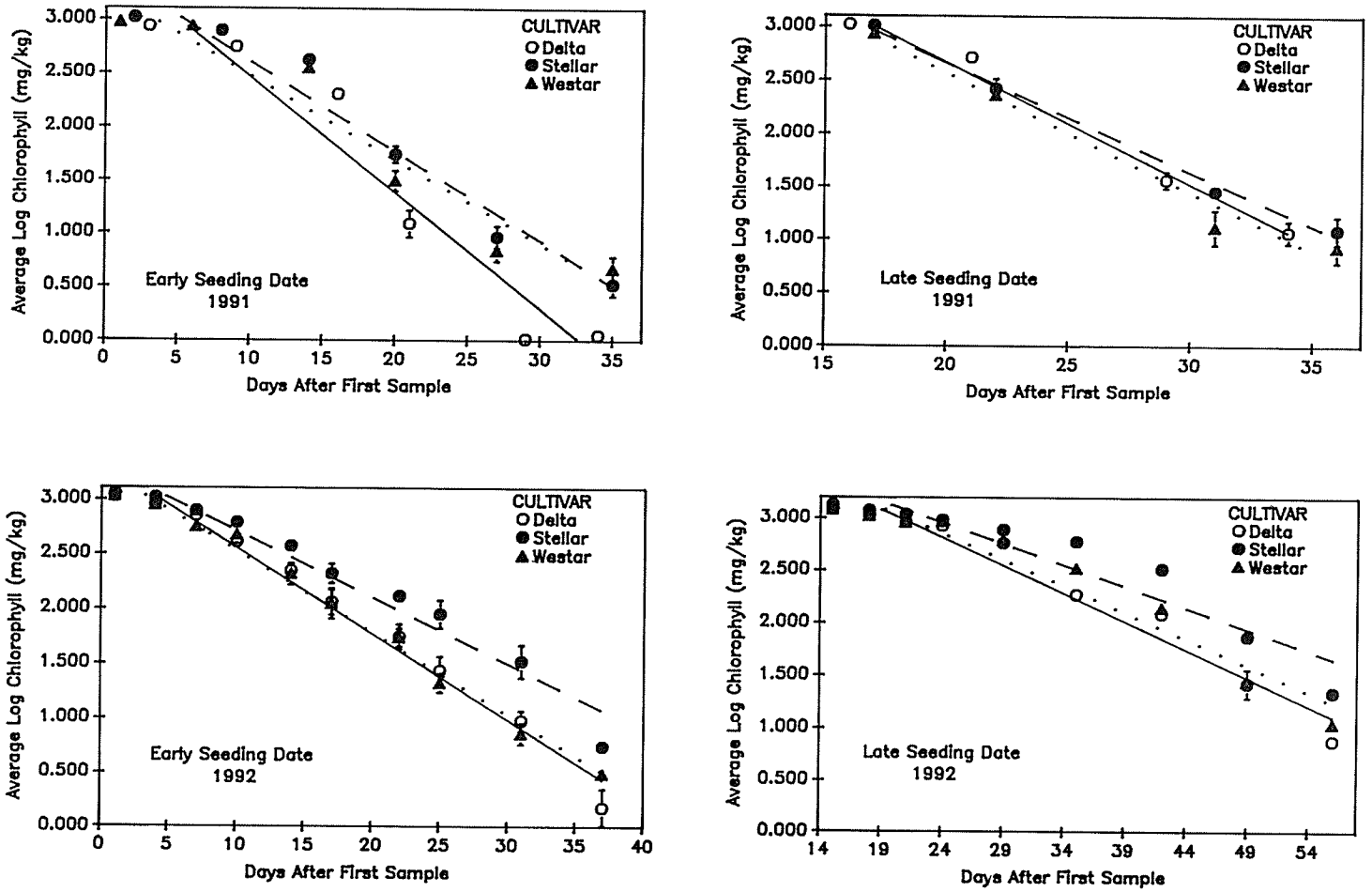


Figure 3: The regressions of log chlorophyll (mg kg^{-1}) against days after first sampling for the cultivars Stellar (----), Delta (——) and Westar (....). (Error bars are standard errors. Each value was averaged over 4 replicates in 1991 and 5 replicates in 1992.)

Table 21: Paired T-Test Results Comparing Rates of Seed Chlorophyll Breakdown in Three Cultivars of B. napus (Stellar, Delta and Westar) Based on Regressions of Log Chlorophyll (mg kg^{-1}) vs. Days After First Sample

Year	Seeding Date	Comparison	df	|T|	Sig.
1991	Early	Delta x Stellar	44	3.058	**(1)
1991	Early	Delta x Westar	44	3.552	**
1991	Early	Stellar x Westar	44	0.739	ns
1991	Late	Delta x Stellar	28	1.397	ns
1991	Late	Delta x Westar	25	0.228	ns
1991	Late	Stellar x Westar	25	0.834	ns
1992	Early	Delta x Stellar	96	4.436	**
1992	Early	Delta x Westar	96	0.961	ns
1992	Early	Stellar x Westar	96	3.875	**
1992	Late	Delta x Stellar	86	3.526	**
1992	Late	Delta x Westar	86	0.248	ns
1992	Late	Stellar x Westar	86	3.219	**

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

In the early seeding date of 1991 there were significant differences (p=.01) between the rates of seed chlorophyll breakdown for the cultivars Delta x Stellar and Delta x Westar, with Delta having the faster rate of chlorophyll breakdown. However there was no significant difference (p=0.05) in the rate of chlorophyll breakdown between Stellar x Westar. In the late seeding date of 1991 there were no significant differences (p=0.05) in rates of seed chlorophyll breakdown between any of the three cultivars. In 1991, the late seeding date ripened during unusually warm temperatures, so rapid chlorophyll degradation in all cultivars may

explain the lack of significantly different rates of seed chlorophyll breakdown. Temperature data is provided in the Appendix (Tables A2 and A3). In 1992, in both the early and late seeding dates, the cultivars Delta x Westar had the same rate of seed chlorophyll breakdown ($p=0.05$), while Delta x Stellar and Westar x Stellar were both significantly different ($p=.01$), with Stellar having the slower rate of seed chlorophyll breakdown. Rates of seed chlorophyll breakdown varied with the external environment. In general, however, Delta had the fastest rate of seed chlorophyll breakdown, followed by Westar, followed by Stellar. These cultivar differences indicate that genetic variability does exist for this trait, so it might be possible to select for rapid seed chlorophyll breakdown in a breeding program to reduce the incidence of green seed at harvest.

Paired T-tests compared rates of seed chlorophyll breakdown in the early and late seeding dates each year (Table 22). Based on the regressions of log chlorophyll (mg kg^{-1}) versus days after first sample, in 1991, the cultivars Stellar and Westar had faster rates of seed chlorophyll breakdown in the later seeding date ($p=0.05$), while the rate of seed chlorophyll breakdown for Delta was not affected by seeding date ($p=0.05$). In 1992, all cultivars had significantly different rates of seed chlorophyll breakdown in the early and late seeding dates ($p=0.01$), with a faster rate of chlorophyll degradation in the early seeding date.

Table 22: Paired T-Test Results Comparing Rates of Seed Chlorophyll Breakdown Within the Same Cultivar Between Early and Late Seeding Dates. Comparison of Regressions of Log Chlorophyll (mg kg^{-1}) vs. Days After First Sample and vs. Growing Degree Days (GDD)

Year	Cultivar	df	|t| (days)	Sig. (days)	|T| (GDD)	Sig. (GDD)
1991	Delta	36	0.461	ns(1)	0.190	ns
1991	Stellar	36	2.056	*	1.349	ns
1991	Westar	33	2.797	**	2.092	*
1992	Delta	91	6.632	**	1.193	ns
1992	Stellar	91	5.664	**	1.006	ns
1992	Westar	91	7.894	**	1.459	ns

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

The weather was considerably different in the two years of the study and even between the different seeding dates within the same year (temperature and precipitation data in Tables A2 and A3 in the Appendix). In general, in 1991 the weather was hot and dry and remained so until late August, by which time the plots in both seeding dates had reached full maturity (HB 5.5). In 1992, the weather was cooler with more precipitation, particularly late in the growing season, which delayed the ripening of plots in the late seeding date until early October.

To determine the contribution of temperature to the differences in rates of chlorophyll breakdown between different seeding dates and years, growing degree days (GDD) were substituted for days in the cultivar regressions (Tables 22 and 23). These regressions are illustrated in Figure 4. GDD provide a measure of the heat units accumulated during the

ripening period, calculated by taking the daily mean temperature minus 5°C and summing over the entire sampling period. The base 5°C was shown to be the minimum temperature required for the growth of oilseed rape (Morrison et al.,1989).

Table 23: Regression Analysis Results for Three Cultivars of B. napus (Stellar, Delta and Westar) Based on Log Chlorophyll (mg kg^{-1}) vs. Growing Degree Days (GDD)

Year	Cultivar	Seeding Date	Slope	MS Error	df	Adj. r^2	Sig.
1991	Delta	Early	-0.006669	0.09615	22	0.94	0.0001
1991	Stellar	Early	-0.005180	0.05292	22	0.95	0.0001
1991	Westar	Early	-0.004881	0.08186	22	0.92	0.0001
1991	Delta	Late	-0.006548	0.03428	14	0.95	0.0001
1991	Stellar	Late	-0.005822	0.03433	14	0.95	0.0001
1991	Westar	Late	-0.006265	0.09245	11	0.90	0.0001
1992	Delta	Early	-0.007615	0.09166	48	0.89	0.0001
1992	Stellar	Early	-0.005842	0.07880	48	0.85	0.0001
1992	Westar	Early	-0.007298	0.06442	48	0.92	0.0001
1992	Delta	Late	-0.006954	0.07225	43	0.87	0.0001
1992	Stellar	Late	-0.005340	0.05371	43	0.85	0.0001
1992	Westar	Late	-0.006623	0.04948	43	0.90	0.0001

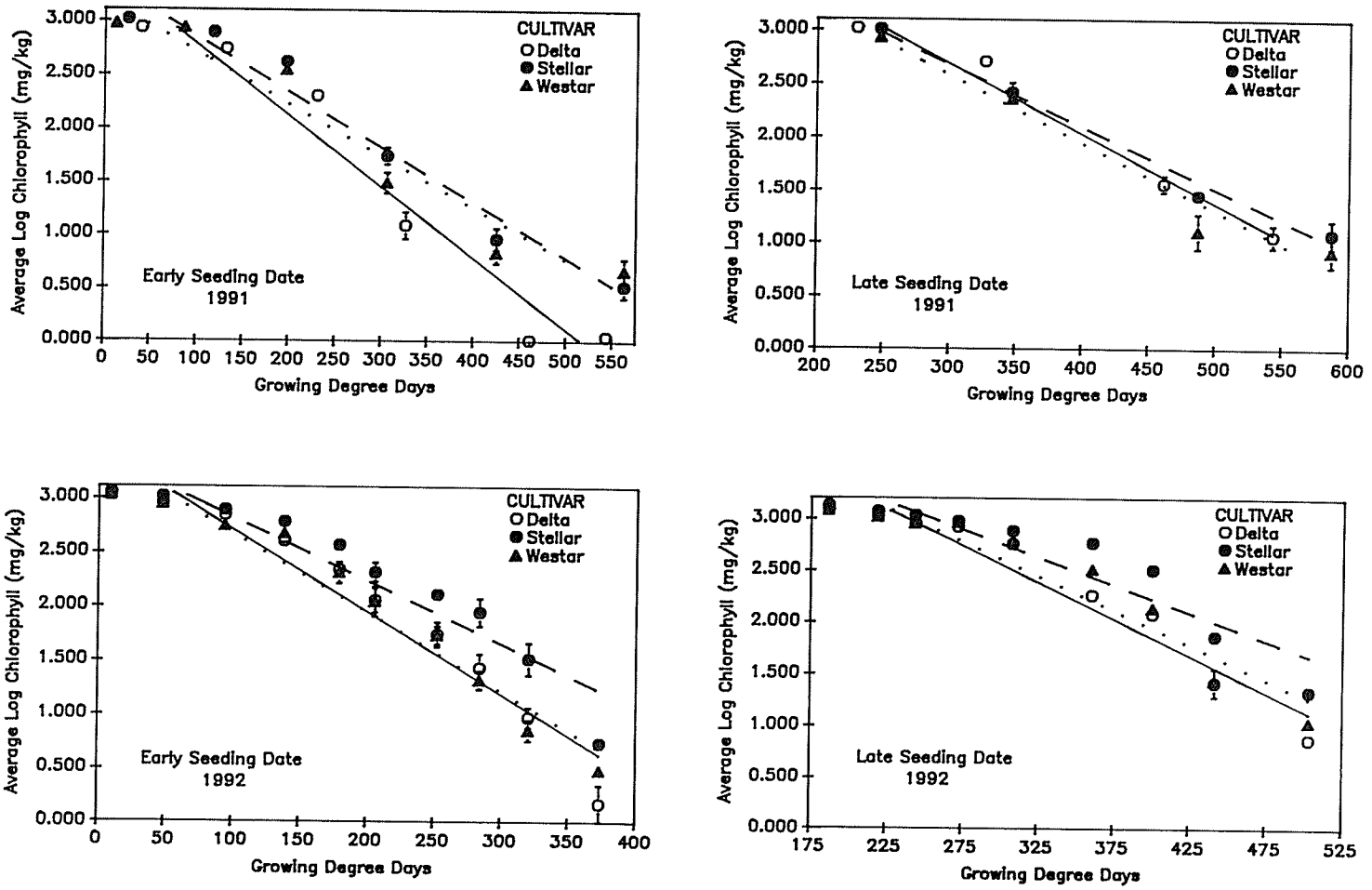


Figure 4: The regressions of lof chlorophyll (mg kg^{-1}) against growing degree days for the cultivars Stellar (----), Delta (——) and Westar (....). (Error bars are standard errors. Each value was averaged over 4 replicates in 1991 and 5 replicates in 1992.)

The conversion to GDD did not alter the significance of the comparisons made between the early and late seeding dates in 1991 substantially (Table 22). The rates of seed chlorophyll breakdown for the cultivar Delta were not significantly different (p=0.05) in the early and late seeding dates. For all cultivars, the conversion to GDD reduced the |T|, but rates of seed chlorophyll breakdown for Westar were still significantly different at the p=.05 level. The unusually warm late season temperatures in 1991 may explain why the conversion to GDD had little effect on the results. Both seeding dates ripened during warm weather, conducive to rapid seed chlorophyll loss. In 1992, the conversion to GDD eliminated the differences in rates of seed chlorophyll breakdown between the early and late seeding dates for all three cultivars (p=0.05) (Table 22). Therefore, the slower rates of seed chlorophyll breakdown observed in the late seeding date of 1992 can be explained by the lower temperatures during seed ripening later in the growing season.

When the original regressions based on log chlorophyll versus days after first sample are considered, all cultivars had significantly different rates of seed chlorophyll breakdown in the two years of the study (1991 and 1992) (p=0.01), except the difference between the early seeding date of Westar in 1991 and 1992 was not significant (p=0.05) (Table 24). Rates of seed chlorophyll degradation were faster in 1991 than 1992, likely due to the hot, dry weather conditions. The conversion to GDD eliminated the differences between years (p=0.05), with the exception of the early seeding date of Westar where a difference was created (p=0.01) (Table 24). This confirms that temperature had a significant effect on rates of seed chlorophyll breakdown.

Table 24: Paired T-Test Results Comparing Rates of Seed Chlorophyll Breakdown in 1991 and 1992. Comparison of Regressions of Log Chlorophyll (mg kg^{-1}) vs. Days After First Sample and vs. Growing Degree Days (GDD)

Seeding Date	Cultivar	df	|T| (days)	Sig. (days)	|T| (GDD)	Sig. (GDD)
Early	Delta	70	4.849	**(1)	1.821	ns
Early	Stellar	70	4.558	**	1.485	ns
Early	Westar	70	0.813	ns	5.549	**
Late	Delta	57	7.446	**	0.637	ns
Late	Stellar	57	8.728	**	0.899	ns
Late	Westar	57	8.026	**	0.599	ns

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

4.2.2 Rates of Moisture Loss in Three Cultivars of Brassica napus

A regression analysis of seed moisture versus days from first sample was carried out for each cultivar in both the early and late seeding dates each year (Table 25). In 1991, plots that were sprayed with Cerone were not included in this study.

Table 25: Regression Analysis Results for Three Cultivars of B. napus (Stellar, Delta and Westar) Based on Seed Moisture Content (%) vs. Days After First Sample.

Year	Cultivar	Seeding Date	Slope	MS Error	df	Adj. r^2	Sig.
1991	Delta	Early	-1.1982	9.0462	22	0.95	0.0001
1991	Stellar	Early	-1.2664	12.7639	22	0.94	0.0001
1991	Westar	Early	-1.3334	16.2716	22	0.94	0.0001
1991	Delta	Late	-0.9334	19.1093	14	0.70	0.0001
1991	Stellar	Late	-0.9773	42.5111	13	0.57	0.0007
1991	Westar	Late	-1.1120	79.0112	14	0.46	0.0023
1992	Delta	Early	-1.1396	24.8744	48	0.87	0.0001
1992	Stellar	Early	-1.1816	17.9543	48	0.91	0.0001
1992	Westar	Early	-1.2783	24.9543	48	0.89	0.0001
1992	Delta	Late	-1.2524	27.2092	43	0.92	0.0001
1992	Stellar	Late	-1.2103	23.4044	43	0.92	0.0001
1992	Westar	Late	-1.2843	35.8389	43	0.90	0.0001

The data fit a linear regression well, therefore seed moisture loss progressed at a constant rate throughout the entire ripening period. In the late seeding date of 1991, the goodness of fit was considerably lower than in the other seeding dates and years, but all cultivars in all seeding dates and years did fit a linear regression ($p > 0.99$). The poorer fit in the late seeding date of 1991 was likely a result of the rapid seed ripening that occurred, which resulted in only four samples per cultivar being harvested. The larger number of samples collected in the other seeding dates resulted in a better fit to a straight line.

Paired T-tests determined that there were no significant differences ($p=0.05$) between the rates of moisture loss for any of the cultivars

tested (Table 26). Unlike seed chlorophyll breakdown, moisture loss occurred at a constant rate throughout the entire ripening period, and this rate was the same in all three cultivars tested.

Table 26: Paired T-Test Results Comparing Rates of Seed Moisture Loss (% day^{-1}) in Three Cultivars of B. napus (Stellar, Delta and Westar) During Ripening

Year	Seeding Date	Comparison	df	|T|	Sig. (p=.05)
1991	Early	Delta x Stellar	44	0.783	ns(1)
1991	Early	Delta x Westar	44	1.473	ns
1991	Early	Stellar x Westar	44	0.695	ns
1991	Late	Delta x Stellar	27	0.162	ns
1991	Late	Delta x Westar	28	0.508	ns
1991	Late	Stellar x Westar	27	0.353	ns
1992	Early	Delta x Stellar	96	0.511	ns
1992	Early	Delta x Westar	96	1.564	ns
1992	Early	Stellar x Westar	96	1.175	ns
1992	Late	Delta x Stellar	86	0.538	ns
1992	Late	Delta x Westar	86	0.365	ns
1992	Late	Stellar x Westar	86	0.874	ns

(1) ns = non significant

The weather was considerably different in the two years of the study and even between the different seeding dates within the same year (temperature and precipitation data in Tables A2 and A3 in the Appendix). Despite this, no significant differences in rates of moisture loss were observed (p=0.05), either between the early and late seeding dates of the

same year (Table 27) or between the studies conducted in 1991 and 1992 (Table 28).

Table 27: Paired T-Test Results Comparing Rates of Seed Moisture Loss (% day^{-1}) Within the Same Cultivar Between Early and Late Seeding Dates

Year	Cultivar	df	|T|	Sig. (p=.05)
1991	Delta	36	1.811	ns(1)
1991	Stellar	36	1.547	ns
1991	Westar	33	0.884	ns
1992	Delta	91	1.325	ns
1992	Stellar	91	0.379	ns
1992	Westar	91	0.0655	ns

(1) ns = non significant

Table 28: Paired T-Test Results Comparing Rates of Seed Moisture Loss (% day^{-1}) Within the Same Cultivar and Seeding Date Between Years (1991 and 1992)

Seeding Date	Cultivar	df	|T|	Sig. (p=.05)
Early	Delta	70	0.577	ns(1)
Early	Stellar	70	0.945	ns
Early	Westar	70	0.543	ns
Late	Delta	57	1.692	ns
Late	Stellar	56	1.248	ns
Late	Westar	57	0.686	ns

(1) ns = non significant

Therefore, in our study the environment did not affect the rate of seed moisture loss. The temperature regimes were distinct in 1991 and 1992, as

reflected in accumulation of GDD, although seed moisture loss did not reflect these differences. It is possible that the external environment might affect the rate of moisture loss during more extreme temperature conditions. Cenkowski et al. (1993) found that rainfall combined with high humidity raised seed moisture, but this increase was temporary. Our study indicates that moisture loss during seed ripening may be genetically determined to occur at a certain rate regardless of the external environment.

A highly significant positive correlation was found between the moisture content of ripening canola seed and its chlorophyll level (+0.85, p=0.0001, N=401). The relationship between the moisture content of each seed sample and its chlorophyll level was plotted in a series of regressions for each cultivar in both the early and late seeding dates of each year (Figures 5 to 8). In most cases the data fit a fourth order regression well, particularly the data from 1992 when a larger number of samples were collected for each cultivar. Seed chlorophyll breakdown did not proceed at a constant rate at all seed moisture levels. Seed chlorophyll was rapidly degraded between 65 and 35% seed moisture, with the rate slowing at lower seed moisture contents. However, seed chlorophyll breakdown did continue below 35% seed moisture. The graphs on the right hand side of Figures 5 to 8 illustrate seed chlorophyll breakdown at lower moisture contents which are difficult to distinguish on the intact graphs due to the low levels of chlorophyll remaining at this stage. Chlorophyll breakdown slowed below 35% seed moisture, but continued until all or most of the chlorophyll had disappeared, at a moisture content of approximately 10%.

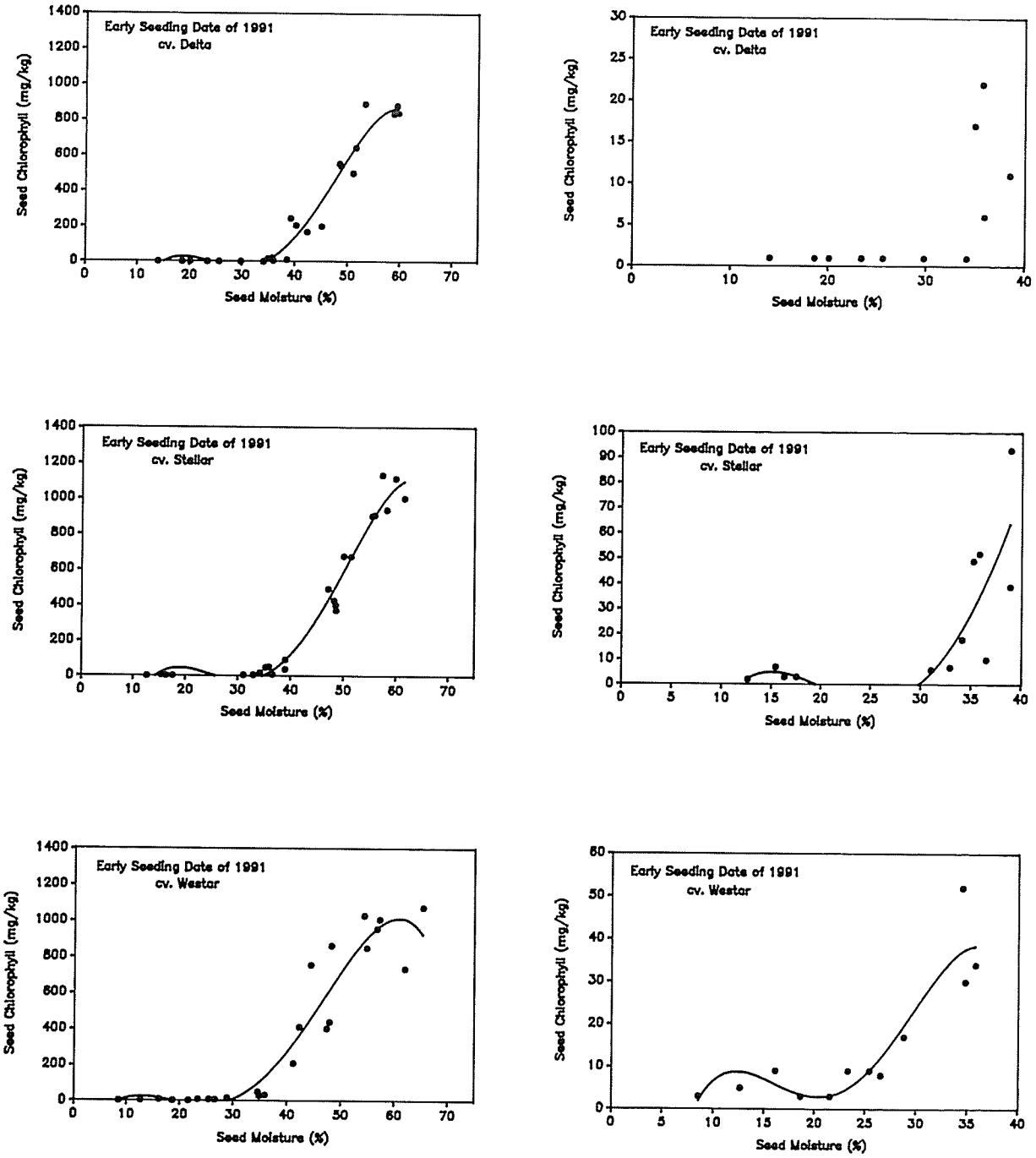


Figure 5: The relationship between the level of seed chlorophyll (mg kg^{-1}) and seed moisture (%) in 3 cultivars of ripening B. napus seed (Delta, Stellar and Westar) in the early seeding date of 1991. (The graphs on the right illustate this relationship at low chlorophyll and moisture contents that are difficult to distinguish on the graphs on the left).

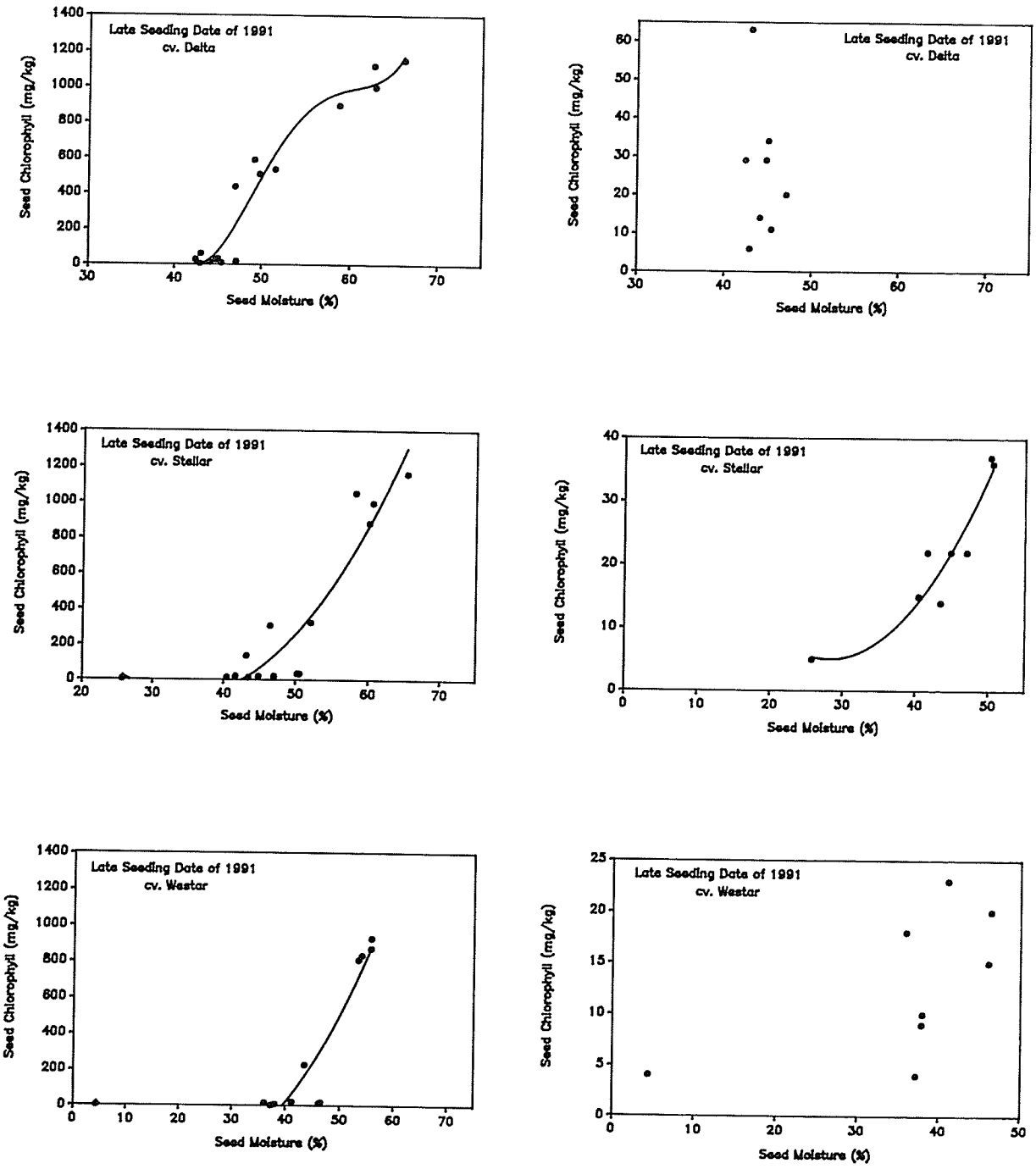


Figure 6: The relationship between the level of seed chlorophyll (mg kg^{-1}) and seed moisture (%) in 3 cultivars of ripening B. napus seed (Delta, Stellar and Westar) in the late seeding date of 1991. (The graphs on the right illustate this relationship at low chlorophyll and moisture contents that are difficult to distinguish on the graphs on the left).

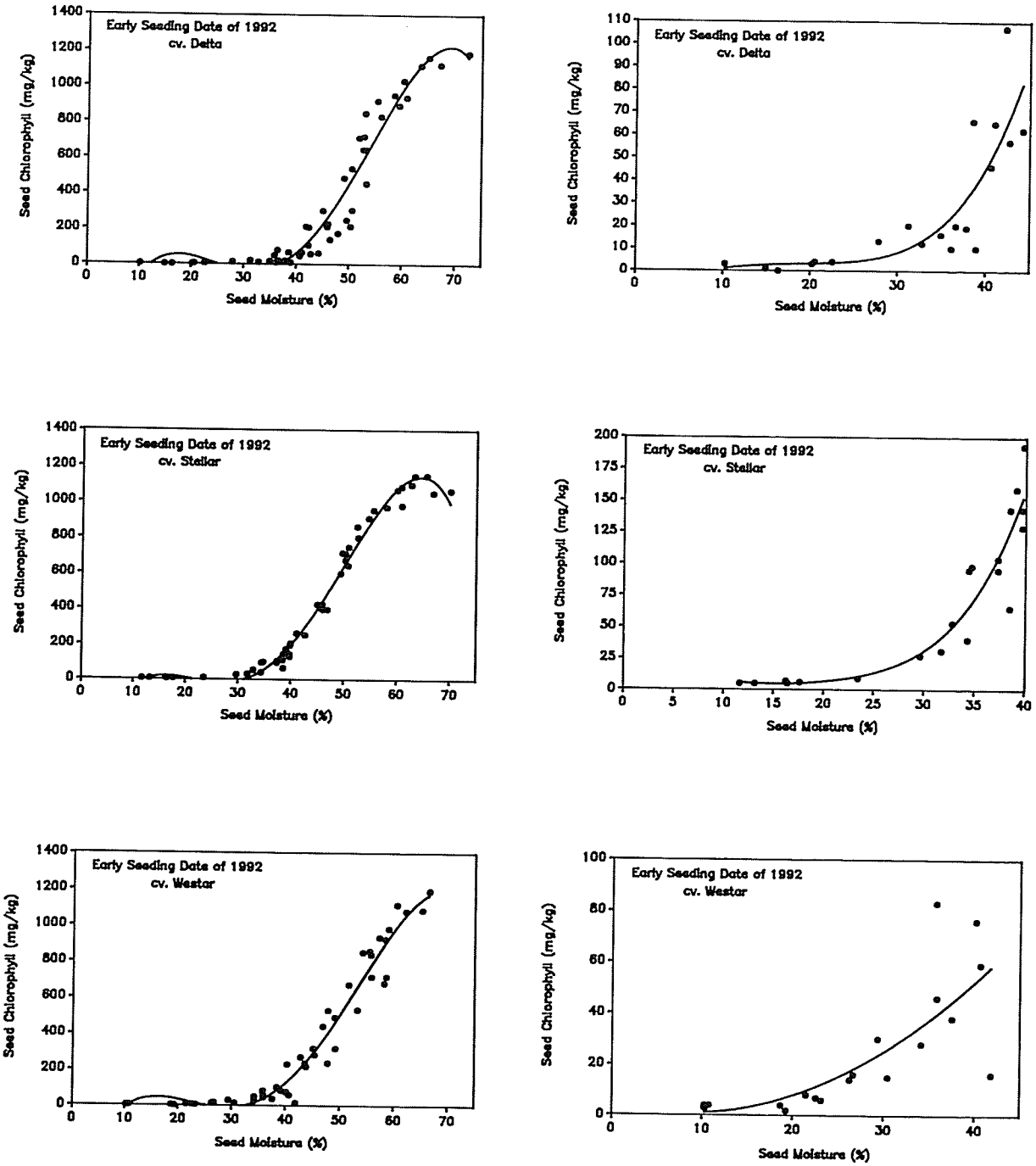


Figure 7: The relationship between the level of seed chlorophyll (mg kg^{-1}) and seed moisture (%) in 3 cultivars of ripening B. napus seed (Delta, Stellar and Westar) in the early seeding date of 1992. (The graphs on the right illustrate this relationship at low chlorophyll and moisture contents that are difficult to distinguish on the graphs on the left).

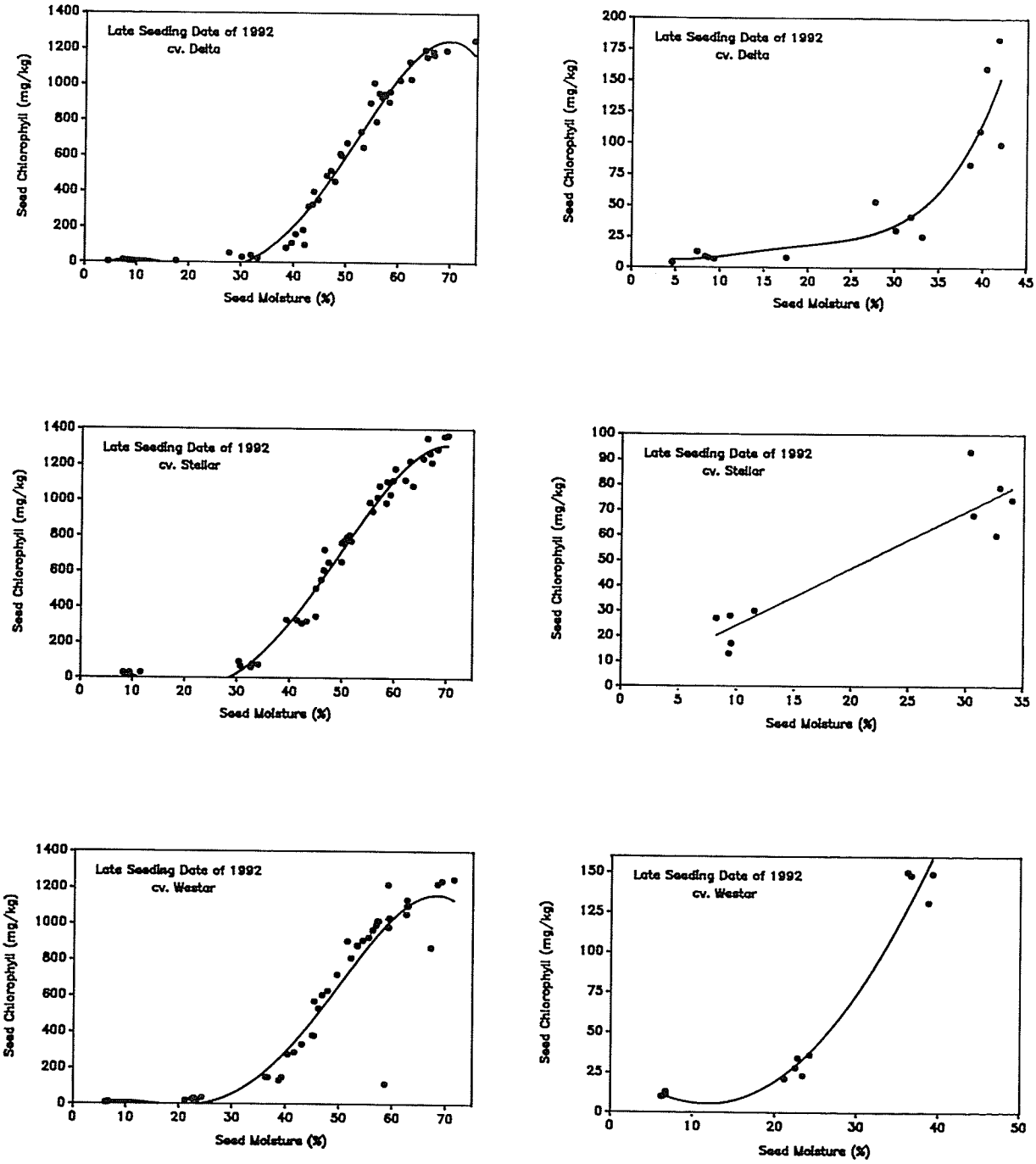


Figure 8: The relationship between the level of seed chlorophyll (mg kg^{-1}) and seed moisture (%) in 3 cultivars of ripening B. napus seed (Delta, Stellar and Westar) in the late seeding date of 1992. (The graphs on the right illustate this relationship at low chlorophyll and moisture contents that are difficult to distinguish on the graphs on the left).

Other researchers, working with cultured canola embryos, have found little or no chlorophyll loss below 35% seed moisture (Johnson-Flanagan, pers. comm.). When relating work from a field study to results from in vitro experiments it is important to remember that canola is an indeterminate crop. The moisture contents reported for our study are based on entire seed samples, not on individual seeds whose precise age and moisture content can be determined. In a seed sample containing 35% moisture many of the seeds will be at higher moisture levels, while others will contain less than 35% moisture. The seeds at higher moisture levels may be the ones that are carrying out chlorophyll breakdown. Therefore our study neither supports nor refutes previous in vitro studies. Rather, it provides new information - that seed chlorophyll breakdown does continue when the canola crop contains less than 35% seed moisture.

Drought or early swathing of the canola crop during hot, dry weather can lead to rapid dry down which "fixes" the chlorophyll level of the seed resulting in high seed chlorophyll at harvest. Because of rapid desiccation, moisture is unavailable, so metabolism within the seed stops and the seed fails to ripen (Ward et al.,1992a). Several studies have shown that a decrease in seed chlorophyll after harvest is only possible if the seed contains adequate moisture (Larsson and Gottfridsson,1974; Salankhe and Desai,1986; Cenkowski et al.,1993). However, under normal field conditions there does not appear to be a critical moisture content at which chlorophyll breakdown stops. Seed chlorophyll had reached low levels by the time the final seed samples were harvested in both years of our study, but the moisture content of the mature seed was quite variable (Table 29).

Table 29: Final Moisture (%) and Chlorophyll (mg kg^{-1}) Levels Reached in Fully Mature Canola Seed (HB 5.5) in 1991 and 1992. (Chl. = total chlorophyll)

Year	Seeding Date	Final Moisture Content (%)	Final Chl. Content (mg kg^{-1})
1991	Early	9 - 23	1 - 9
1991	Late	26 - 47	5 - 22
1992	Early	10 - 23	0 - 7
1992	Late	5 - 12	4 - 30

Studies by Norton and Harris (1975) have described canola seed maturation in three stages. Stage one occurs from 1 to 23 DPA and encompasses the period of rapid cell division. Phase two occurs from 24 to 49 DPA and this is the phase of rapid cell expansion, increases in fresh and dry weight and oil and protein deposition. Stage three occurs between 50 and 90 DPA and at this stage both chlorophyll and moisture are lost as the seed dries down.

The time of onset of each of these stages will vary with the growing conditions and cultivar. For instance, in Western Canada the canola crop is generally harvested by approximately 60 DPA. Therefore although this pattern of seed development is uniform the speed at which it progresses will vary.

Canola seed development has been divided by other authors into predesiccation and desiccation periods, with the switch occurring at approximately 55% seed moisture (Finkelstein et al.,1985). It has been suggested that a rapid switch to the desiccation phase may deprive the seed of time to degrade chlorophyll (Johnson-Flanagan et al.,1991).

However our work clearly shows that significant chlorophyll breakdown occurs below 55% seed moisture.

Crouch and Sussex (1981) found that in cultured canola embryos, chlorophyll loss was first apparent at 30 DPA and continued to 60 DPA. In our study the earliest samples were collected at 31 to 39 DPA depending upon the seeding date and year, but both chlorophyll breakdown and moisture loss were occurring at this time. Under field conditions the time required for chlorophyll to fully degrade and for the seed to dry down cannot be predicted to occur uniformly at a certain number of DPA as seed ripening is highly dependent on the external environment.

During seed ripening, both moisture and chlorophyll levels declined. The rate of chlorophyll loss varied with the stage of maturity of the seed, with very rapid chlorophyll breakdown occurring when chlorophyll levels were high, but the rate slowed as more and more chlorophyll was lost (Figure A2 in the Appendix). This agrees with the results of Cenkowski et al. (1993) who also observed that seed chlorophyll declined exponentially as the canola crop ripened. As the seed ripened chlorophyll loss slowed down.

Cenkowski et al. (1993) found that the moisture content of ripening canola seed could be used to predict its chlorophyll level. However, Cenkowski's study was conducted using a single cultivar (Westar) and we found that different cultivars degrade seed chlorophyll at different rates. In our study, the rate of moisture loss could not be used to predict the rate of chlorophyll breakdown with enough accuracy to detect differences in chlorophyll breakdown rates between cultivars, seeding dates or years. Significantly different rates of seed chlorophyll

breakdown were found between the three cultivars ($p=0.01$), but no significant differences ($p=0.05$) were observed in the rates of moisture loss. Chlorophyll breakdown rates were significantly different ($p=0.01$) between seeding dates and years, yet rates of moisture loss remained the same ($p=0.05$). This suggests that although moisture and chlorophyll both decline as seed matures, the rate of seed chlorophyll breakdown does not depend on the rate of moisture loss, or vice versa when canola seed ripens under typical field conditions.

4.2.3 The Effect of Applied Cerone on Rates of Seed Chlorophyll Breakdown

The objective of this study was to determine whether Cerone would enhance chlorophyll degradation in physiologically mature but green canola seed (HB 5.3). Seed samples contained approximately 55 to 65% moisture at this stage of development. Each seed sample was analyzed for chlorophyll and a regression analysis was performed based on the logarithm of the chlorophyll content (mg kg^{-1}) vs. days after first sample for each cultivar in each spray treatment of each seeding date (Table 30). Chlorophyll values were converted to the logarithmic scale in order to linearize the data to allow seed chlorophyll breakdown rates to be compared.

Table 30: Regression Analysis Results for Three Cultivars of B. napus (Stellar, Delta and Westar) and Four Cerone Treatments Based on Log chlorophyll (mg kg^{-1}) vs. Days After First Sample.

Seeding Date	Trt.*	Cultivar	Slope	MS Error	df	Adj. r^2	Sig.
Early	A	Delta	-0.1085	0.1178	22	0.92	0.0001
Early	A	Stellar	-0.08414	0.06449	22	0.93	0.0001
Early	A	Westar	-0.07894	0.09025	22	0.91	0.0001
Early	B	Delta	-0.1029	0.1234	22	0.91	0.0001
Early	B	Stellar	-0.08335	0.08067	22	0.92	0.0001
Early	B	Westar	-0.08214	0.07399	22	0.93	0.0001
Early	C	Delta	-0.1086	0.1032	22	0.93	0.0001
Early	C	Stellar	-0.08524	0.05582	22	0.94	0.0001
Early	C	Westar	-0.08275	0.1079	22	0.90	0.0001
Early	D	Delta	-0.1054	0.1248	22	0.91	0.0001
Early	D	Stellar	-0.07826	0.06399	22	0.93	0.0001
Early	D	Westar	-0.07963	0.08212	22	0.92	0.0001
Late	A	Delta	-0.1139	0.02899	14	0.96	0.0001
Late	A	Stellar	-0.1020	0.03101	14	0.95	0.0001
Late	A	Westar	-0.1113	0.08004	11	0.91	0.0001
Late	B	Delta	-0.1144	0.03040	13	0.96	0.0001
Late	B	Stellar	-0.1097	0.03809	14	0.95	0.0001
Late	B	Westar	-0.1120	0.04188	12	0.95	0.0001
Late	C	Delta	-0.1156	0.04096	14	0.94	0.0001
Late	C	Stellar	-0.1116	0.02445	14	0.97	0.0001
Late	C	Westar	-0.1162	0.06792	11	0.93	0.0001
Late	D	Delta	-0.1164	0.02019	13	0.97	0.0001
Late	D	Stellar	-0.09871	0.06862	14	0.89	0.0001
Late	D	Westar	-0.1156	0.03990	11	0.96	0.0001

(*) A = control
B = 1/4 L Cerone ha^{-1}
C = 1/2 L Cerone ha^{-1}
D = 1 L Cerone ha^{-1}

Paired T-tests indicated no significant difference (p=0.05) in the rate of seed chlorophyll breakdown between any of the spray treatments for any of the cultivars tested. The results for one cultivar, Westar, are presented in Table 31.

Table 31: Paired T-Test Results Comparing Spray Treatments for One Cultivar, Westar, in 1991. Based on the Regressions of Log Chlorophyll (mg kg^{-1}) vs. Days From First Sample.

Sowing	Cultivar	Treatment Comparison	df	|T|	p=.05
Early	Westar	AxB(*)	44	0.452	ns(1)
Early	Westar	AxC	44	0.490	ns
Early	Westar	AxD	44	0.0951	ns
Early	Westar	BxC	44	0.0819	ns
Early	Westar	BxD	44	0.364	ns
Early	Westar	CxD	44	0.410	ns
Late	Westar	AxB	23	0.0579	ns
Late	Westar	AxC	22	0.362	ns
Late	Westar	AxD	22	0.353	ns
Late	Westar	BxC	23	0.366	ns
Late	Westar	BxD	23	0.361	ns
Late	Westar	CxD	22	0.0520	ns

(*) A = control
B = 1/4 L Cerone ha^{-1}
C = 1/2 L Cerone ha^{-1}
D = 1 L Cerone ha^{-1}

(1) ns = non significant

Therefore, Cerone, at the levels and stage of seed development applied, did not enhance seed chlorophyll breakdown. When rates of ethylene evolution from the siliques were measured, the levels were quite high (over 0.075 μL g $tissue^{-1}$ $hour^{-1}$ in some green seed samples), suggesting

that the endogenous ethylene level in the seed may already be high enough to stimulate ripening. Additional applied ethylene was ineffective. Most hormonally controlled processes show a threshold response; that is when the hormone reaches the critical level a response is initiated, with higher levels of the hormone causing no additional response. This is only one possible reason for the lack of response to applied ethylene. Other possibilities are that the level applied was not in the physiological range to control chlorophyll breakdown, the pods and seed coats may be barriers to the uptake and utilization of the applied Cerone, the time of application may be too late to affect seed chlorophyll breakdown, or chlorophyll breakdown in B. napus seed may simply not be controlled by ethylene.

4.2.4 Rates of Ethylene Evolution From Ripening B. napus Siliques Containing Seed

Patterns of ethylene evolution have been studied during plant senescence (Mattoo and Aharoni,1988; Aharoni and Lieberman,1979; Kao and Yang,1983) and fruit ripening (Knee et al.,1988) but there are no reports of ethylene evolution patterns during pod, silique or seed maturation in any species. Rates of ethylene evolution from canola siliques were measured once or twice a week as the crop ripened. In 1991, plots that had been sprayed with Cerone were not included in this study. Canola silique ethylene production peaked early during seed ripening, then declined. The peak of ethylene production is clearly visible in most cultivars in both years. In the late seeding date of the cultivar Westar

in 1991 and in the late seeding date of the cultivar Delta in 1992, sampling did not begin soon enough to observe this. In both years of the study, the peak of ethylene was produced after moisture loss and seed chlorophyll breakdown had already begun (Figures 9 to 20). The moisture content of the seed samples ranged from 45 to 61% at the time of maximal ethylene production from the siliques. Therefore, ethylene was produced during the ripening period, but changes in the amount of ethylene produced can not be shown to trigger either the initiation of seed chlorophyll breakdown or moisture loss. However, an increase in sensitivity to ethylene during seed ripening cannot be ruled out. The role of this ethylene is not clear; it might be produced in response to chlorophyll loss or to stress during senescence, or it may control some other aspect of seed ripening or plant senescence.

In all cultivars where sampling began early enough, a burst of ethylene was apparent. The period of time after the ethylene peak was designated as the period of active ethylene evolution. Significant positive correlations were found between the chlorophyll content of the seed and the rate of ethylene evolution during this period, in all cultivars, seeding dates and years of the study (Table 32).

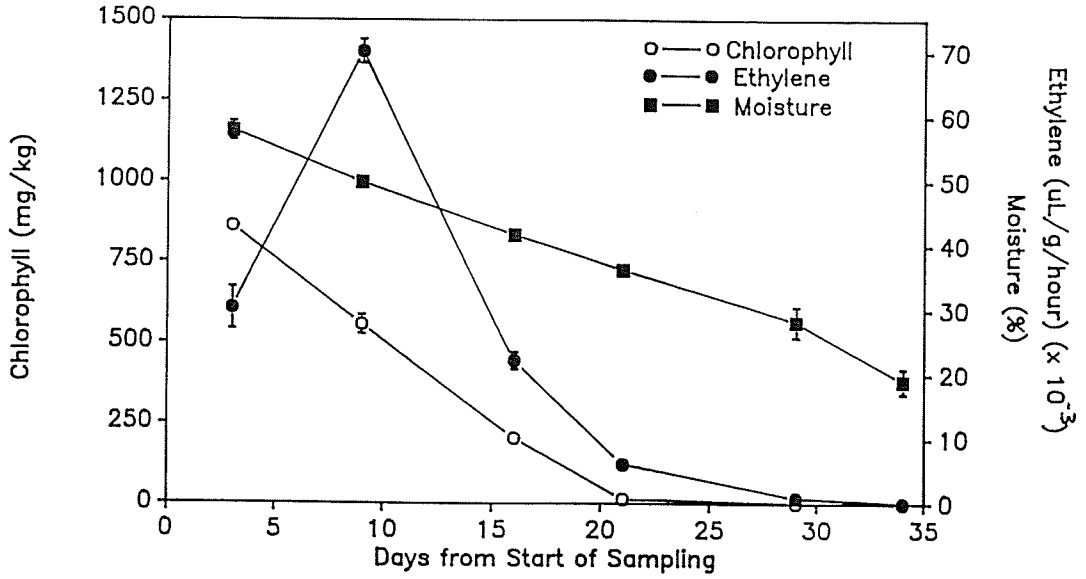


Figure 9: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1991 (cv. Delta). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

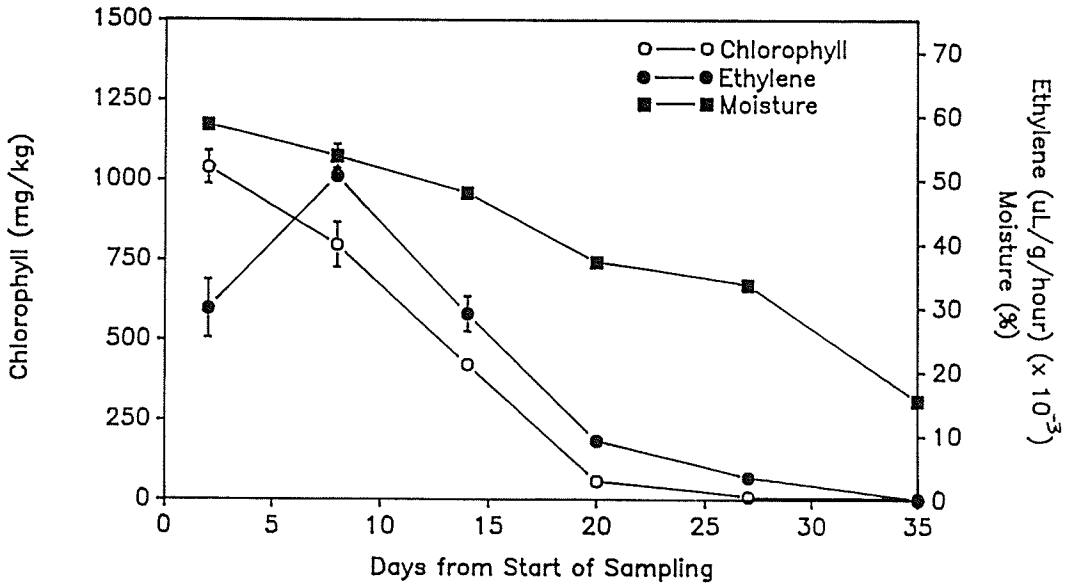


Figure 10: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1991 (cv. Stellar). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

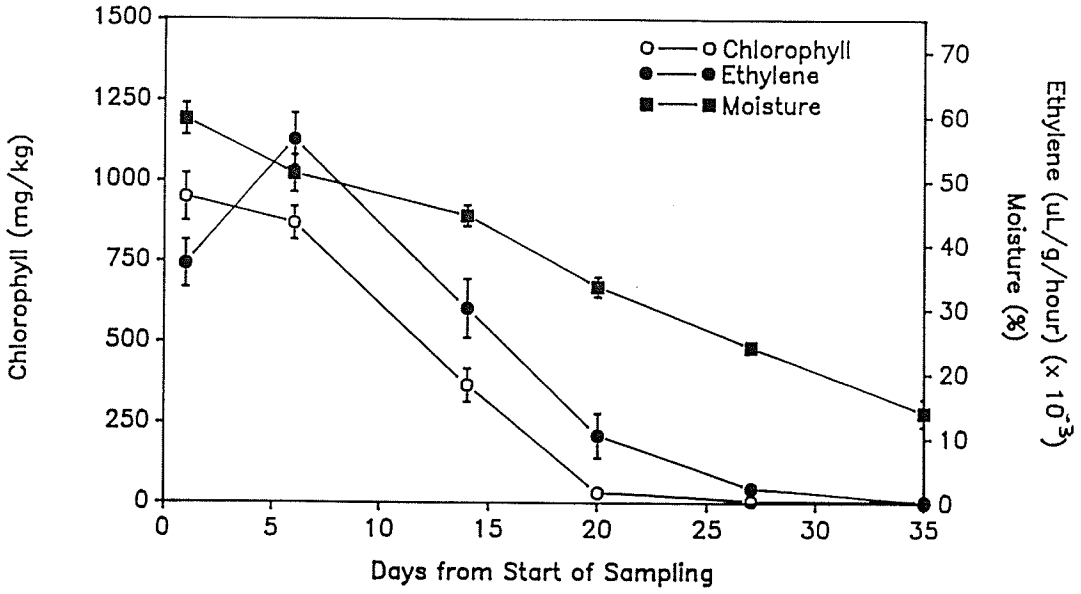


Figure 11: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1991 (cv. Westar). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

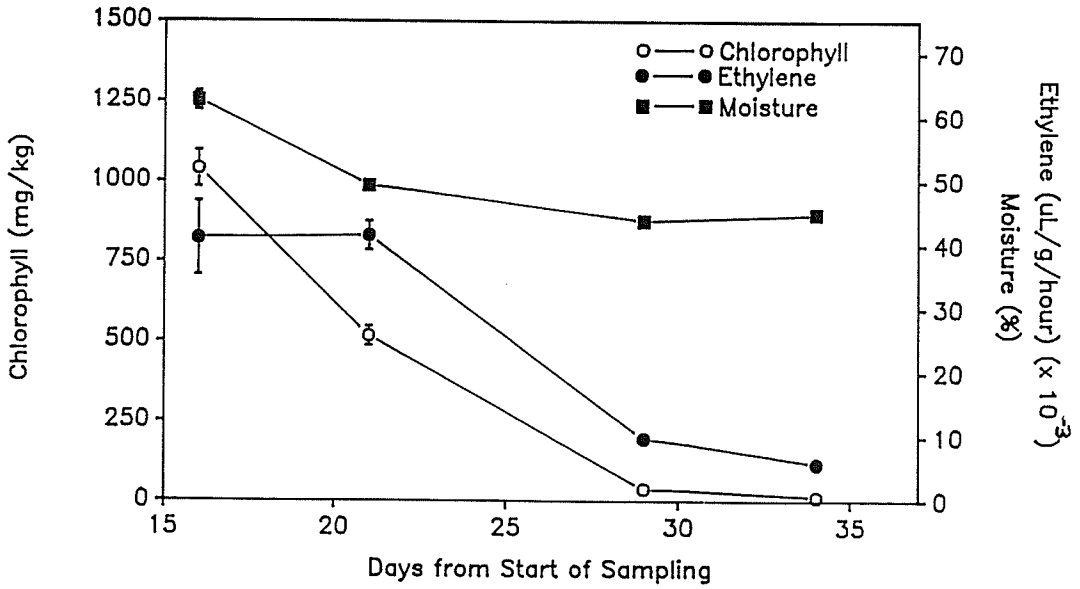


Figure 12: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1991 (cv. Delta). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

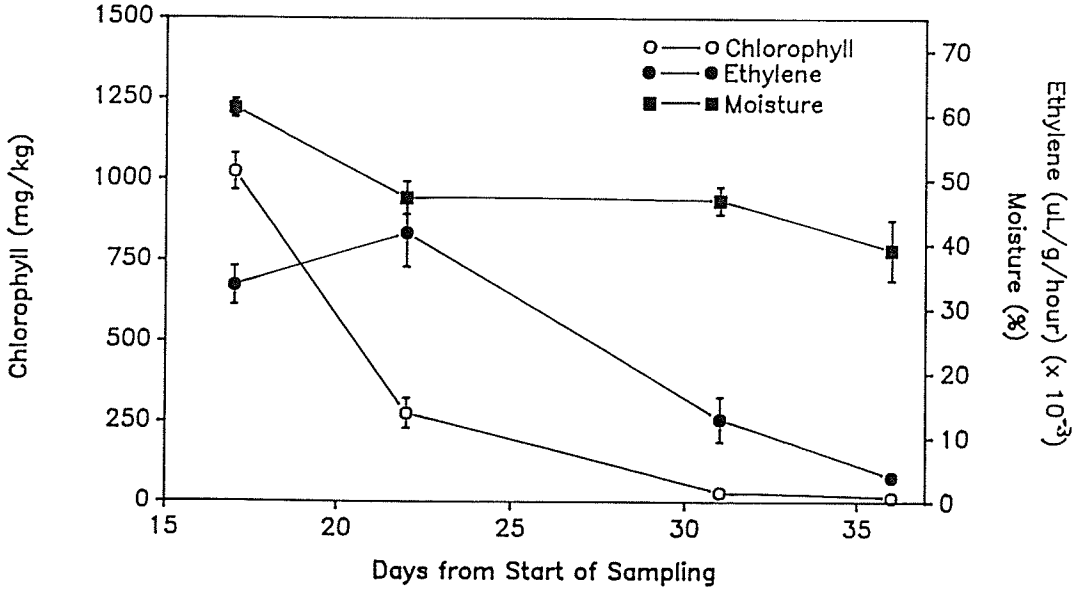


Figure 13: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1991 (cv. Stellar). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

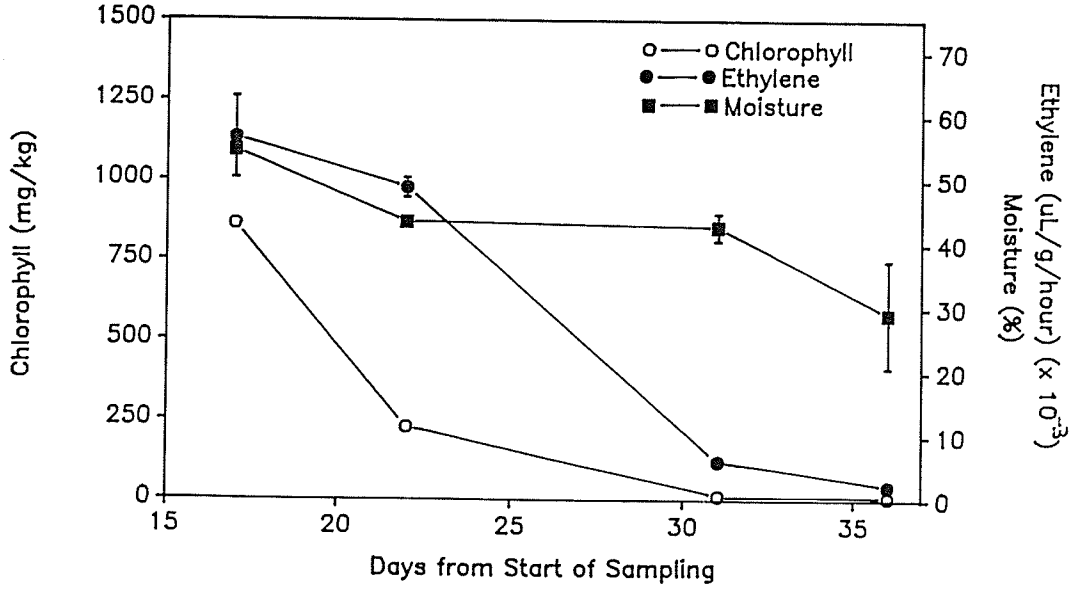


Figure 14: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1991 (cv. Westar). (Error bars represent standard errors. Each point represents the average of 4 replicates.)

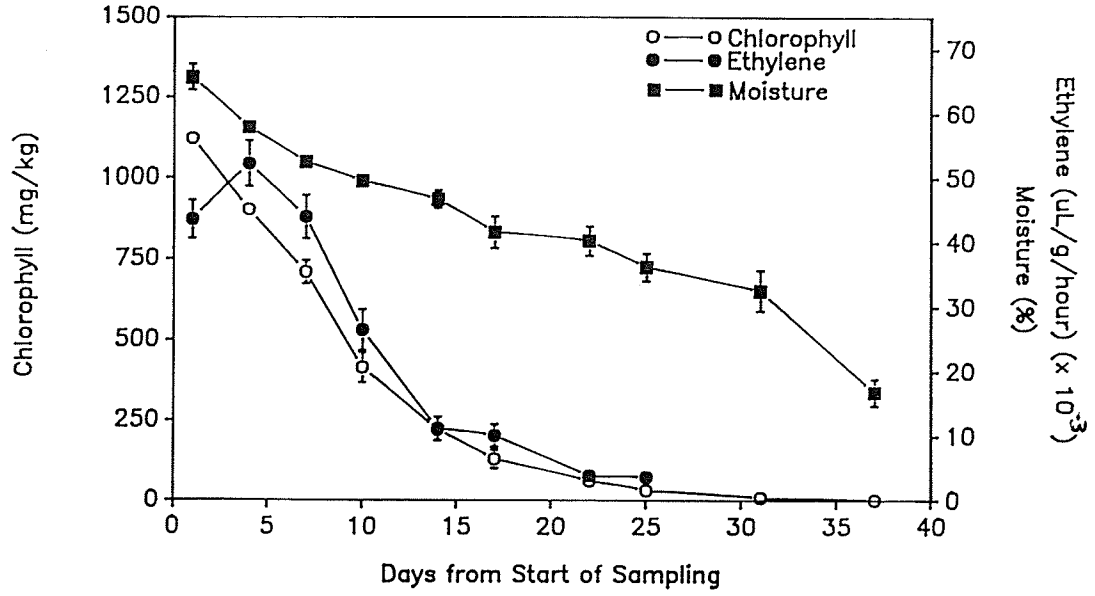


Figure 15: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1992 (cv. Delta). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

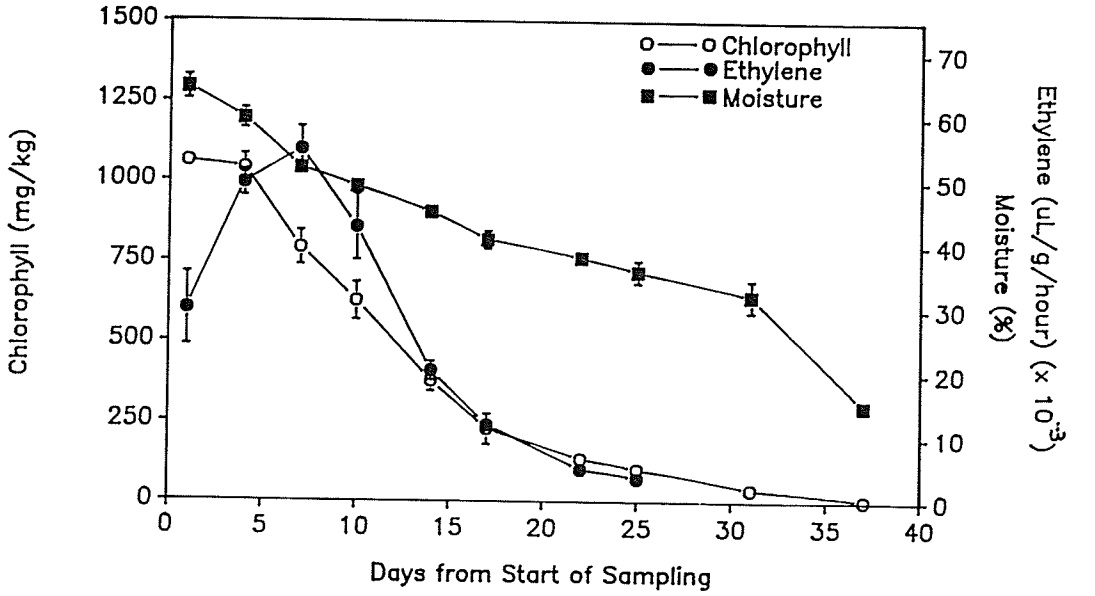


Figure 16: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1992 (cv. Stellar). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

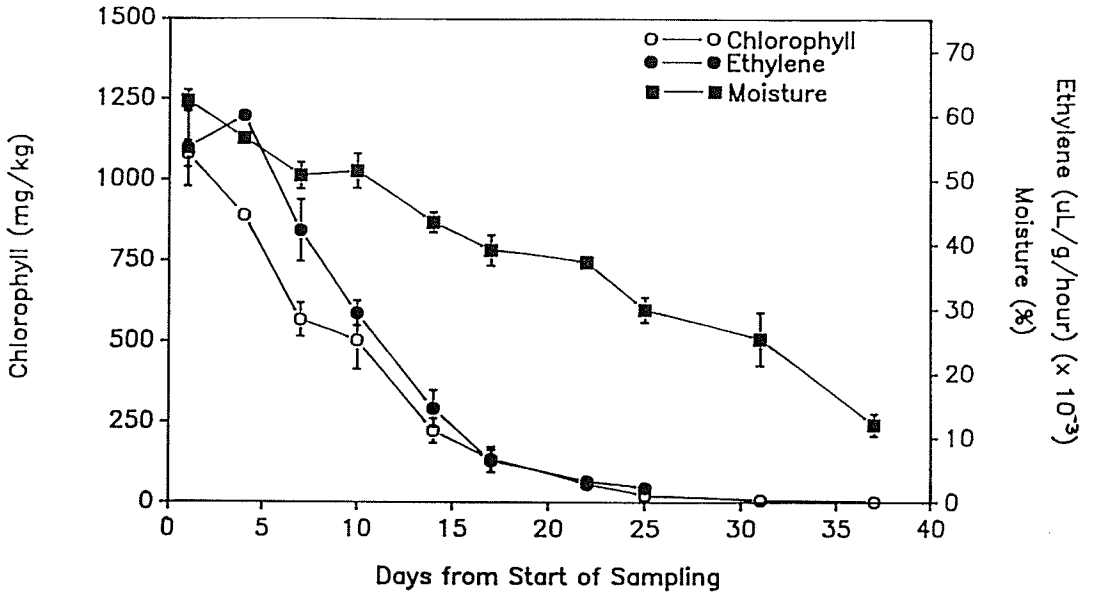


Figure 17: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the early seeding date of 1992 (cv. Westar). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

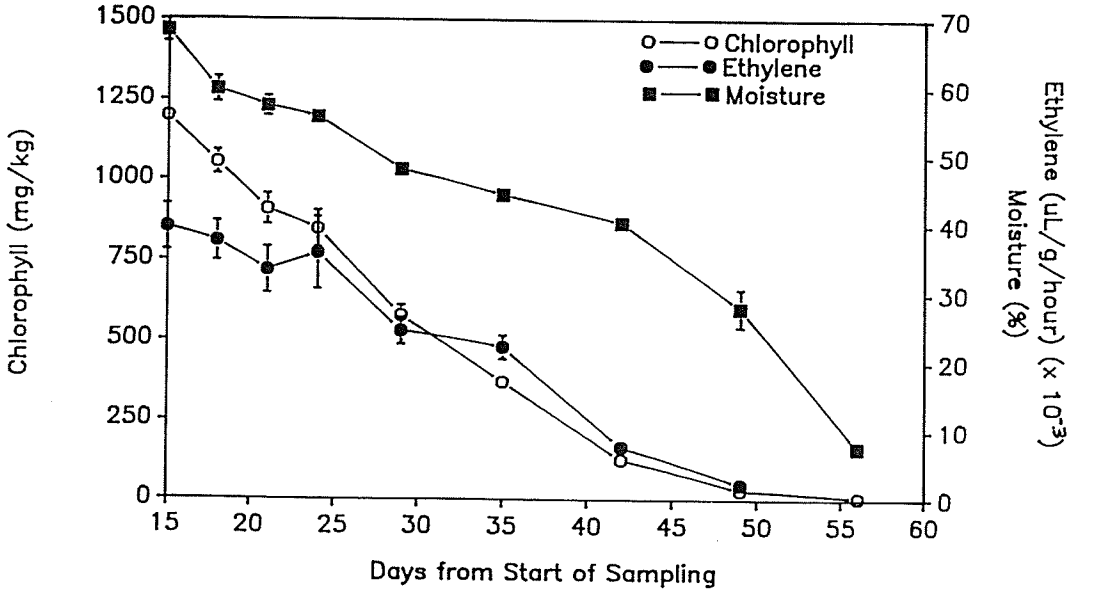


Figure 18: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1992 (cv. Delta). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

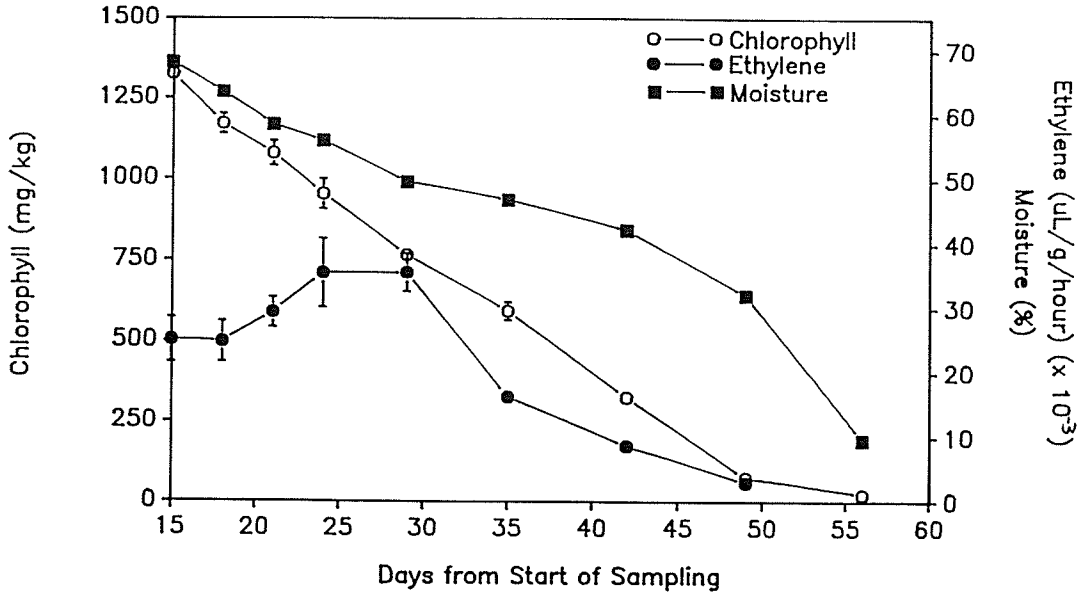


Figure 19: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1992 (cv. Stellar). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

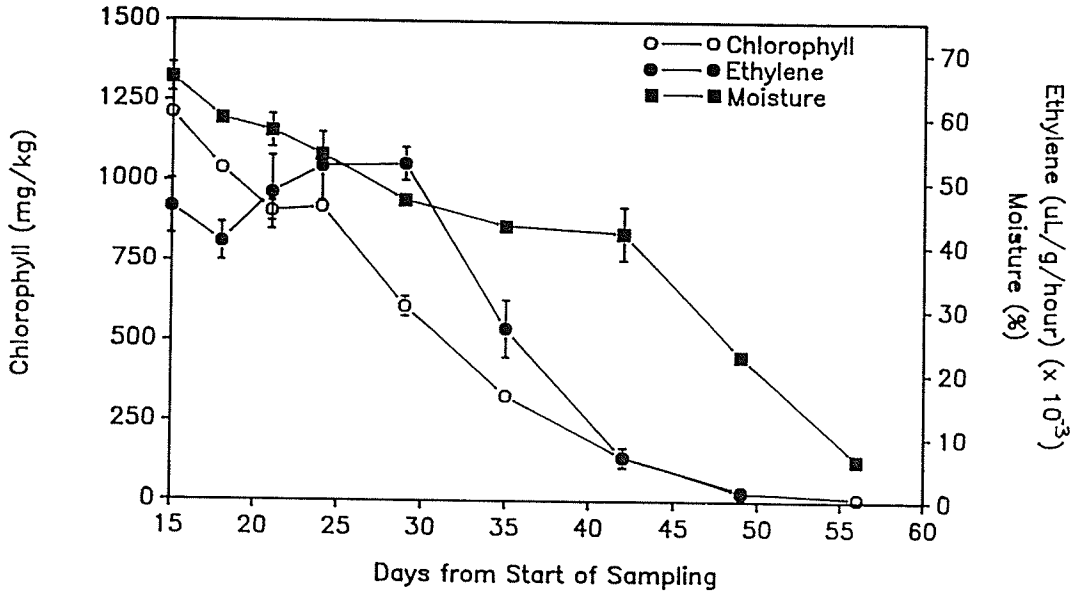


Figure 20: Relationship between seed chlorophyll (mg kg^{-1}), ethylene (μL/g fresh wt/hour) and moisture (%) in the late seeding date of 1992 (cv. Westar). (Error bars represent standard errors. Each point represents the average of 5 replicates.)

Table 32: Correlations Between Rates of Ethylene Evolution from Siliques (μL/g fresh wt./hour) and Chlorophyll Content (mg kg^{-1}) of the Seed

Year	Sowing	Cultivar	Correlation	N	Sig.
1991	Early	Delta	+ 0.99024	16	0.0001
1991	Early	Stellar	+ 0.98081	16	0.0001
1991	Early	Westar	+ 0.92884	16	0.0001
1991	Late	Delta	+ 0.98651	12	0.0001
1991	Late	Stellar	+ 0.85924	12	0.0003
1991	Late	Westar	+ 0.98918	9	0.0001
1992	Early	Delta	+ 0.95051	35	0.0001
1992	Early	Stellar	+ 0.96805	30	0.0001
1992	Early	Westar	+ 0.92267	35	0.0001
1992	Late	Delta	+ 0.88896	25	0.0001
1992	Late	Stellar	+ 0.88461	20	0.0001
1992	Late	Westar	+ 0.96907	20	0.0001

In order to determine whether cultivars had significantly different ($p=0.05$) rates of change in the rate of ethylene evolution from ripening siliques , a regression analysis for ethylene evolution was performed for each cultivar in both the early and late seeding dates of each year, during the period of active ethylene evolution. Logarithms were used to linearize the response (Table 33).

Table 33: Regression Analysis Results for Three Cultivars of B. napus (Stellar, Delta and Westar) Based on Log Ethylene (μL/g fresh wt./hour) vs. Days After First Sample

Year	Cultivar	Seeding Date	Slope	MS Error	df	Adj. r^2	Sig.
1991	Delta	Early	-0.093383	0.02585	13	0.95	0.0001
1991	Stellar	Early	-0.064348	0.01000	14	0.96	0.0001
1991	Westar	Early	-0.067366	0.03463	14	0.89	0.0001
1991	Delta	Late	-0.068741	0.01524	10	0.91	0.0001
1991	Stellar	Late	-0.072385	0.02928	10	0.87	0.0001
1991	Westar	Late	-0.097852	0.01351	10	0.96	0.0001
1992	Delta	Early	-0.062174	0.02664	33	0.88	0.0001
1992	Stellar	Early	-0.069041	0.00946	28	0.95	0.0001
1992	Westar	Early	-0.073006	0.02841	33	0.91	0.0001
1992	Delta	Late	-0.046513	0.02905	23	0.86	0.0001
1992	Stellar	Late	-0.053373	0.01733	18	0.91	0.0001
1992	Westar	Late	-0.081500	0.05620	18	0.87	0.0001

Paired T-tests determined that some cultivar pairs had significantly different rates of change in the rate of ethylene evolution, while others did not (Table 34) and no consistent pattern was evident.

Table 34: Paired T-Test Results Comparing Rates of Change in the Rate of Ethylene Evolution (μL/g fresh wt./hour) day^{-1} From Canola Siliques and Chlorophyll Breakdown (mg kg^{-1}) day^{-1} in Three Cultivars of B. napus Seed (Stellar, Delta and Westar)

Year	Seeding Date	Cultivar Comparison+	[a]log chl (mg kg^{-1}) d^{-1} |T|	[b] log ethylene (μL $g^{-1}h^{-1}$) d^{-1} |T|
1991	Early	D x S	3.058 **(1)	4.279 **
1991	Early	D x W	3.552 **	3.046 **
1991	Early	S x W	0.739 ns	0.421 ns
1991	Late	D x S	1.397 ns	0.333 ns
1991	Late	D x W	0.228 ns	3.307 **
1991	Late	S x W	0.834 ns	2.470 *
1992	Early	D x S	4.436 **	1.343 ns
1992	Early	D x W	0.961 ns	1.956 ns
1992	Early	S x W	3.875 **	0.756 ns
1992	Late	D x S	3.526 **	1.190 ns
1992	Late	D x W	0.248 ns	4.634 **
1992	Late	S x W	3.219 **	3.477 **

(+) D = Delta, S = Stellar, W = Westar
(1) ** = significant at p=0.01
* = significant at p=0.05
ns = non significant at p=0.05

Each ethylene result was compared to the results for seed chlorophyll breakdown (Table 34) to determine whether differences in rates of seed chlorophyll breakdown paralleled differences in rates of change in the rates of ethylene evolution from the siliques. We wished to determine whether a cultivar having a faster rate of seed chlorophyll breakdown than other cultivars would also have a faster rate of change in the rate of ethylene evolution from the siliques containing seed. This would

establish a link between ethylene production and chlorophyll breakdown in the seed. Few parallels were observed. In many cases, if the rates of seed chlorophyll breakdown were significantly different, the rates of change in rates of ethylene evolution from the siliques were not, or vice versa. This suggests that an increase in silique ethylene evolution during seed ripening does not control chlorophyll breakdown.

In most cases there were significant differences (p=0.05) in the rates of change in the rates of ethylene evolution from the siliques between the early and late seeding dates within the same year (Table 35).

Table 35: Paired T-Test Results Comparing Rates of Change in the Rates of Ethylene Evolution Between Early and Late Seeding Dates. Log ethylene vs. Days After First Sample.

Year	Cultivar	df	|t|	Significance
1991	Delta	23	2.590	*[1]
1991	Stellar	24	0.979	ns
1991	Westar	24	3.191	**
1992	Delta	56	2.895	**
1992	Stellar	46	3.367	**
1992	Westar	51	1.142	ns

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

In 1991, rates were different (p=0.05) for both the cultivars Delta and Westar; with Delta, the early seeding date had a faster rate of change in the rate of ethylene evolution, while for Westar the late seeding date evolved ethylene more quickly. In 1992, both Delta and Stellar had faster

rates of change in rates of ethylene evolution in the early seeding date. Therefore, the external environment affected the rate of change in the rate of ethylene evolution from the siliques.

These results were compared to those for seed chlorophyll breakdown (Table 23), but once again, no consistent pattern was observed. Three of the results were the same as for seed chlorophyll, while the other three were not. Once again this suggests that seed chlorophyll breakdown and ethylene evolution from the siliques are independent events which occur at the same time.

To determine whether temperature influenced the rate of ethylene evolution from siliques, growing degree days (GDD) were substituted for days in the regressions (Table 36).

Table 36: Regression Analysis Results for Three Cultivars of B napus (Stellar, Delta and Westar) Based on Log Ethylene vs. Growing Degree Days (GDD)

Year	Cultivar	Seeding Date	Slope	MS Error	df	Adj. r^2	Sig.
1991	Delta	Early	-0.005628	0.02219	13	0.95	0.0001
1991	Stellar	Early	-0.003948	0.00761	14	0.97	0.0001
1991	Westar	Early	-0.004181	0.02859	14	0.91	0.0001
1991	Delta	Late	-0.004117	0.01500	10	0.91	0.0001
1991	Stellar	Late	-0.004277	0.02643	10	0.88	0.0001
1991	Westar	Late	-0.005716	0.01702	10	0.95	0.0001
1992	Delta	Early	-0.005659	0.02889	33	0.87	0.0001
1992	Stellar	Early	-0.006704	0.01228	28	0.94	0.0001
1992	Westar	Early	-0.006615	0.03403	33	0.89	0.0001
1992	Delta	Late	-0.006595	0.03992	23	0.81	0.0001
1992	Stellar	Late	-0.008056	0.01950	18	0.89	0.0001
1992	Westar	Late	-0.012166	0.07018	18	0.84	0.0001

In 1991, the conversion to GDD had no effect on the results (Table 37).

Table 37: Paired T-test Results Comparing Rates of Change in Rates of Ethylene Evolution (μL/g fresh wt./hour) in Three Cultivars of B. napus (Stellar, Delta and Westar) Between Early and Late Seeding Dates. Based on Log ethylene vs. GDD.

Year	Cultivar	df	|T|	Significance
1991	Delta	23	2.799	*[1]
1991	Stellar	24	0.727	ns
1991	Westar	24	2.815	**
1992	Delta	56	1.306	ns
1992	Stellar	46	2.060	*
1992	Westar	51	5.113	**

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

This is not surprising, as the 1991 growing season was ideal for rapid seed ripening. The weather remained warm until the end of August, therefore, both seeding dates ripened under similar temperature regimes, which explains why the conversion to GDD had no effect. In 1992, the difference in rates of change in rates of ethylene evolution between seeding dates disappeared (p=0.05) for the cultivar Delta when the GDD conversion was used, the |T| value for the cultivar Stellar was still significant at the 5% level, but not at the 1% level, and a significant difference was created (p=0.01) for the cultivar Westar. Therefore, temperature did affect rates of ethylene evolution from the siliques in

amounts of rain and chilling temperatures during the 1992 growing season may have triggered the production of stress ethylene.

Some cultivars had significantly different (p=0.05) rates of change in rates of ethylene evolution in 1991 and 1992, while others did not (Table 38).

Table 38: Paired T-Test Results Comparing Rates of Change in Rates of Ethylene Evolution (μL/g fresh wt./hour) in Three Cultivars of B. napus in 1991 and 1992. Based on Log Ethylene vs. Days After First Sample.

Seeding Date	Cultivar	df	|T|	Sig.
Early	Delta	46	4.393	**[1]
Early	Stellar	42	1.044	ns
Early	Westar	47	0.809	ns
Late	Delta	33	2.414	*
Late	Stellar	28	2.227	*
Late	Westar	28	1.391	ns

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

The conversion to GDD created differences (p=0.01) in the rates of change in rates of ethylene evolution between 1991 and 1992, with the exception of the early seeding date of the cultivar Delta where the difference was eliminated (p=0.05) (Table 39), confirming that temperature had a significant effect on the rate of ethylene evolution from the siliques.

Table 39: Paired T-Test Results Comparing Rates of Change in Rates of Ethylene Evolution (μL/g fresh wt./hour) in Three Cultivars of B. napus in 1991 and 1992. Log Ethylene vs. Growing Degree Days.

Seeding Date	Cultivar	df	|T|	Sig.
Early	Delta	46	0.061	ns[1]
Early	Stellar	42	7.471	**
Early	Westar	47	4.604	**
Late	Delta	33	2.997	**
Late	Stellar	28	4.728	**
Late	Westar	28	5.287	**

(1) ** = significant at p=.01
* = significant at p=.05
ns = non significant at p=.05

4.2.5 Summary

The three cultivars tested had significantly different (p=0.05) rates of seed chlorophyll breakdown that could not be explained by temperature variation, indicating that genetic variability does exist for this trait. Rates of seed chlorophyll breakdown were affected by the external environment, specifically by temperature. Moisture contents and chlorophyll levels were positively correlated in ripening B. napus seed. Seed of all cultivars had the same rate of moisture loss and there were no differences between seeding dates or years. Therefore, under the conditions encountered in this study, the external environment had no effect on the rate of moisture loss from the seed. This suggests that the rate of moisture loss may be genetically programmed to occur at a constant rate.

There were differences in the rates of change in the rates of ethylene evolution between different cultivars and seeding dates, and in some cultivars, between the two years of the study. Temperature affected the rate of change in the rate of ethylene evolution from the siliques. Seed chlorophyll levels and the rates at which the siliques containing seed evolved ethylene were positively correlated over the period of active ethylene evolution, but increases in ethylene production did not appear to control the rate of chlorophyll breakdown. The phase of rapid ethylene evolution by the siliques began after moisture loss and chlorophyll breakdown had begun, suggesting that endogenous ethylene production is not the trigger for the initiation of these events.

During seed ripening, chlorophyll was lost in an exponential pattern with rapid chlorophyll loss at high chlorophyll levels, but becoming slower as more and more pigment was lost, continuing until almost all of the pigment had disappeared, by the time the seed moisture content had fallen to approximately 10%. Moisture was lost at a constant rate throughout the entire ripening period, and ethylene evolution from the siliques peaked early during ripening, then declined. Chlorophyll breakdown, moisture loss and ethylene evolution are three events occurring simultaneously during seed ripening, but they do not appear to control one another. It cannot be shown that the rate at which any one of these processes occurs is dependent on either of the other two. Cultivars with the same rate of seed chlorophyll loss did not have the same rate of change in rates of ethylene production, and all cultivars had the same rate of moisture loss regardless of the rate of seed chlorophyll breakdown.

Cerone, applied to physiologically mature green canola seed (HB 5.2) at levels ranging from 1/4 to 1 L ha^{-1}, had no effect on the rate of seed chlorophyll breakdown.

4.3 Chlorophyll Derivatives in Ripening Canola Seed

Seed samples of three cultivars Westar, Stellar and Delta, harvested once a week as the crop ripened, were analyzed by HPLC to determine the type and quantity of chlorophyll pigments present in each seed sample. The study was conducted over two years, 1990 and 1991, with early and late seeding dates each year, to examine the influence of environment on chlorophyll pigment composition.

Typical chromatograms from each year of the study are presented in Figures 21 and 22. The HPLC method used gave good separation of PHO A, METHYL A, CHL B, CHL A, PHY B, PHY A and PYRO A. The absolute amount (mg kg^{-1}) of each chlorophyll pigment in each sample, including epimers, was determined for each seed sample and these data were combined over replicates. The epimers are artifacts of the main pigments formed during seed storage, pigment extraction or analysis (Endo et al.,1992). To simplify the results, the epimers were combined with the main pigments from which they were derived, for example CHL A' was summed with CHL A. The relative proportion (%) of each chlorophyll derivative in each seed sample was calculated (pigment/total chlorophyll), to allow comparison between seed samples having a wide range of chlorophyll levels (Tables 40 to 51). The ranges observed for the level of each pigment in each sample are available in Appendix Tables A4 to A15.

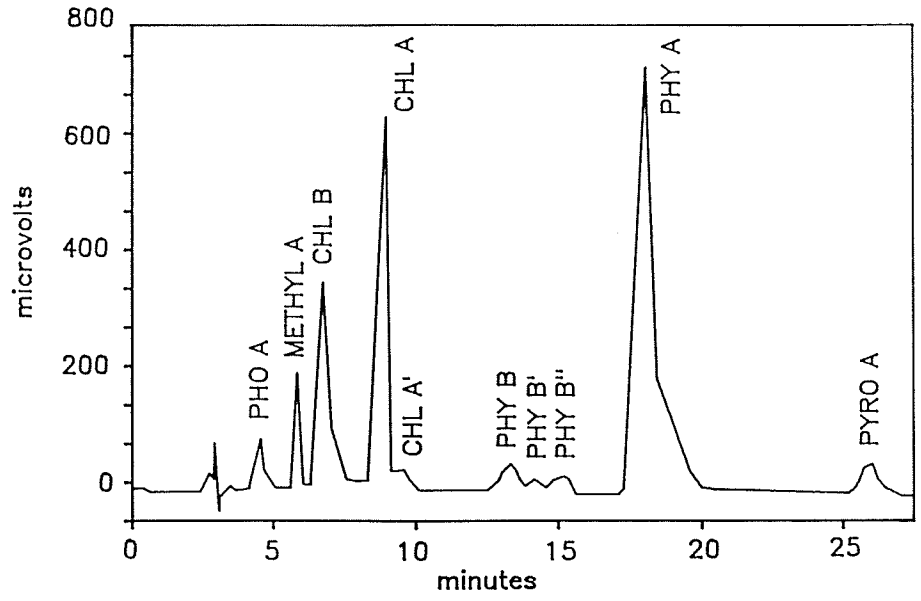


Figure 21: Typical chromatogram of the chlorophyll derivatives detected in canola seed in 1991. (PHO = pheophorbide, METHYL = methylpheophorbide, CHL = chlorophyll, PHY = pheophytin, and PYRO = pyropheophytin.)

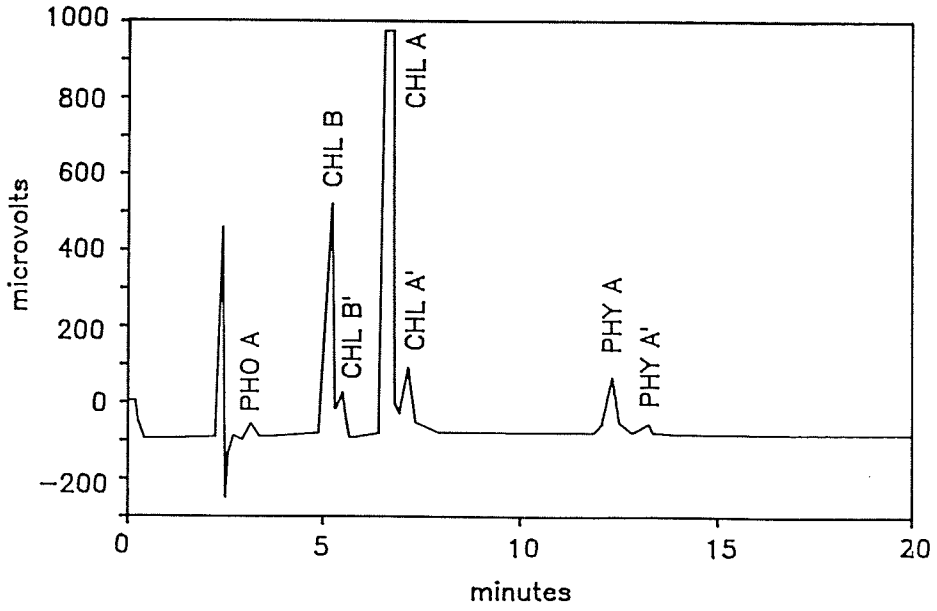


Figure 22: Typical chromatogram of the chlorophyll derivatives detected in canola seed in 1992. (PHO = pheophorbide, CHL = chlorophyll and PHY = pheophytin).

Table 40: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Westar) from the Early Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling					
	1	6	14	20	27	35
PHO A	2.2%	1.7%	0.6%	1.5%	-	-
METHYL A	3.2%	3.5%	1.8%	0.5%	3.0%	0.5%
CHL B	22.2%	22.1%	15.8%	27.3%	54.1%	17.1%
CHL A	19.5%	23.4%	27.2%	58.7%	41.9%	82.4%
PHY B	8.5%	7.4%	11.3%	2.0%	-	-
PHY A	43.1%	39.8%	40.9%	10.0%	1.1%	-
PYRO A	1.2%	2.3%	2.5%	trace	-	-
Total CHL (mg kg^{-1})	1239	906	463	48	8	4
% "A" der.	69.3	70.6	72.9	70.7	46.0	82.9
CHL A:B	0.88	1.06	1.72	2.15	0.77	4.82
PHY A:B	5.07	5.38	3.62	5.00	PHY A	-
PHY B:CHL B	0.38	0.33	0.72	0.073	CHL	CHL
PHY A:CHL A	2.21	1.70	1.50	0.17	0.026	CHL

Table 41: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Westar) from the Late Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling			
	17	22	31	36
PHO A	2.8%	2.6%	-	-
METHYL A	5.5%	0.7%	-	1.0%
CHL B	22.4%	29.5%	32.4%	25.9%
CHL A	21.3%	52.1%	67.0%	70.9%
PHY B	7.7%	-	-	trace
PHY A	38.5%	15.1%	0.6%	2.1%
PYRO A	1.7%	-	-	-
Total CHL (mg kg^{-1})	882	126	20	9
% "A" der.	69.7	70.5	67.6	74.1
CHL A:B	0.95	1.77	2.07	2.74
PHY A:B	5.00	PHY A	PHY A	PHY A
PHY B:CHL B	0.34	CHL	CHL	CHL
PHY A:CHL A	1.81	0.29	0.0090	0.030

Table 42: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Stellar) from the Early Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling					
	2	8	14	20	27	35
PHO A	2.0%	1.6%	0.9%	3.0%	0.8%	-
METHYL A	0.8%	3.5%	2.6%	0.6%	6.2%	0.6%
CHL B	32.5%	22.8%	18.2%	29.2%	49.4%	28.0%
CHL A	31.8%	26.8%	31.9%	51.0%	39.9%	71.4%
PHY B	2.0%	7.2%	9.5%	-	-	-
PHY A	29.8%	35.9%	34.8%	16.1%	3.8%	-
PYRO A	1.0%	2.2%	2.1%	0.2%	-	-
Total CHL (mg kg^{-1})	1164	1007	505	96	10	5
% "A" der.	65.5	70.0	72.3	70.8	50.6	72.0
CHL A:B	0.98	1.18	1.75	1.75	0.81	2.55
PHY A:B	14.90	4.99	3.66	PHY A	PHY A	-
PHY B:CHL B	0.062	0.32	0.52	CHL	CHL	CHL
PHY A:CHL A	0.94	1.34	1.09	0.32	0.10	CHL

Table 43: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Stellar) from the Late Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling			
	17	22	31	36
PHO A	4.5%	4.4%	2.0%	1.0%
METHYL A	17.7%	0.4%	1.9%	0.6%
CHL B	21.1%	34.4%	23.5%	24.6%
CHL A	19.4%	43.3%	60.7%	69.9%
PHY B	8.1%	0.1%	3.1%	-
PHY A	27.4%	17.4%	8.9%	3.9%
PYRO A	1.9%	-	-	-
Total CHL (mg kg^{-1})	1073	358	44	15
% "A" der.	70.8	65.5	73.4	75.4
CHL A:B	0.92	1.26	2.58	2.84
PHY A:B	3.38	174.00	2.87	PHY A
PHY B:CHL B	0.38	0.0029	0.13	CHL
PHY A:CHL A	1.41	0.40	0.15	0.056

Table 44: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Delta) from the Early Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling					
	3	9	16	21	29	34
PHO A	6.0%	1.4%	3.3%	-	-	-
METHYL A	2.6%	2.2%	-	0.2%	-	trace
CHL B	31.3%	20.8%	25.1%	41.3%	-	56.0%
CHL A	24.1%	31.8%	44.0%	51.1%	-	44.0%
PHY B	3.0%	8.3%	2.1%	-	-	-
PHY A	32.0%	33.2%	24.8%	7.4%	-	-
PYRO A	0.9%	2.1%	0.7%	-	-	-
Total CHL (mg kg^{-1})	921	704	212	19	-	0.2
% "A" der.	65.7	70.8	72.8	58.7	-	44.0
CHL A:B	0.77	1.53	1.75	1.24	-	0.79
PHY A:B	10.67	4.00	11.81	PHY A	-	-
PHY B:CHL B	0.10	0.40	0.084	CHL	CHL	CHL
PHY A:CHL A	1.33	1.04	0.56	0.14	CHL	CHL

Table 45: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Delta) from the Late Seeding Date of 1991. (PHY A indicates that no PHY B remained, CHL indicates that only chlorophylls remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin).

Pigment	Days from Start of Sampling			
	16	21	29	34
PHO A	3.4%	5.2%	1.1%	-
METHYL A	4.0%	1.2%	0.1%	-
CHL B	26.6%	35.9%	22.0%	41.0%
CHL A	24.4%	33.9%	66.6%	57.4%
PHY B	6.0%	0.3%	-	-
PHY A	34.4%	23.0%	10.2%	1.6%
PYRO A	1.2%	0.6%	-	-
Total CHL (mg kg^{-1})	1009	592	62	13
% "A" der.	67.4	63.8	78.0	59.0
CHL A:B	0.92	0.94	3.03	1.40
PHY A:B	5.73	76.67	PHY A	PHY A
PHY B:CHL B	0.23	0.0084	CHL	CHL
PHY A:CHL A	1.41	0.68	0.15	0.028

Table 46: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Westar) from the Early Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling					
	1	7	14	22	31	37
PHO A	0.4%	-	-	-	-	-
METHYL A	-	-	-	-	-	3.5%
CHL B	28.1%	25.2%	22.2%	17.9%	12.6%	24.4%
CHL A	64.4%	73.7%	75.3%	82.1%	87.4%	72.1%
PHY A	7.0%	1.1%	2.6%	-	-	-
Total CHL (mg kg^{-1})	2375	1023	350	67	11	5
% "A" der.	71.9	74.8	77.8	82.1	87.4	75.6
CHL A:B	2.29	2.92	3.39	4.59	6.94	2.95
PHY A:CHL A	0.11	0.015	0.035	CHL	CHL	CHL

Table 47: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replciates) Detected in Ripening Canola Seed (cv. Westar) from the Late Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling						
	15	21	29	35	42	49	56
PHO A	-	-	0.5%	-	-	-	-
METHYL A	-	-	-	-	-	-	-
CHL B	26.2%	23.9%	31.2%	37.5%	23.7%	16.7%	15.1
CHL A	65.4%	71.5%	64.0%	59.0%	75.9%	83.3%	84.9
PHY A	8.4%	4.7%	4.3%	3.5%	0.4%	-	-
Total CHL (mg kg^{-1})	2081	1612	855	364	187	35	10
% "A" der.	73.8	76.1	68.8	62.5	76.3	83.3	84.9
CHL A:B	2.50	2.99	2.05	1.57	3.20	4.99	5.62
PHY A:CHL A	0.13	0.066	0.067	0.059	0.0052	CHL	CHL

Table 48: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Stellar) from the Early Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling					
	1	7	14	22	31	37
PHO A	0.4%	-	-	-	-	-
METHYL A	0.1%	-	-	-	-	1.6%
CHL B	30.3%	27.0%	30.9%	24.2%	25.9%	21.0%
CHL A	64.1%	70.2%	64.0%	74.6%	73.1%	77.4%
PHY A	5.1%	2.9%	5.1%	1.2%	1.0%	-
PYRO A	-	trace	-	-	-	-
Total CHL (mg kg^{-1})	2218	1828	550	188	61	9
% "A" der.	69.7	73.0	69.1	75.8	74.1	79.0
CHL A:B	2.12	2.60	2.07	3.08	2.82	3.69
PHY A:CHL A	0.080	0.041	0.080	0.016	0.014	CHL

Table 49: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Stellar) from the Late Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling						
	15	21	29	35	42	49	56
PHO A	-	-	0.7%	1.3%	-	0.1%	-
METHYL A	-	-	-	0.2%	-	-	-
CHL B	28.2%	25.4%	34.1%	38.0%	37.8%	26.5%	21.2%
CHL A	61.3%	67.8%	59.3%	51.9%	59.6%	71.6%	78.8%
PHY A	10.5%	6.9%	5.9%	8.7%	2.7%	1.7%	-
Total CHL (mg kg^{-1})	2139	1855	991	588	352	102	26
% "A" der.	71.8	74.6	65.9	62.0	62.2	73.5	78.8
CHL A:B	2.17	2.67	1.74	1.37	1.58	2.70	3.72
PHY A:CHL A	0.17	0.10	0.099	0.17	0.045	0.024	CHL

Table 50: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Delta) from the Early Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling					
	1	7	14	22	31	37
PHO A	-	-	-	-	-	1.8%
METHYL A	-	-	-	-	-	1.1%
CHL B	29.4%	24.0%	22.7%	20.2%	20.8%	15.1%
CHL A	65.3%	73.7%	75.5%	79.9%	79.2%	81.2%
PHY A	5.4%	2.3%	1.7%	-	-	0.8%
Total CHL (mg kg^{-1})	2223	1666	400	116	13	3
% "A" der.	70.6	76.0	77.3	79.8	79.2	84.9
CHL A:B	2.22	3.07	3.33	3.96	3.81	5.38
PHY A:CHL A	0.083	0.031	0.023	CHL	CHL	0.0099

Table 51: Relative Proportion (%) of Each Chlorophyll Pigment (averaged over replicates) Detected in Ripening Canola Seed (cv. Delta) from the Late Seeding Date of 1992. (CHL indicates that only CHL A remained). (PHO=pheophorbide, METHYL= methylpheophorbide, CHL=chlorophyll, PHY=pheophytin).

Pigment	Days from Start of Sampling						
	15	21	29	35	42	49	56
PHO A	0.2%	-	0.8%	1.3%	-	-	-
METHYL A	-	-	-	-	-	-	-
CHL B	27.9%	22.3%	28.3%	37.4%	24.4%	19.7%	22.4
CHL A	62.3%	72.0%	67.3%	57.0%	75.0%	80.4%	77.6
PHY A	9.6%	5.7%	3.7%	4.4%	0.6%	-	-
Total CHL (mg kg^{-1})	2110	1566	743	495	181	22	6
% "A" der.	72.1	77.7	71.7	62.6	75.6	80.3	77.6
CHL A:B	2.23	3.23	2.38	1.52	3.07	4.08	3.46
PHY A:CHL A	0.15	0.079	0.055	0.077	0.0080	CHL	CHL

The three cultivars tested, Stellar, Delta and Westar, all contained the same types of chlorophyll pigments - chlorophylls, pheophytins, pheophorbides, methylpheophorbides and pyropheophytins. No pigments were detected that were unique to any one cultivar and none of the pigments occurred at exceptionally high or low levels in a single cultivar. This suggests that chlorophyll likely degrades by the same pathway in all cultivars tested, as the same breakdown intermediates appear. There were also no differences in pigment composition between the early and late seeding dates within the same year.

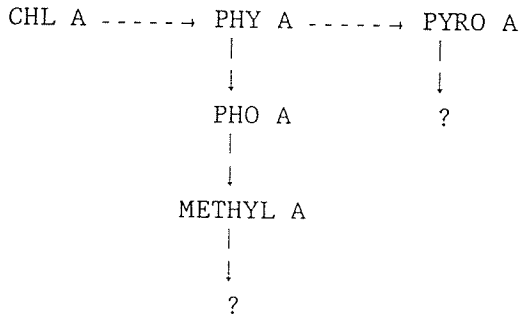
There were differences in pigment composition between the two years of the study. In seeds harvested in 1991, the main pigments detected were CHL A, CHL B, PHY A and PHY B. Minor pigments which appeared in some samples included PHO A, METHYL A and PYRO A, as well as numerous epimers - PHO A′, METHYL A′, CHL B′, CHL A′, PHY B′, PHY B′′ (a second distinct PHY B epimer) and PHY A′. No unidentified peaks were detected, except for two samples which contained trace peaks that eluted between CHL A and PHY B. In 1992, CHL A, CHL B and PHY A appeared as major pigments, but no PHY B was detected. Low levels of PHO A and METHYL A were detected in some samples, but not PYRO A. Epimers of CHL B, CHL A and PHY A appeared as minor components. No unidentified peaks appeared in any samples in the second year of the study.

The differences in pigment composition between the two years of the study could be due to seed aging in storage. The freeze-dried samples harvested in 1991 were stored at room temperature (~22°C) for 18 to 21 months prior to analysis, while samples collected in 1992 were only stored for 3 to 8 months. A previous study indicated that frozen (-20°C) canola

seed could be stored for up to one month with no change in total chlorophyll, measured by spectrophotometric absorbance (Ward et al.,1992b), but chlorophyll pigment composition was not examined. Prolonged storage of freeze-dried seed at room temperature might have allowed the seed to deteriorate. Mouldy, heated or otherwise damaged seeds have been shown to contain elevated levels of pheophytins (Johansson and Appelqvist,1984). PHY B was only detected in the 1991 seed samples and PHY A was present at much higher levels in samples collected in 1991 than in 1992.

Environmental effects might also explain the differences in pigment composition between the two years of the study. In 1991, the seed ripened quickly during hot, dry weather, with the late seeding date fully ripe by the end of August. In 1992, cool wet weather delayed ripening and prolonged the sampling period until early October for the late seeding date. Temperature and precipitation data is presented in the Appendix (Tables A2 and A3). From this study, it cannot be determined whether the differences in pigment composition between the two years were caused by seed aging in storage or environmental influences.

The chlorophyll decomposition products detected by the HPLC system were PHO A, METHYL A, CHL B, CHL A, PHY B, PHY A or PYRO A. No unidentified peaks were detected at chlorophyll specific wavelengths. It is possible that other breakdown products could also have been produced and escaped detection by the HPLC system. However any pigments that were structurally similar to the chlorophylls should have been detected. This suggests that the initial steps in the chlorophyll breakdown pathway in ripening canola seed may be:



and CHL B ------→ PHY B ------→ ?

A series of investigations by Johnson-Flanagan and colleagues have attempted to determine the pathway(s) and enzymes involved in chlorophyll breakdown in ripening canola seed. CHL A and CHL B were the main pigments detected with low levels of pheophytins, pheophorbides and chlorophyllides. During ripening, both CHLLIDE A and PHY A accumulated, indicating that two separate pathways operate simultaneously in degreening canola seed (Johnson-Flanagan and Thiagarajah,1990; Johnson-Flanagan and McLachlan,1990a,b). We were unable to detect chlorophyllide.

It is clear that pigment composition depends on seed maturity. Green seed (HB 5.2-5.3) from the earliest samples harvested contained significant amounts of CHL A, CHL B, PHY A and PHY B, but at maturity (HB 5.5) canola seed contained mainly CHL A and CHL B. The minor components (PHO A, METHYL A and PYRO A) were detected sporadically as the seed matured, but most had disappeared when the seed reached full maturity (HB 5.5) (Tables 40 to 51). These minor components were likely breakdown intermediates that appeared as CHL A degraded. PHY B and PHY A were also breakdown intermediates formed from CHL B or CHL A and then further degraded. PHY B appeared only in green seed in the first year of the

study, disappearing within two to three weeks. PHY A was present at significant levels in green seed, but gradually disappeared as the seed ripened (Tables 40 to 51). In fully mature seed (HB 5.5), PHY A was either absent or present at low levels. The transient nature of the pheophytins is confirmed by following the ratios of PHY B:CHL B and PHY A:CHL A during seed maturation (Tables 40 to 51). In all cultivars, seeding dates and years tested, the ratio of PHY B:CHL B and of PHY A:CHL A declined as the seed matured from physiologically mature green (HB 5.2) to full maturity (HB 5.5). In the final seed samples taken from each plot, only chlorophylls remained. It is also possible that some of the pheophytins detected may have formed during aging of the freeze-dried seed in storage.

Endo et al. (1992) characterized the chlorophyll pigment composition of mature canola seed from a crushing plant and found CHL A and B to be the major pigments present, with PHY A present as a minor component and traces of PHO A and METHYL A in some samples. Cenkowski et al. (1993) detected mainly CHL A and CHL B with lesser amounts of PHY A in extracts from ripening B. napus seed collected daily during ripening of a field grown crop. We also found CHL A and B to be the major pigments in ripe canola seed, but seed that was not fully mature also contained PHY A and B and traces of PHO A, METHYL A and PYRO A. Ours is the first report of PHY B and PYRO A in canola seed. Since these pigments were only observed in one year of the study, it is uncertain whether these compounds occurred naturally in ripening seed, or whether they were formed during seed storage.

The percentage of "A" derivatives detected varied with the stage of

seed maturity from 44 to 87%. In general, the percentage of "A" derivatives in fully mature seed (HB 5.5) was higher than in green seed (HB 5.2-5.3), but there was not a steady increase during seed ripening (Tables 40 to 51). PHY A and B appeared as temporary breakdown intermediates, so their appearance and disappearance may explain some of the variability observed. Results were more consistent when the ratio of CHL A:B was followed during seed ripening. In both seeding dates of 1991 and the early seeding date of 1992, the ratio of CHL A:B increased steadily as the seed matured, with the exception of occasional samples. In the late seeding date of 1992 the increase was not as consistent but the ratio of CHL A:B was considerably higher in mature seed (HB 5.5) than in green seed (HB 5.2) (Tables 40 to 51). The variation observed in the late seeding date of 1992 may be due to the extremely cool wet weather in which the crop matured, which resulted in a slow and prolonged ripening period.

In 1991, the CHL A:B ratio in green seed (HB 5.3) was approximately 1:1 while in mature seed (HB 5.5) it was close to 2:1. In 1992, green seed (HB 5.2) averaged a CHL A:B ratio of approximately 2:1 while in mature seed (HB 5.5) this value was close to 4:1. Therefore, the CHL A:B ratio approximately doubled as canola seed ripened from physiologically mature green (HB 5.2) to full maturity (HB 5.5). Previous studies by Daun and Thorsteinson (1989) and Endo et al. (1992) have found the CHL A:B ratio in commercial samples of canola seed to be 3:1. Our study shows that the ratio of CHL A:B depends on the stage of maturity of the seed.

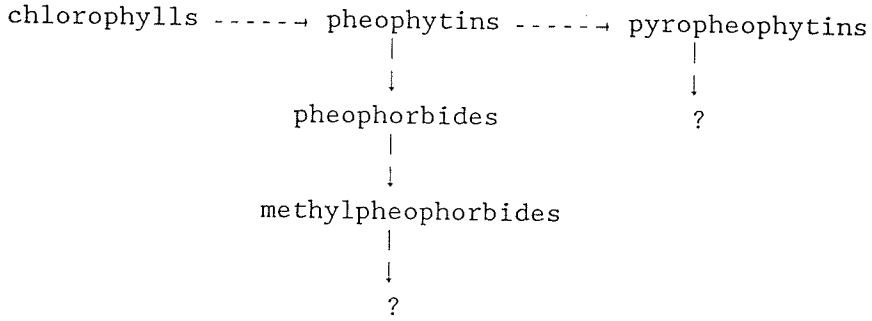
CHL B seemed to degrade faster than CHL A, suggesting enzymatic reactions, as non-enzymatic photobleaching degrades CHL B and CHL A at the

same rate (Jen and Mackinney,1970). Researchers working with other plant species, have found that during senescence the ratio of CHL A:B decreases, with CHL A being degraded more quickly (Simpson et al.,1976). Johnson-Flanagan and Thiagarajah (1990) found that as the moisture content of B. napus embryos fell from 70 to 50%, the ratio of CHL A:B decreased. However, in our study involving field grown ripening B. napus seed this was not the case. Between the physiologically mature green stage of seed development (HB 5.2) and full seed maturity (HB 5.5) CHL B degraded faster than CHL A, with an accompanying rise in the CHL A:B ratio (Tables 40 to 51). The PHY A:B ratio showed a similar pattern to the chlorophylls in 1991 where this could be determined, with PHY B disappearing sooner than PHY A.

4.3.1 Summary

The major pigments detected in canola seed were chlorophylls A and B and pheophytins A and B. Small amounts of PHO A, METHYL A and PYRO A occurred in some samples. There were no differences in pigment composition between different cultivars, or between seeding dates within the same year. There were differences in pigment composition between the two years of the study, that may be the result of either seed aging in storage or environmental influences. Pigment composition was dependent on seed maturity, with green seeds (HB 5.2-5.3) containing both chlorophylls and pheophytins, but mainly chlorophylls remaining in ripe seed (HB 5.5). The pheophytins and the minor components were breakdown intermediates, formed from the chlorophylls and subsequently degraded. Ripe canola seed (HB 5.5) had a CHL A:B ratio double that of green seed (HB 5.2-5.3). The

"B" derivatives seemed to degrade faster than the "A" derivatives, suggesting enzymatic reactions. These observations suggest that during seed ripening in canola:



4.4 Effects of Processing and Storage on the Chlorophyll Derivatives in Commercially Extracted Canola Oil

The type and amount of each chlorophyll derivative in pressed, solvent extracted, crude and degummed canola oil, from three separate batches, obtained from the same crushing plant, was measured when the oil was fresh and after storage for various periods of time. The absolute amount (mg kg^{-1}) of each chlorophyll derivative detected, in each oil sample, including the epimers, was calculated. To simplify the results, the epimers were combined with the main pigments from which they were derived, for example CHL A′ was summed with CHL A, and the relative proportion of each chlorophyll derivative in each oil sample was calculated (Tables 52 to 63).

The structure of each chlorophyll derivative is illustrated in Figure 1 in the literature review. The HPLC system separated PHO A, METHYL A, CHL B, CHL A, PHY B, PHY A and PYRO A. Figure 23 illustrates typical chromatograms for pressed, solvent extracted, crude and degummed oils. The small peaks which appeared immediately adjacent to the main peaks are due to epimers - for example CHL A and CHL A′, which were formed either during oil extraction, storage or analysis. In the case of PHY B, two different epimers were detected. Epimers were summed with the main pigments from which they were derived as there was no practical reason to consider them separately.

Table 52: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Fresh Canola Oil Samples from Batch 1. (CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL was determined by HPLC).

Type of Oil	Chlorophyll Derivative					
	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL (mg kg^{-1})
Pressed	-	-	-	50.6%	49.4%	24.3
Extracted	10.4%	27.9%	-	21.2%	28.8%	26.8
Crude	12.4%	37.2%	-	21.6%	28.8%	34.7
Degummed	4.5%	-	-	62.9%	32.6%	17.8

Table 53: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Canola Oil Samples from Batch 1 Stored for 8 Days at Room Temperature in the Light or Dark. (CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL was determined by HPLC).

Type of Oil	Chlorophyll Derivative					
	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL (mg kg^{-1})
Light Bench						
Pressed	3.2%	16.2%	-	45.8%	34.6%	27.7
Extracted	4.6%	9.2%	2.8%	31.9%	51.5%	28.5
Crude	-	-	3.6%	51.3%	45.1%	30.4
Degummed	-	-	5.9%	46.2%	48.0%	34.0
Dark Bench						
Pressed	10.8%	30.0%	-	35.5%	23.8%	32.4
Extracted	8.7%	13.2%	-	38.5%	39.4%	33.2

Table 54: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 1 Stored for 15 Days. (CHL= chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL was determined by HPLC).

Storage Conditions	Chlorophyll Derivative					
	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL ($mg\ kg^{-1}$)
Pressed Oil						
Freezer	14.1%	35.7%	1.5%	25.8%	22.7%	32.5
Fridge	11.9%	29.4%	-	29.3%	29.4%	34.4
Dark Bench	11.1%	17.3%	1.9%	37.5%	32.2%	36.0
Light Bench	-	6.1%	1.3%	48.7%	43.8%	22.8
Extracted Oil						
Freezer	10.0%	21.0%	1.6%	22.5%	45.1%	32.0
Fridge	11.3%	18.0%	1.0%	25.2%	44.6%	29.4
Dark Bench	7.5%	6.3%	1.8%	32.6%	51.8%	33.4
Light Bench	4.1%	2.5%	1.3%	32.7%	59.4%	23.9
Crude Oil						
Freezer	8.0%	0.9%	1.8%	44.5%	44.8%	32.6
Fridge	5.0%	-	3.7%	48.0%	43.3%	32.3
Dark Bench	1.9%	0.3%	5.7%	44.3%	47.7%	31.4
Light Bench	0.4%	0.4%	4.4%	50.5%	44.3%	27.1
Degummed Oil						
Freezer	0.9%	0.3%	4.4%	42.9%	51.6%	34.3
Fridge	1.1%	-	6.0%	40.9%	52.0%	35.2
Dark Bench	1.1%	0.3%	8.6%	39.8%	50.3%	36.2
Light Bench	0.6%	0.3%	6.7%	41.9%	50.5%	32.9

Table 55: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 1 Stored for 25 Days. (CHL= chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL was determined by HPLC).

Storage Conditions	Chlorophyll Derivative					
	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL ($mg\ kg^{-1}$)
Pressed Oil						
Freezer	9.9%	31.5%	-	27.0%	31.7%	38.2
Fridge	10.8%	24.8%	-	32.4%	32.0%	37.9
Dark Bench	10.1%	10.6%	1.3%	41.1%	37.0%	39.7
Light Bench	2.6%	2.2%	0.4%	46.8%	48.1%	23.1
Extracted Oil						
Freezer	8.6%	18.0%	-	20.1%	53.3%	38.3
Fridge	8.1%	12.9%	0.8%	23.9%	54.3%	38.1
Dark Bench	6.9%	2.6%	2.0%	31.7%	56.7%	38.8
Light Bench	1.3%	0.3%	3.2%	35.3%	60.0%	31.5
Crude Oil						
Freezer	7.0%	0.5%	1.6%	41.4%	49.4%	37.2
Fridge	4.8%	0.3%	1.1%	42.7%	51.1%	35.6
Dark Bench	-	0.5%	6.8%	39.8%	53.0%	38.7
Light Bench	1.2%	0.6%	4.8%	44.3%	48.9%	32.9
Degummed Oil						
Freezer	1.6%	0.3%	8.4%	38.3%	51.5%	38.4
Fridge	0.8%	0.5%	5.8%	38.5%	54.3%	39.2
Dark Bench	-	0.2%	9.6%	36.5%	53.7%	41.7
Light Bench	1.5%	0.3%	14.7%	39.4%	44.2%	39.6

Table 56: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Fresh Canola Oil Samples from Batch 2. (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL was determined by HPLC).

Type of Oil	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL (mg kg^{-1})
Pressed	0.5	0.5	17.6	37.1	0.5	35.2	8.6	61.0
Extract	-	0.2	12.0	8.3	-	23.0	55.3	49.8
Crude	0.2	0.5	14.5	23.0	1.0	29.7	31.1	60.8
Degum	-	0.4	3.6	-	5.9	58.9	31.2	54.6

Table 57: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 2 Stored for 7 Days. (PHO= pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	0.3	0.2	18.5	34.5	-	37.5	9.0	65.7
Fridge	0.3	0.5	18.1	33.1	0.6	38.1	9.3	64.9
Dark	0.2	0.6	18.2	25.8	0.6	45.2	9.4	67.1
Light	0.4	0.7	16.7	23.5	0.8	47.9	10.1	59.4
Extracted Oil								
Freezer	-	0.1	12.1	7.2	-	23.1	56.1	52.7
Fridge	-	-	10.1	5.8	-	24.7	58.0	52.9
Dark	-	-	10.4	2.5	2.1	27.5	57.5	52.4
Light	-	0.2	8.4	2.0	-	28.2	60.2	46.5
Crude Oil								
Freezer	-	0.3	16.6	27.2	0.9	34.2	20.8	60.5
Fridge	-	-	15.3	20.8	-	33.6	29.7	56.0
Dark	-	0.2	14.9	13.1	1.6	40.8	29.4	57.7
Light	-	0.2	12.7	11.1	1.7	41.6	32.6	54.3
Degummed Oil								
Freezer	-	0.2	3.3	-	6.7	60.5	29.5	55.9
Fridge	0.1	0.2	3.5	-	6.9	60.4	28.9	56.8
Dark	-	0.1	2.1	-	7.6	60.6	29.6	55.9
Light	1.2	-	1.0	-	14.6	56.2	26.9	77.6

Table 58: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 2 Stored for 14 Days. (PHO= pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	0.5	1.1	24.7	33.7	0.4	33.1	6.6	59.1
Fridge	1.0	0.7	22.0	29.2	0.7	39.2	7.1	66.0
Dark	0.8	0.5	20.0	19.0	0.7	51.1	7.9	71.7
Light	1.0	0.6	13.8	14.4	1.3	58.4	10.5	67.8
Extracted Oil								
Freezer	0.2	0.1	11.1	8.4	2.2	28.0	50.1	42.3
Fridge	-	-	8.4	5.8	2.2	30.2	53.4	50.6
Dark	0.2	0.3	7.0	1.1	2.1	36.1	53.3	56.9
Light	0.2	0.2	2.2	0.6	2.5	37.0	57.2	62.9
Crude Oil								
Freezer	-	1.1	17.7	25.0	0.6	33.8	21.8	53.2
Fridge	0.1	0.3	14.0	18.4	0.9	38.3	28.0	63.4
Dark	0.7	0.3	12.8	6.7	1.4	49.3	28.8	64.8
Light	0.5	0.4	7.6	4.8	0.9	54.5	31.2	59.3
Degummed Oil								
Freezer	0.6	0.3	2.0	-	8.5	63.5	25.1	56.3
Fridge	0.7	0.4	1.0	-	8.2	62.8	27.0	77.0
Dark	0.6	0.4	0.3	-	10.3	63.2	25.2	68.6
Light	0.6	0.4	-	-	10.3	63.3	25.4	72.1

Table 59: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 2 Stored for 28 Days. (PHO= pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	0.5	-	16.4	34.3	2.5	38.0	8.3	72.1
Fridge	0.5	-	14.5	27.5	4.0	41.5	12.1	82.7
Dark	1.3	-	13.8	10.8	4.2	56.5	13.4	81.6
Light	1.5	0.7	7.8	6.0	5.2	63.5	15.4	66.8
Extracted Oil								
Freezer	0.5	0.3	8.1	7.5	5.3	23.3	55.1	67.4
Fridge	0.3	0.1	6.5	4.6	7.5	25.9	55.0	75.4
Dark	0.3	0.2	5.0	0.2	6.4	30.8	57.1	71.6
Light	0.1	-	0.6	-	7.6	30.7	60.9	67.4
Crude Oil								
Freezer	0.5	-	11.5	20.7	4.5	32.9	30.0	68.7
Fridge	0.3	0.2	8.0	16.1	6.0	37.7	31.8	73.5
Dark	0.6	0.4	9.4	2.6	6.3	47.3	33.3	79.1
Light	0.7	0.5	3.0	1.2	7.4	50.4	37.0	69.0
Degummed Oil								
Freezer	0.8	-	2.2	-	13.1	53.2	30.7	75.9
Fridge	0.8	-	1.7	-	13.7	53.7	30.1	73.9
Dark	0.6	0.6	-	0.2	15.8	52.1	30.8	74.3
Light	0.7	0.8	-	-	14.7	52.8	31.0	74.0

Table 60: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Fresh Canola Oil Samples from Batch 3. (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Type of Oil	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed	-	3.7	16.6	23.0	1.7	51.0	4.1	117.6
Extracted	-	2.2	11.6	5.0	6.0	57.2	18.1	77.3
Crude	1.4	1.6	14.9	14.6	4.4	52.5	10.6	95.6
Degummed	0.9	1.0	1.8	-	12.9	55.4	28.2	88.0

Table 61: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 3 Stored for 7 Days. (PHO= pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	1.8	1.3	18.1	19.9	2.2	52.4	4.4	117.8
Fridge	2.2	1.6	17.3	16.6	3.5	54.5	4.5	117.0
Dark	1.8	1.9	15.1	5.2	4.2	67.1	4.7	113.2
Light	2.2	2.1	12.2	4.1	4.4	70.1	5.0	102.9
Extracted Oil								
Freezer	0.9	0.9	12.0	4.2	6.6	57.6	17.9	81.3
Fridge	0.6	0.9	10.4	3.2	7.4	59.3	18.2	85.2
Dark	0.6	1.2	9.6	0.4	7.7	62.6	17.8	83.4
Light	0.6	1.4	7.8	0.3	8.0	63.6	18.2	84.6
Crude Oil								
Freezer	1.4	1.4	14.8	13.3	5.3	53.8	10.1	99.4
Fridge	1.6	1.5	15.3	12.3	4.6	56.3	8.5	105.8
Dark	1.8	1.9	13.8	3.4	5.2	66.6	7.4	105.7
Light	1.7	2.1	9.2	2.0	6.0	68.8	10.1	94.3
Degummed Oil								
Freezer	0.8	0.8	1.6	-	14.1	56.0	26.8	93.1
Fridge	0.7	1.1	0.7	-	14.0	57.2	26.2	88.5
Dark	0.8	1.4	0.1	-	15.0	56.0	26.7	92.2
Light	2.6	2.9	-	-	19.2	50.9	24.5	115.9

Table 62: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 3 Stored for 14 Days. (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	-	5.0	25.6	18.7	1.5	46.3	2.9	97.2
Fridge	2.3	1.2	21.8	12.9	2.9	55.4	3.6	114.2
Dark	2.2	1.3	17.1	1.0	4.0	70.2	4.4	114.4
Light	2.2	1.4	10.3	0.4	3.8	76.9	5.1	106.1
Extracted Oil								
Freezer	1.5	0.7	16.2	4.6	5.2	57.4	14.5	71.6
Fridge	1.2	0.5	14.0	2.2	5.7	60.9	15.6	82.4
Dark	1.1	0.7	10.2	-	6.5	65.0	16.6	92.1
Light	1.0	0.7	5.3	-	6.8	68.1	18.1	91.1
Crude Oil								
Freezer	2.2	1.0	21.3	14.0	3.2	51.3	7.1	93.5
Fridge	1.6	0.8	18.1	8.2	4.0	58.3	8.9	108.1
Dark	1.8	1.3	15.1	0.3	4.6	69.7	7.2	106.7
Light	1.5	1.0	8.3	0.1	5.3	72.7	11.1	100.5
Degummed Oil								
Freezer	1.2	0.5	0.4	-	15.0	59.5	23.5	78.3
Fridge	0.9	0.4	0.2	-	14.0	59.3	25.2	103.1
Dark	1.0	0.5	-	-	15.2	58.6	24.7	100.4
Light	0.9	0.5	-	-	15.5	58.0	25.1	103.7

Table 63: Relative Proportion (%) of Each Chlorophyll Derivative Detected in Four Types of Canola Oil Samples (pressed, solvent extracted, crude and degummed) from Batch 3 Stored for 28 Days. (PHO= pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin, PYRO=pyropheophytin). (TOTAL CHL (mg kg^{-1}) was determined by HPLC).

Storage	Chlorophyll Derivative							
	PHO A	METHYL A	CHL B	CHL A	PHY B	PHY A	PYRO A	TOTAL CHL
Pressed Oil								
Freezer	1.9	1.0	16.5	18.2	5.8	52.5	4.2	129.3
Fridge	2.5	-	15.3	9.3	7.9	59.3	5.8	130.2
Dark	2.3	1.8	8.9	0.3	10.8	69.2	6.8	128.1
Light	2.7	2.2	3.0	0.3	11.1	72.7	8.0	120.6
Extracted Oil								
Freezer	1.0	0.4	9.0	4.4	12.1	54.6	18.4	100.0
Fridge	1.1	0.8	7.6	1.6	13.3	55.9	19.7	109.2
Dark	1.4	1.1	4.8	0.2	14.5	57.5	20.5	108.8
Light	1.2	1.1	1.0	0.3	15.4	59.5	21.5	102.9
Crude Oil								
Freezer	1.9	1.0	13.2	11.8	9.1	52.5	10.6	112.8
Fridge	1.8	1.2	11.2	4.9	10.3	58.4	12.3	115.3
Dark	2.2	1.7	7.4	-	12.6	64.1	12.1	125.0
Light	2.2	2.0	1.7	0.2	12.6	65.9	15.3	113.5
Degummed Oil								
Freezer	1.4	-	1.1	0.2	18.7	50.2	28.4	112.2
Fridge	1.2	0.8	0.4	0.2	19.0	50.1	28.2	117.5
Dark	1.2	0.8	-	0.3	19.9	49.4	28.3	117.7
Light	1.1	1.2	-	0.3	20.0	49.2	28.3	114.1

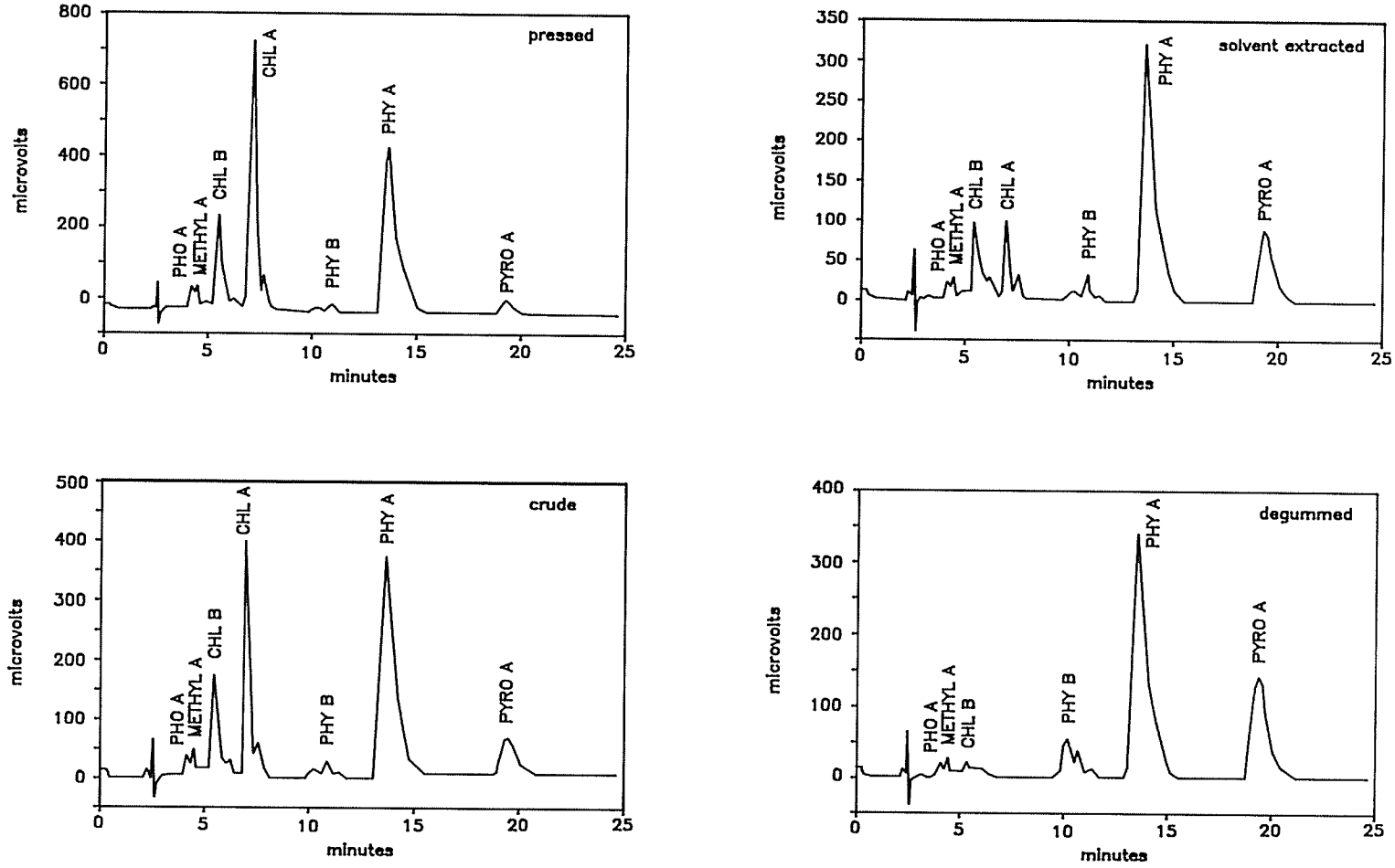


Figure 23: Chromatograms of the chlorophyll derivatives detected in pressed, solvent extracted, crude and degummed canola oil samples. (PHO=pheophorbide, METHYL=methylpheophorbide, CHL=chlorophyll, PHY=pheophytin and PYRO=pyropheophytin.)

Five main pigments were detected in commercially extracted canola oil - PHY A, PYRO A, CHL A, CHL B and PHY B. Small quantities of PHO A and METHYL A also appeared in some samples. These results agree with those of Endo et al. (1992) who found the main pigments in crude and degummed canola oil to be PHY A, PYRO A and PHY B, and also with the results of Suzuki and Nishioka (1993) who concluded that the major pigments detected in commercially extracted crude canola oil were PHY A, PHY B, PYRO A and PYRO B. The HPLC system in these experiments did not resolve PYRO B from PHY A, and any small amounts of this pigment would be included in the PHY A results.

4.4.1 Effect of Oil Storage on Chlorophyll Derivatives

Duration of oil storage and storage conditions affected the composition of chlorophyll derivatives in the oil. In all types of oil, CHL B was converted to PHY B and CHL A was converted first to PHY A, then to PYRO A during storage. Conversion was the most rapid in oils stored at room temperature in the light, followed by storage at room temperature in the dark and refrigerated storage, respectively. Only minor changes occurred in oil samples that were frozen (Tables 52 to 63).

In the fresh oil samples, levels of PHY A ranged from 21.2 to 62.9% of total chlorophyll pigments, PYRO A from 4.1 to 55.3% and PHY B from 0 to 12.9%. Significant levels of CHL A and CHL B were also detected in fresh oil samples and these had not been reported by either Endo et al. (1992) or Suzuki and Nishioka (1993). CHL B levels ranged from 1.8 to 17.6% of total chlorophyll pigments and CHL A levels ranged from 5.0 to 37.2%, depending upon the type of oil tested. A possible reason for the

discrepancy between our results and those of previous studies is the conversion of chlorophylls A and B to pheophytins and pyropheophytins during oil storage, noted in the present study. In the study by Endo et al. (1992) the samples had been stored for up to two years under cool, dark conditions prior to analysis, and the study by Suzuki and Nishioka (1993) did not specify how long the commercially extracted oil was stored prior to testing. The minor components detected included PHO A, present at 0.2 to 1.4% of total chlorophyll pigments in fresh oil samples, and METHYL A, present at 0.2 to 3.7% in fresh oil samples, as well as epimers of all five of the main pigments. These results are in agreement with those of Endo et al. (1992) who reported traces of PHO A and METHYL A, and both Endo et al. and Suzuki and Nishioka (1993) reported traces of various epimers.

4.4.2 Effects of Oil Processing on Chlorophyll Derivatives

Suzuki and Nishioka (1993) reported a lower proportion of A:B derivatives in solvent extracted oil than in pressed oil, while Endo et al. (1992) reported similar compositions. In our study, the proportion of A:B derivatives was quite variable. In batch one, the solvent extracted oil contained a lower proportion of "A" derivatives than the pressed oil, in batch two the solvent extracted oil contained more "A" derivatives than the pressed oil, and in batch three the two were almost identical (Table 64).

Table 64: Percentage of "A" Derivatives in Fresh Oil Samples

Oil Type	Batch 1 (28 mg kg^{-1})	Batch 2 (54 mg kg^{-1})	Batch 3 (79 mg kg^{-1})
Pressed	100.0	81.9	81.7
Solvent Extracted	89.6	86.9	82.4
Crude	87.6	84.5	80.7
Degummed	95.5	90.5	86.8

There were differences in the types and amounts of chlorophyll pigments detected in pressed, solvent extracted, crude and degummed canola oils. This study did not follow the same batch of oil through the refinery, but general trends in the pigment composition of each type of oil were apparent. The predominant chlorophyll pigments detected in fresh pressed, solvent extracted and crude oils were PHY A, PYRO A, CHL A, CHL B and, in some cases, PHY B. In the fresh degummed oils, CHLS A and B were either absent or present as minor components, while the main pigments detected were PHY A, PYRO A and PHY B (Figure 23). Therefore, degumming caused chlorophylls to be converted to pheophytins. Endo et al. (1992) concluded that commercial oil extraction converted chlorophylls to pheophytins and degumming converted pheophytins to pyropheophytins. They found a higher ratio of PYRO A:PHY A in the degummed oil than in the crude oil. We did not observe this. In our results, degumming converted chlorophylls to pheophytins but there was no apparent conversion of pheophytins to pyropheophytins. We observed decreases in the ratios of CHL B:PHY B and CHL A:PHY A, and an increase in the ratio of PHY A:PYRO A between the crude and degummed oils (Table 65), with the exception of

batch three where the PHY A:PYRO A ratio was lower in the degummed oil than in the crude oil.

Table 65: Ratios of Chlorophyll Derivatives in Three Batches of Fresh Crude and Degummed Canola Oils

Oil Type	Ratio	Batch 1	Batch 2	Batch 3
Crude	CHL B:PHY B	all CHL B	14.5 : 1	3.4 : 1
Degummed		all CHL B	0.61 : 1	0.14 : 1
Crude	CHL A:PHY A	1.7 : 1	0.77 : 1	0.28 : 1
Degummed		all PHY A	all PHY A	all PHY A
Crude	PHY A:PYRO A	0.75 : 1	1 : 1	5 : 1
Degummed		1.9 : 1	1.9 : 1	2 : 1

Differences in processing conditions such as cooking temperature and duration might account for the differences between our results and those of Endo et al. (1992). Suzuki and Nishioka (1993) found that high cooking temperatures converted pheophytins to pyropheophytins. The duration and conditions of oil storage prior to analysis in Endo's study might explain the observed conversion of pheophytin to pyropheophytin.

The "A" type pigments comprised 81 to 100% of total chlorophyll pigments found in fresh commercially extracted canola oil (Table 64). This is in agreement with the results of Suzuki and Nishioka (1993) who reported a A:B ratio of 5:1 and with the results of Endo et al. (1992) who found 90% of the pigments in crude and degummed canola oils to be of the "A" type.

The type of oil and the total chlorophyll content of the oil both

affected the proportion of A:B derivatives detected. For the three batches of oil that we examined, which averaged 28, 54 and 79 mg kg^{-1} total chlorophyll, the lower the total chlorophyll content of the oil, the larger proportion of "A" derivatives it contained. Fresh oil samples from batch one (28 mg kg^{-1}) contained 88 to 100% "A" derivatives, batch two (54 mg kg^{-1}) contained 82 to 91% "A" derivatives, and batch three (79 mg kg^{-1}) contained 81 to 87% "A" derivatives. In batches two and three, the degummed oil contained a slightly higher proportion of "A" derivatives than the pressed, solvent extracted or crude oils (Table 64). This may be explained by the conversion of chlorophylls to pheophytins and pyropheophytins during degumming, which utilizes acidic treatments. CHL B would be converted to PHY B and PYRO B, which we were unable to resolve. This likely accounts for the apparently lower proportion of "B" derivatives detected in degummed oils.

Pressed oils generally contained a higher proportion of CHLS A and B and less PHY B and PYRO A than did solvent extracted oils. Therefore, solvent extraction may convert CHL B to PHY B and CHL A to PHY A and PYRO A. However, unlike Suzuki and Nishioka (1993), who found solvent extracted oil to contain mainly pyropheophytins, we observed all five of the main pigments (CHL B, CHL A, PHY B, PHY A and PYRO A) in solvent extracted oils. Suzuki and Nishioka (1993) used hexane as the extraction solvent which may not fully extract the more polar chlorophylls. Therefore, solvent extraction is likely responsible for some, but not all, of the chlorophyll pigment conversion that occurs during oil processing. The other possibility is that pheophytins and pyropheophytins may be more difficult to extract from seed than chlorophylls. This might account for

the higher levels of pheophytins and pyropheophytins in the solvent extracted oil compared to the pressed oil.

The amount and composition of chlorophyll derivatives present in commercially extracted canola oil has important implications for bleaching. There is no information on the relative ease of removal of chlorophylls versus pheophytins, but Suzuki and Nishioka (1993) have found that, using activated earth, PHY A and PYRO A were removed from the oil six times more readily than PHY B or PYRO B. Results differed with activated carbon however. Knowledge of the composition of chlorophyll derivatives in canola oil will make it easier to choose the correct amount and type of bleaching earth for efficient colour removal. Knowledge of how extraction and processing conditions cause these pigments to interconvert should eventually allow us to manipulate processing conditions to yield an oil that can be bleached efficiently.

4.4.3 Summary

The main pigments in fresh, commercially extracted canola oil are PHY A, PYRO A, CHL A, CHL B and PHY B. The "A" derivatives comprised 81 to 100% of total chlorophyll pigments in fresh oil samples. Solvent extraction and degumming both had appreciable effects on the composition of chlorophyll derivatives in the oil. During degumming chlorophylls were converted to pheophytins and pyropheophytins. During oil storage, both light and high temperature affected the composition of chlorophyll derivatives; CHL B was converted to PHY B and CHL A was converted first to PHY A, then to PYRO A.

4.5 Relationship Between Total Chlorophyll as Measured By HPLC and Spectrophotometry

4.5.1 Total Chlorophyll Measured in Commercially Extracted Canola Oil

In addition to measuring the chlorophyll components in each batch of oil by HPLC, as described in section 4.4, total chlorophyll levels were also measured using a spectrophotometer. A small apparent decrease in total chlorophyll was observed in the pressed, solvent extracted and crude oils during storage for one month (Figures 24 and 25). The apparent decrease was greatest for the oils stored at room temperature in the light, followed by storage at room temperature in the dark, in the refrigerator, and in the freezer, respectively. In the degummed oils, on the other hand, there was no apparent decrease in total chlorophyll during oil storage under any of the conditions tested (Figures 24 and 25).

A decrease in total chlorophyll during oil storage has never been reported. In the previous section we showed that during oil storage CHL B was converted to PHY B and CHL A was converted to PHY A and PYRO A. This conversion occurred the most quickly in oils stored at room temperature in the light, followed by storage at room temperature in the dark and refrigerated storage respectively. Only minor changes occurred in oil samples that were frozen.

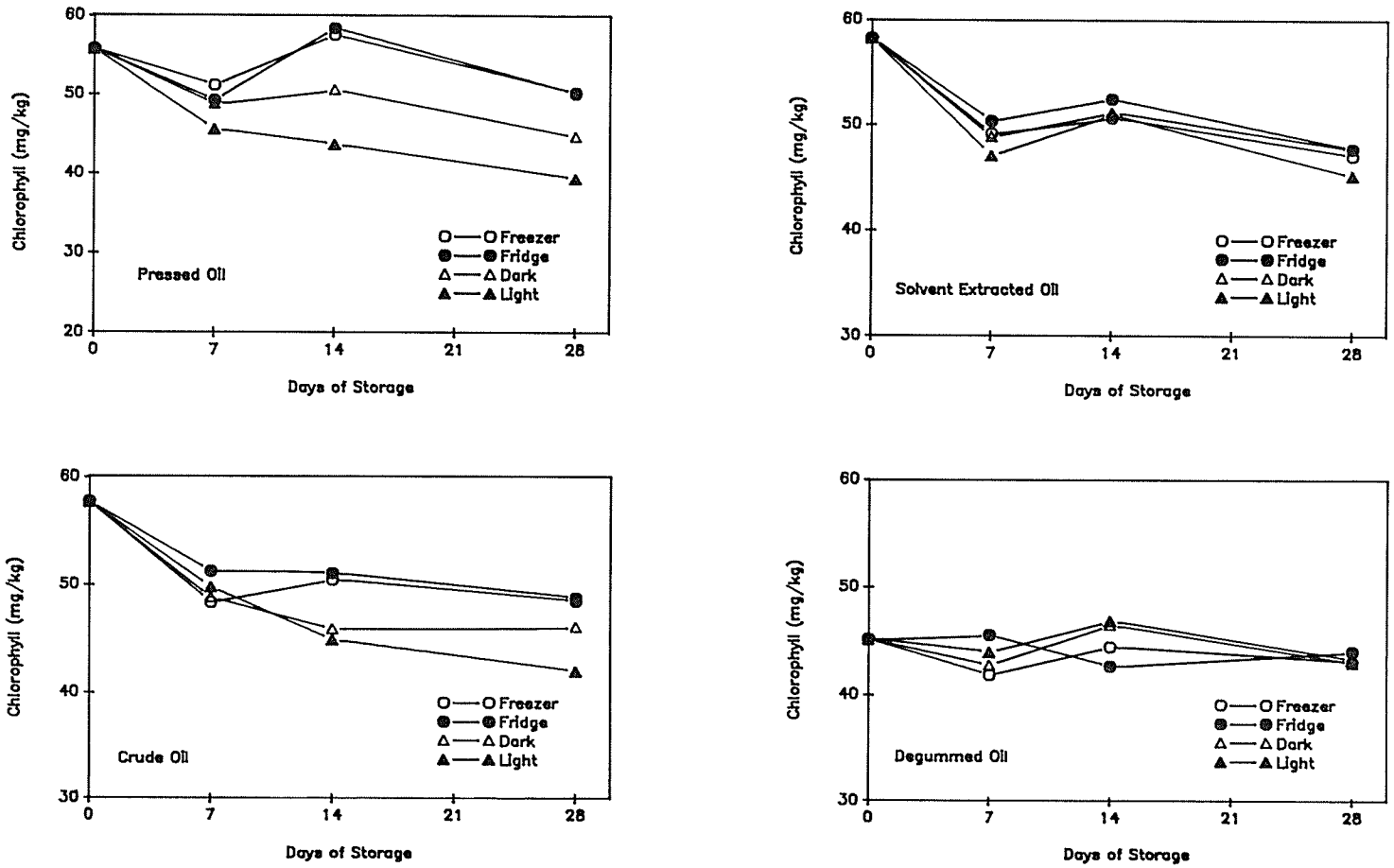


Figure 24: Total chlorophyll (mg kg^{-1}) measured spectrophotometrically in batch 2 of pressed, solvent extracted, crude and degummed canola oils stored for 28 days in a freezer, refrigerator or at room temperature in either the light or dark.

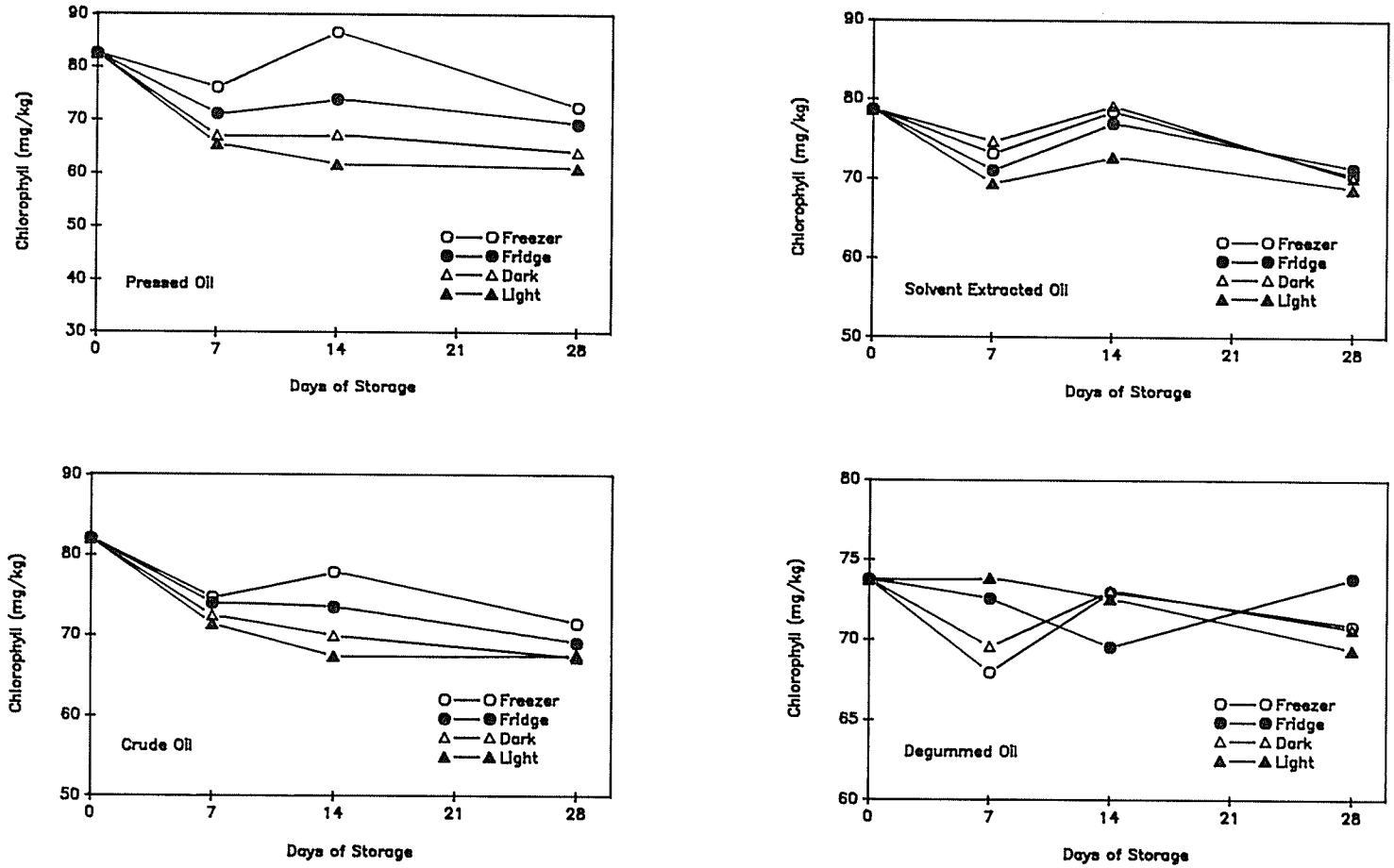


Figure 25: Total chlorophyll (mg kg^{-1}) measured spectrophotometrically in batch 3 of pressed, solvent extracted, crude and degummed canola oils stored for 28 days in a freezer, refrigerator or at room temperature in either the light or dark.

Therefore, the apparent decrease in total chlorophyll during storage as measured by the spectrophotometic method was not a real decrease. During storage, chlorophylls were converted to pheophytins and some pyropheophytins. The spectrophotometric method of chlorophyll measurement is calibrated using only CHL A, which has a much higher extinction coefficient (at 665 nm) than PHY A, PYRO A or any of the other chlorophyll derivatives (Table 1, section 3.3). CHL A, PHY A and PYRO A all absorb maximally in the same range (663-667 nm), but the molar extinction coefficient of CHL A (75 000) is approximately 1.7 and 1.5 times higher than those of PHY A (44 500) and PYRO A (49 000) respectively. Thus pheophytins and pyropheophytins in the stored oil samples, although present at the same concentration as the original chlorophylls, will produce a lower absorbence reading on the spectrophotometer, leading to an underestimate of total chlorophyll. In the pressed, solvent extracted and crude oils, the conversion of CHL A to PHY A and PYRO A was responsible for the apparent decrease in total chlorophyll observed. The degummed oil contained mainly pheophytins and pyropheophytins to begin with, so little conversion was possible and there was no decrease in absorbence.

Several researchers have noted that spectrophotometric methods which are calibrated with CHL A tend to underestimate total chlorophyll levels if the extract contains significant quantities of pheophytins or pyropheophytins (Tkachuk et al.,1988; Daun and Thorsteinson,1989; Davies et al.,1990; Suzuki and Nishioka,1993). Suzuki and Nishioka (1993) discussed this apparent discrepancy between pigment concentrations measured using the AOCS spectrophotometric method Cc 13d-55 and an HPLC method. They detected concentrations of pheophytins and pyropheophytins

1.4 times higher using HPLC than by the spectrophotometric method. They state that the calibration of the spectrophotometric method with CHL A accounted for this observation. Daun and Thorsteinson (1989) recalibrated the AOCS spectrophotometric method using PHY A, which increased the level of "chlorophyll" detected 1.5 times.

In our study, total chlorophyll determined by HPLC, calculated as the sum of all chlorophyll derivatives detected, did not agree with total chlorophyll measured using the spectrophotometric method. There was a strong positive correlation between total chlorophyll levels determined by HPLC and spectrophotometry ($r=0.73$, Prob.$> |r| = 0.0001$). The mean ratio of HPLC chlorophyll/spectrophotometer chlorophyll was 1.40 with a standard deviation of 0.27. Therefore the HPLC method detected, on average, 1.4 times more total chlorophyll per sample than did the spectrophotometric method. However this value varied a great deal between samples.

4.5.2 Total Chlorophyll Measured in Ripening Canola Seeds

The relationship between total chlorophyll measured by HPLC, calculated as the sum of all chlorophyll derivatives detected in a seed sample, and total chlorophyll measured by the AOCS spectrophotometric method AK 2-92, was compared for seed samples ranging from green (HB 5.2) to fully ripe (HB 5.5).

Again, the HPLC method detected, on average, 1.4 times more total chlorophyll pigments than the spectrophotometric method. There was a high positive correlation (+0.93) between total chlorophyll measured by HPLC and spectrophotometry ($N=232$), significant at the $p=0.0001$ level. The ratio between the HPLC measurement of total chlorophyll pigments and the

spectrophotometric readings varied between 0.3 to 3.0 for individual samples. Over the entire study (N=216), the ratio of HPLC/spectrophotometer chlorophyll was 1.37 with a standard deviation of 0.43. Any samples which contained less than 3.0 mg kg^{-1} total chlorophyll as measured by the spectrophotometric method were not included in the calculations. The spectrophotometric method only detects chlorophyll to an accuracy of within 3 mg kg^{-1}, therefore, samples containing lower levels of chlorophyll are below the limits of accurate detection. Once again, the discrepancy in total chlorophyll as measured by HPLC and spectrophotometry is explained by the calibration of the spectrophotometric method using only CHL A, leading to an underestimate of any other pigments present.

The above results illustrate a potential problem in chlorophyll analysis studies. For any given study, the same method of chlorophyll measurement must be used throughout the entire study, as results from different methods cannot be directly compared. The results of separate studies which have been carried out using different methods of chlorophyll measurement should not be directly compared either.

The spectrophotometric and HPLC methods of chlorophyll measurement were not designed for the same use. The spectrophotometric method gives a relatively rapid measurement of total chlorophyll and was designed to compare samples of the same type. The more diverse the samples that are being compared, the greater the degree of error that may be introduced. For example, a high quality seed sample which contained 15 mg kg^{-1} CHL A would produce a higher absorbence reading than a damaged seed sample which contained 15 mg kg^{-1} PHY A. Therefore, this method of chlorophyll

measurement should be used only for its intended purpose - that is to compare chlorophyll levels within a group of similar samples.

The HPLC method, on the other hand, can accurately determine the levels of each chlorophyll pigment in a sample. Therefore, it can be used to make comparisons between different types of samples. It is, however, much more time consuming and expensive than the spectrophotometric method if one is only interested in total chlorophyll. The other drawback to HPLC is that there is, at present, no standard HPLC method available to measure chlorophyll pigments. Each lab operates with different equipment, columns, solvents, flow rates, etc. which makes it impossible to directly compare results between labs. As more laboratories purchase HPLC equipment, a standardized method for chlorophyll determination in canola seed and oil would be useful.

4.5.3 Summary

In conclusion, when the spectrophotometric and HPLC methods of chlorophyll measurement were compared, the HPLC method detected, on average, 1.4 times as much total chlorophyll as did the spectrophotometric method, when either commercially extracted canola oil or seed extracts were considered, and there was a highly significant positive correlation between the two methods. The discrepancies between these two methods are explained by the fact that the spectrophotometric method is calibrated using only CHL A. The HPLC method, on the other hand, detects each pigment separately and sums them. Total chlorophyll measured by different methods should not be directly compared. There appears to be a need for a standardized method of chlorophyll pigment measurement by HPLC.

5.0 SUMMARY AND CONCLUSIONS

These studies have investigated several aspects of chlorophyll breakdown in ripening canola seed. Seed collected from one B. rapa and three B. napus cultivars grown in the provincial "Agroman" trials was analyzed to determine the contribution of genotype and environment to the level of chlorophyll in canola seed at harvest. Both the genotype of the plant and the location at which it was grown contributed significantly to the chlorophyll content of the seed at harvest. The cultivar Stellar consistently produced the highest seed chlorophyll levels, followed by Westar, Delta and Tobin, respectively. The cultivars containing the highest levels of seed chlorophyll at harvest are the most likely to be harvested prematurely or have the most uneven maturity. The location of the trial had a greater effect on seed chlorophyll levels than the year in which the trial was conducted. Minimal genotype by environment interaction was observed with the four cultivars examined in this study, but G x E interactions were significant when a larger number of cultivars were tested (Ward,1990). There was a strong negative correlation between oil and protein contents, but the correlation between oil and chlorophyll or protein and chlorophyll at harvest was inconsistent. Therefore, it should be possible to select for high oil content and low seed chlorophyll simultaneously.

Rates of seed chlorophyll breakdown during ripening were investigated in three cultivars of B. napus - Stellar, Delta and Westar. These three cultivars had significantly different rates of seed chlorophyll breakdown that could not be explained by temperature variation, indicating that

genetic variability does exist for this trait. Rates of seed chlorophyll breakdown were slower at lower temperatures.

There was a strong positive correlation between moisture and chlorophyll levels in ripening B. napus seed. All cultivars had the same rate of seed moisture loss and the external environment had no effect on this rate. Moisture loss may be genetically programmed to occur at a constant rate regardless of the external environment.

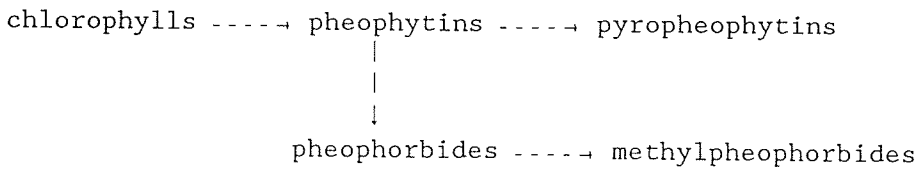
Rates of ethylene evolution from the siliques were measured as the crop ripened, as ethylene is well established as a ripening hormone. There were differences in the rates of change in the rates of ethylene evolution between the three cultivars tested, and between early and late seeding dates, but not between the two years of the study. Temperature affected the rate of ethylene evolution from the siliques. Seed chlorophyll levels and ethylene evolved by the siliques were positively correlated, but ethylene did not seem to control the rate of chlorophyll breakdown. There was a phase of rapid ethylene evolution by the siliques containing seed after moisture loss and chlorophyll breakdown had begun, suggesting that an increase in endogenous ethylene production is not the trigger for the initiation of either of these events.

During seed ripening, chlorophyll was lost in an exponential pattern with rapid chlorophyll loss at high chlorophyll levels, but becoming slower as more and more pigment was lost. Moisture was lost at a constant rate throughout the entire ripening period, and the rate of ethylene evolution from the siliques peaked early during ripening, then declined. Chlorophyll breakdown, moisture loss and ethylene evolution appear to be three independent events; the rate at which any one of these processes

occurs was not shown to be dependent on the other two.

Cerone, which breaks down to release ethylene, was applied to physiologically mature green canola (HB 5.3) at levels ranging from 1/4 to 1 L ha^{-1} (120 to 480 g active ingredient ha^{-1}). There was no effect on the rate of seed chlorophyll breakdown.

HPLC was used to monitor the chlorophyll pigments in canola seed throughout the ripening period. Major pigments detected included chlorophylls A and B and pheophytins A and B. Small amounts of PHO A, METHYL A and PYRO A were also detected in some samples. There were no differences in pigment composition between the three cultivars examined or between early and late seeding dates within the same year. However, there were differences in pigment composition between the two years of the study, that may be the result of either seed aging in storage or environmental influences. Pigment composition was dependent on seed maturity, with green seed (HB 5.2-5.3) containing large amounts of chlorophylls and pheophytins, but only chlorophylls remaining in ripe seed (HB 5.5). Both the pheophytins and the minor components (PHO A, METHYL A, PYRO A) appeared as breakdown intermediates, being formed from the chlorophylls, then being degraded themselves. The ratio of chlorophyll A:B increased during seed ripening, with ripe canola seed (HB 5.5) having a CHL A:B ratio double that of green seed (HB 5.2-5.3). The "B" derivatives seemed to degrade faster than the "A" derivatives, suggesting enzymatic reactions. These results suggest that the initial steps in the chlorophyll breakdown pathway in ripening canola seed may be:



HPLC was also used to characterize the chlorophyll pigments in canola oil immediately after commercial extraction and following oil storage, in order to determine the best storage conditions for analytical samples and to examine the changes that chlorophyll derivatives undergo during oil processing and storage. Samples of pressed, solvent extracted, crude and degummed canola oils obtained from a commercial crusher were stored for one month under various storage conditions and chlorophyll pigments were measured on a weekly basis. The main pigments in commercially extracted canola oil were PHY A, PYRO A, CHL A and CHL B. The "A" derivatives comprised 81 to 100% of total chlorophyll pigments in fresh oil samples. The percentage of "A" derivatives was dependent on the type of oil and the total chlorophyll content of the oil. During degumming chlorophylls were converted to pheophytins and pyropheophytins. During oil storage, both exposure to light and high temperatures affected the composition of chlorophyll derivatives; CHL B was converted to PHY B and CHL A was converted first to PHY A, then to PYRO A.

When spectrophotometric and HPLC methods to measure total chlorophyll were compared, the HPLC method detected, on average, 1.4 times as much total chlorophyll as did the spectrophotometric method, when either commercially extracted canola oil or seed extracts were tested. This discrepancy results from the fact that the spectrophotometric method is

calibrated using only CHL A, thereby underestimating all other chlorophyll pigments that have lower extinction coefficients.

In conclusion, the green seed problem causes a significant loss of revenue for Canadian farmers every year. Chlorophyll breakdown is poorly understood and only a few studies have been carried out to determine the pigment composition of canola seed and oil. It is hoped that a better understanding of the exact chlorophyll pigments present in canola seed and oil will improve our knowledge of how chlorophyll is degraded, how it causes problems during oil extraction and processing and, in turn, how these problems can best be solved, either through plant breeding or by modifying oil processing.

Many questions about chlorophyll breakdown in canola seed remain unanswered and could be investigated in future studies. If one wished to select for low seed chlorophyll in a breeding program it would first be necessary to choose a simple, rapid, accurate, cost effective method for seed chlorophyll analysis and then screen available germplasm for lines with low seed chlorophyll at harvest. In general, however, low seed chlorophyll at harvest correlates well with early maturity, and maturation time is already a selection factor in canola breeding programs. The advantage of selecting directly for low seed chlorophyll is questionable.

These studies have characterized the chlorophyll pigment composition of canola seed and oil, but the question of how best to remove these chlorophyll pigments remains unanswered. Different processing methods available for canola seed, for example, cooking temperature, extraction solvents, physical versus alkali refining, oil storage and other variables should be compared for their effects on chlorophyll derivatives. More

efficient, cost effective methods are needed to remove chlorophyll from canola oil. The effectiveness of different types of bleaching clay on the removal of the various chlorophyll derivatives (chlorophylls, pheophytins etc.) should be compared.

Finally, there is a need to elucidate the rest of the chlorophyll breakdown pathway, not only in canola seed, but in plants in general. Little is known beyond the production of pheophytins, pheophorbides, methylpheophorbides and pyropheophytins. The enzymes controlling chlorophyll degradation remain largely unknown, and nothing is known about how chlorophyll breakdown is controlled. In canola seed, it will be necessary to clarify whether one pathway or two operate in the initial breakdown of chlorophyll (to pheophytin, chlorophyllide or both), which enzyme(s) control the reaction(s) and how the entire process is controlled.

LITERATURE CITED

Abeles F.B. 1973. Ethylene in Plant Biology. Academic Press, New York.

Abeles F.B. and Dunn L.J. 1989. Role of peroxidase during ethylene - induced chlorophyll breakdown in Cucumis sativus cotyledons. J. Plant Growth Regul. 8:319-325.

Abraham V. and deMan J.M. 1986. Hydrogenation of canola oil as affected by chlorophyll. JAOCS 63(9):1185-1188.

Aharoni N. and Lieberman M. 1979. Patterns of ethylene production in senescing leaves. Plant Phys. 64:796-800.

Aljuburi H., Huff A. and Hshich M-L. 1979. Enzymes of chlorophyll catabolism in orange flavedo. Plant Phys. 68:854-856.

Amir-Shapira D., Goldschmidt E.E. and Altman A. 1987. Chlorophyll catabolism in senescing plant tissues: In vivo breakdown intermediates suggest different degradative pathways for Citrus fruit and parsley leaves. Proc. Natl. Acad. Sci. 84:1901-1905.

AOCS Official Method Cc 13d-55. Revised 1989. Official Methods and Recommended Practices of the American Oil Chemists' Society, 3rd edn. edited by V.C. Mehlenbacher, T.H. Hopper, E.M. Smallee, W.E. Link, R.O. Walker and R.C. Walker, American Oil Chemists' Society, Champaign, IL, USA.

AOCS Official Method Ak 2-92. 1992. Official Methods and Recommended Practices of the American Oil Chemists' Society, 3rd edn. edited by V.C. Mehlenbacher, T.H. Hopper, E.M. Smallee, W.E. Link, R.O. Walker and R.C. Walker, American Oil Chemists' Society, Champaign, IL, USA.

Apelbaum A., Goldschmidt E.E. and Ben-Yehoshua S. 1976. Involvement of endogenous ethylene in the induction of color change in Shamouti oranges. Plant Phys. 66:624-627.

Appelqvist L-A. and Ohlson R. (editors). 1971. Rapeseed. Elsevier Publishing Company, New York. pg. 174-197.

Barmore C.R. 1975. Effect of ethylene on chlorophyllase activity and chlorophyll content in calamondin rind tissue. HortScience 10(6): 595-596.

Beach D.H.C. 1983. Ch, 8 - Rapeseed Crushing and Extraction. in Kramer J.K.G., Sauer F.D. and Pigden W.J. (eds). High and Low Erucic Acid Rapeseed Oils. Production, Usage, Chemistry and Toxilogical Evaluation. Academic Press, Toronto. pp. 181-195.

Bidwell R.G.S. 1979. Plant Physiology, 2nd edition. Macmillan Publishing Co., Inc., New York. p.421-422.

Canadian General Standards Board. 1987. CAN/CGSB-32.300-N87. Canola oil, crude, degummed and refined. Canadian General Standards Board, Ottawa, Canada.

Canadian Grain Commission, Official Grain Grading Guide, 1991 Edn., revised 1992, amended 1993/94, Office of the Chief Grain Inspector, Inspection Division, Winnipeg, Manitoba, Canada.

Castelfranco P.A. 1983. Chlorophyll Biosynthesis: Recent advances and areas of current interst. Ann. Rev. Plant Physiol. 34:241-278.

Cenkowski S., Jayas D.S. and Daun J.K. 1993. Potential of in-field and low temperature drying for reducing chlorophyll contents in canola (Brassica napus L.). J. Sci. Food Agric. 63:377-383.

Choe H.T. and Whang M. 1986. Effects of ethephon on aging and photosynthetic activity in isolated chloroplasts. Plant Phys. 80:305-309.

Cochran, William G. and Cox, Gertrude M. 1957. Experimental Design 2nd edition. John Wiley and Sons Inc., New York.

Crouch M.L. and Sussex I.M. 1981. Development and storage-protein synthesis in Brassica napus L. embryos in vivo and in vitro. Planta 153:64-74.

Dahlen J.A.H. 1973. Chlorophyll content monitoring of Swedish rapeseed and its significance in oil quality. JAOCS 50: 312A-327A.

Daun J.K. 1982. The relationship between rapeseed chlorophyll, rapeseed oil chlorophyll and percentage green seeds. JAOCS 59(1):15-18.

Daun J.K. 1987. Chlorophyll in Canadian canola and rapeseed and its role in grading. 7th International Rapeseed Congress, Poznan, Poland.

Daun J.K. and Thorsteinson C.T. 1989. Determination of chlorophyll pigments in crude and degummed canola oils by HPLC and spectrophotometry. JAOCS 66(8):1124-1127.

Davies M.E., Shanks P.R., Anderson D.S. and Taylor R.S. 1990. Investigations on the measurement and removal of chlorophyll derivatives from edible oils. in Erickson D.R. (ed.). World Conference Proceedings - Edible Fats and Oils Processing: Basic Principles and Modern Practices. AOCS, Champaign, Il, USA.

Diosady L.L. 1991. Chlorophyll removal from canola oil (two parts). 1. Ion Exchange. Research on canola seed, oil and meal. 9th Project Report. Canola Council of Canada.

Endo Y., Usuki R. and Kaneda T. 1984a. Prooxidant activities of chlorophylls and their decomposition products on the photooxidation of methyl linoleate. JAOCS 61(4):781-784.

Endo Y., Usuki R. and Kaneda T. 1984b. The photooxidative alteration of chlorophylls in methyl linoleate and prooxidant activity of their decomposition products. Agric. Biol. Chem. 48(4):985-989.

Endo Y., Thorsteinson C.T. and Daun J.K. 1992. Characterization of chlorophyll pigments present in canola seed, meal and oil. JAOCS 69(6):564-568.

Finkelstein R.R. and Crouch M.L. 1986. Rapeseed embryo development in culture on high osmoticum is similar to that in seeds. Plant Phys. 81:907-912.

Fraser M.S. and Frankl G. 1985. Detection of chlorophyll derivatives in soybean oil by HPLC. JAOCS 62(1):113-121.

Goldschmidt E.E. 1980. Ch. 9 - Pigments changes associated with fruit maturation and their control. in Senescence in Plants. Kenneth V. Thiamann (editor). CRC Press, Boca Raton, Florida.

Gomez, Kwanchai A. and Gomez, Arturo A. 1984. Statistical Procedures for Agricultural Research 2nd edition. John Wiley and Sons Inc., New York.

Grami B. and Stefansson B.R. 1977a. Gene action for protein and oil content in summer rape. Can. J. Plant Sci. 57:625-631.

Grami B. and Stefansson B.R. 1977b. Paternal and maternal effects on protein and oil content in summer rape. Can. J. Plant Sci. 57:945-949.

Grami B., Baker R.J. and Stefansson B.R. 1977. Genetics of protein and oil content in summer rape: heritability, number of effective factors, and correlations. Can. J. Plant Sci. 57:937-943.

Harper F.R. and Berkenkamp B. 1975. Revised growth stage key for Brassica campestris amd B. napus. Can. J. Plant Sci. 55:657-658.

Harris, Dawn. 1988. Manage to avoid green seed. Country Guide: Jan. 1988: 23-23.

Helwig J.T. and Council K.A. (editors). 1979. SAS User's Guide. SAS Institute Inc., Cary, North Carolina.

Hendry G.A.F, Houghton J.D. and Brown S.B. 1987. Tansley Review No. 11 - The Degradation of Chlorophyll - a Biological Enigma. New Phytologist 107:255-302.

Hirschfeld K.R. and Goldschmidt E.E. 1983. Chlorophyllase activity in chlorophyll-free chromoplasts. Plant Cell Reports 2:117-118.

Horodyski A. and Tabola P. 1983. Qualitative and quantitative changes during the ripening process of three winter rape cultivars. 6th International Rapeseed Congress, Paris. pp. 675-679.

Huff, Albert. 1982. Peroxidase-catalysed oxidation of chlorophyll by hydrogen peroxide. Phytochem. 21(2):261-265.

Humphrey A.M. 1980. Chlorophyll. Food Chemistry 5:57-67.

Hyannien P. and Ellfolk N. 1973. Chlorophylls. 1. Separation and isolation of chlorophylls a and b by multiple liquid-liquid partition. Acta Chem. Scand. 27:1463-1477.

International Standards Organization. 1993. ISO Method 10519:1992(E), revised 1993. Rapeseed - Determination of Chlorophyll Content - Spectrometric Method. International Organization for Standardization, Geneva, Switzerland.

Jen J.J. and MacKinney G. 1970. On the photodecomposition of chlorophyll in vitro - 2. Intermediates and breakdown products. Photochem. Photobiol. 11:303-308.

Johansson S-A. and Appelqvist L-A. 1984. The effect of seed pretreatment and extraction conditions on the amount of minor components in seed oils. II. Chlorophyll and related pigments. Fette Seifen Anstrichmittel 8:304-313.

Johnson-Flanagan A.M. and McLachlan G. 1990a. The role of chlorophyllase in degreening canola (Brassica napus) seeds and its activation by sublethal freezing. Physiologia Plantarum 80:460-466.

Johnson-Flanagan A.M. and McLachlan G. 1990b. Peroxidase-mediated chlorophyll bleaching in degreening canola (Brassica napus) seeds and its inhibition by sublethal freezing. Physiologia Plantarum 80:453-459.

Johnson-Flanagan A.M. and Thiagarajah M.R. 1990. Degreening in canola (Brassica napus cv. Westar) embryos under optimum conditions. J. Plant Physiol. 136:180-186.

Johnson-Flanagan A.M., Singh J. and Thiagarajah M.R. 1990. The impact of sublethal freezing during maturation on pigment content in seeds of Brassica napus. J. Plant Physiol. 136:385-390.

Johnson-Flanagan A.M., Haiwen Z., Geng X.-M., Brown D.C.W., Nykiforuk C.L. and Singh J. 1992. Frost, abscisic acid, and desiccation hasten embryo development in Brassica napus. Plant Phys. 99:700-706.

Jung M.Y., Yoon S.H. and Min D.B. 1989. Effects of processing steps on the contents of minor components and oxidation of soybean oil. JAOCS 66(1):118-120.

Kader A.A. 1985. Ethylene-induced senescence and physiological disorders in harvested horticultural crops. HortScience 20(1):54-56.

Kalmokoff M.L. and Pickard M.D. 1988. Resistance of green pigments in commercial canola oil to enzymatic hydrolysis. Can. Inst. Food Sci. Technol. J. 21(5):534-536.

Kao C.H. and Yang S.F. 1983. Role of ethylene in the senescence of detached rice leaves. Plant Phys. 73:881-885.

Kato M. and Shimuzu S. 1985. Chlorophyll metabolism in higher plants VI. Involvement of peroxidase in chlorophyll degradation. Plant Cell Physiol. 26(7):1291-1301.

Kato M. and Shimizu S. 1987. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. Can. J. Bot. 65:729-735.

Kiritsakis A. and Dugan L.R. 1985. Studies in photooxidation of olive oil. JAOCS 62(5):892-896.

Knee M., Hatfield S.G.S. and Bramlage W.J. 1987. Response of developing apple fruits to ethylene treatment. J. Exp. Bot. 38(191):972-979.

Knee M., Tsantili E. and Hatfield S.G.S. 1988. Promotion and inhibition by ethylene of chlorophyll degradation in orange fruits. Annals Appl. Biol. 113:129-135.

Koseoglu S.S. and Lusas E.W. 1990a. Ch8 - Hydrogenation of Canola Oil. in Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology. Fereidoon Shahidi (editor). Van Nostrand Reinhold, New York. pg. 126-137.

Koseoglu S.S. and Lusas E.W. 1990b. Recent advances in canola oil hydrogenations. JAOCS 67(1):39-47.

Langmeier M., Ginsberg S. and Matile P. 1993. Chlorophyll breakdown in senescent leaves: demonstration of Mg-dechelatase activity. Physiol. Plant 89:347-353.

Larsson R. and Gottfridsson K. 1974. Changes of chlorophyll content in spring rapeseed in relation to moisture content and temperature of the seed after harvest. Proceedings of the 4th International Rapeseed Congress, Giessen, Germany. pg. 261-266.

Levadoux W.L., Kalmokoff M.L., Pickard M.D. and GrootWassink J.W.D. 1987. Pigment removal from canola oil using chlorophyllase. JAOCS 64(1): 139-144.

Looney N.E. and Patterson M.E. 1967. Chlorophyllase activity in apples and bananas during the climacteric phase. Nature 214:1245-1246.

Luthy B., Martinoia E., Matile P. and Thomas H. 1984. Thylakoid-associated "chlorophyll oxidase": distinction from lipoxygenase. Z. Planzen. 113S:423-434.

MacKinney G. 1940. Criteria for purity of chlorophyll preparations. J. Biol. Chem. 132:91-109.

Mag T.K. 1983. Canola oil processing in Canada. JAOCS 60(2):380-384.

Mag T.K. 1990a. Bleaching - Theory and Practice. World Conference Proceedings - Edible Fats and Oils Processing: Basic Principles and Modern Practices. AOCS, Champaign, IL, USA. pg.107-116.

Mag T.K. 1990b. Ch.15 - Further Processing of Canola and Rapeseed Oils. in Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology. Fereidoon Shahidi (editor). Van Nostrand Reinhold, New York. pg. 251-276.

Manitoba Agriculture. 1991. 1991 Field Crop Variety Recommendations for Manitoba. Manitoba Agriculture Publications, Winnipeg, Manitoba.

Mattoo A.K. and Aharoni N. 1988. Ch. 8 - Ethylene and Plant Senescence. in Senescence and Aging in Plants. L.D. Nooden and A.C. Leopold (editors). Academic Press Inc., Toronto. pg. 241-280.

McGregor D.I. 1991. Influence of environment and genotype on rapeseed/ canola seed chlorophyll content. Proceedings of GCIRC 1991 Congress, Saskatoon, Saskatchewan. pp. 1743-1748.

Medlicott A.P., Sigrist J.M.M, Reynolds S.B. and Thompson A.K. 1987. Effects of ethylene and acetylene on mango fruit ripening. Annals Appl. Biol. 111:439-444.

Minguez-Mosquera M.I., Gandul-Rojas B., Garrido-Fernandez J. and Gallardo-Guerrero L. 1990. Pigments present in virgin olive oil. JAOCS 67(3):192-196.

Morrison M.J., McVetty P.B.E. and Shaykewich C.F. 1989. The determination and verification of a baseline temperature for the growth of Westar summer rape. Can. J. Plant Sci. 69:455-464.

Niewiadomski H., Bratkowska I. and Mossakowska E. 1965. Content of chlorophylls and carotenes in rapeseed oil. JAOCS 42(8):731-734.

Norton G. and Harris J.F. 1975. Compositional changes in developing rape seed (Brassica napus L.). Planta 123:163-174.

Oilseeds Sector Profile, International Markets Bureau, Markets and Industry Services Branch, Agriculture Canada, Ottawa, January 1994.

Panford J.A., Williams P.C. and deMan J.M. 1988. Analysis of oilseeds for protein, oil, fiber and moisture by near-infrared reflectance spectroscopy. JAOCS 65(10):1627-1634.

Patterson H.B.W. 1989. Handling and Storage of Oilseeds, Oils, Fats and Meal. Elsevier Applied Science, New York. pg. 1-86, 128-140.

Pennington F.C., Strain H.H., Svec W.A. and Katz J.J. 1964. Preparation and properties of pyrochlorophyll a, methyl pyrochlorophyll a, pyropheophytin a and methyl pheophorbide a derived from chlorophyll by decarbomethoxylation. J. Amer. Chem. Soc. 86:1418-1426.

Prior E.M., Vadke V.S. and Sosulski F.W. 1991. Effect of heat treatments on canola press oils. I. Non-triglyceride components. JAOCS 68(6): 401-406.

Purvis, Albert C. 1980. Sequence of chloroplast degreening in calmondin fruit as influenced by ethylene and $AgNO_3$. Plant Phys. 66:624-627.

Richardson L.L. 1978. Use of bleaching clays in processing edible oils. JAOCS 55(11):777-780.

Sadhu M.K. and Chattopadhyay G. 1989. Effect of a post-harvest fruit dip in ethephon on the ripening of litchi fruits. J. Hort Sci. 64(2): 239-242.

Salunkhe D.K. and Desai B.B. 1986. Rapeseed and Mustard. Postharvest Biotechnology of Oilseeds. CRC Press Inc., Boca Raton, FL, USA. pp. 71-92.

Schoch S., Rudiger W., Luthy B. and Matile P. 1985. ^{13}-Hydroxychlorophyll a, the first product of the reaction of chlorophyll-oxidase. J. Plant Physiol. 115:85-89.

Segers J.C. 1983. Pretreatment of edible oils for physical refining. JAOCS 60(2):262-264.

Shimokawa K. 1981. Purification of ethylene-enhanced chlorophyllase from Citrus ushiu fruits. Agric. Biol. Chem. 45(10):2357-2359.

Shimokawa K., Sakanoshita A. and Horiba K. 1978. Ethylene-induced changes of chloroplast structure in Satsuma mandarin (Citrus unshiu Marc.). Plant Cell Phys. 19(2):229-236.

Simpson K.L., Lee T-C., Rodriguez D.B. and Chichester C.O. 1976. Ch. 17 - Metabolism in Senescent and Stored Tissues. in The Chemistry and Biochemistry of Plant Pigments. 2nd ed. vl. T.W. Goodwin (editor). Academic Press, New York. pg.780-797.

Singh R.D. and Chuaqui C.A. 1991. Chlorophyll removal from canola oil (two parts). 2. Development of a continuous process to remove chlorophylls from canola oil. Research on canola seed, oil and meal. 9th Project Report. Canola Council of Canada, 1991.

Statistics Canada. Field Crop Reporting Series, No. 8, November 30,1993.

Suzuki, Kiyaji and Nishioka, Akira. 1993. Behavior of chlorophyll derivatives in canola oil processing. JAOCS 70:837-841.

Swern D. (editor). 1982. Bailey's Industrial Oil and Fat Products. v.2. 4th ed. John Wiley and Sons, Toronto. pg. 175-327.

Tautorus C.L., Low M.H. and Dyck J. 1993. Chemical aspects of chlorophyll breakdown products and their relevance to canola oil stability. Tenth Project Report - Research on Canola Seed, Oil and Meal. Canola Council of Canada, Winnipeg, MB, Canada. pg.194-213.

Teasdale B.F. and Mag T.K. 1983. Ch. 9 - The Commercial Processing of Low and High Erucic Acid Rapeseed Oils. in Kramer J.K.G., Sauer F.D. and Pigden W.J. (eds). High and Low Erucic Acid Rapeseed Oils. Production, Usage, Chemistry and Toxilogical Evaluation. Academic Press, Toronto. pp. 197-229.

Thomas A. 1982. Desired quality attributes in winter and summer rapeseed. JAOCS 59(1):1-6.

Tkachuk R. and Kuzina F.D. 1982. Chlorophyll analysis of whole rapeseed kernels by near infrared reflectance. Can. J. Plant Sci. 62:875-884.

Tkachuk R., Mellish V.J., Daun J.K. and Macri L.J. 1988. Determination of chlorophyll in ground rapeseed using a modified near infrared reflectance spectrophotometer. JAOCS 65(3):381-385.

Unger E.H. 1990. Ch.14 - Commercial processing of canola and rapeseed: crushing and oil extraction. in Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology. Fereidoon Shahidi (editor). Van Nostrand Reinhold, New York. pg. 235-249.

Usuki R., Endo Y., Suzuki T. and Kaneda T. 1983. Prooxidant activities of chlorophylls and related compounds on autoxidation of edible oils. Proceedings of 16th ISF Congress, Budapest.

Usuki R., Endo Y. and Kaneda T. 1984a. Prooxidant activities of chlorophylls and pheophytins on the photooxidation of edible oils. Agric. Biol. Chem. 48(4):991-994.

Usuki R., Suzuki T., Endo Y. and Kaneda T. 1984b. Residual amounts of chlorophylls and pheophytins in refined edible oils. JAOCS 61(4): 785-787.

Wasielewski M.R. and Svec W.A. 1980. Synthesis of covalently linked dimeric derivatives of chlorophyll a, pyrochlorophyll a, chlorophyll b and bacteriochlorophyll a. J. Org. Chem. 45:1969-1974.

Ward K. 1990. The Effect of Genotype, Environment and Agronomic Practices on the Chlorophyll Level in Harvested Canola Seed. M.Sc. Thesis - University of Manitoba, Winnipeg.

Ward K., Scarth R., Daun J.K. and McVetty P.B.E. 1992a. Effects of genotype and environment on seed chlorophyll degradation during ripening in four cultivars of oilseed rape (Brassica napus). Can. J. Plant Sci. 72:643-649.

Ward K., Scarth R., Daun J.K. and McVetty P.B.E. 1992b. The effect of freezing on the analysis of chlorophyll content of canola seed (Brassica napus L.). JAOCS 69(10):1039-1040.

Warner K., Frankel E.N. and Mounts T.L. 1989. Flavour and oxidative stability of soybean, sunflower and low erucic acid rapeseed oils. JAOCS 66(4):558-564.

Weaver R.J., Adel-Gawad H.A. and Martin G.C. 1972. Translocation and persistence of 1,2-^{14}C-(2-chloroethyl)-phosphonic acid (Ethephon) in Thompson seedless grapes. Physiol. Plant 26:13-16.

Wilde R.C. 1971. Practical applications of (2-chloroethyl)-phosphonic acid in agricultural production. HortScience 6(4):364-367.

Wilson J.R., Nutting M.-D. and Bailey G.F. 1962. Use of tetracyanoethylene for removal of visual carotenoid spectra from solution of pheophytins. Anal. Chem. 34:1331-1332.

6.0 APPENDIX

Table A1: Growth Stage Key for *Brassica napus* and *B. rapa*

Growth Stage	Description of Plant
0	Preemergence
1	Seedling
2	Rosette
2.1	First true leaf expanded
2.2	Second true leaf expanded
2.3	Third true leaf expanded (add 0.1 for each additional leaf)
3	Bud
3.1	Inflorescence visible at centre of rosette
3.2	Inflorescence raised above level of rosette
3.3	Lower buds yellowing
4	Flowering
4.1	First flower open
4.2	Many flowers opened, lower pods elongating
4.3	Lower pods starting to fill
4.4	Flowering complete, seeds enlarging in lower pods
5	Ripening
5.1	Seeds in lower pods full size, translucent
5.2	Seeds in lower pods green
5.3	Seeds in lower pods green-brown mottled
5.4	Seeds in lower pods brown
5.5	Seeds in all pods brown, plant senescent

(Harper and Berkenkamp,1975)

Table A2: Daily Weather Conditions Recorded During the 1991 Field Study

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
05/13	27.1	14.9	21.2	0.5	520.9	-
05/14	32.3	14.0	22.7	0.0	553.2	-
05/15	19.7	15.5	17.0	4.3	81.9	-
05/16	23.1	12.4	17.6	0.0	582.8	-
05/17	21.1	7.6	14.6	0.0	609.9	-
05/18	22.7	4.8	14.6	0.0	624.9	-
05/19	25.1	4.5	16.4	0.0	605.5	-
05/20	25.6	15.6	20.1	0.0	348.2	-
05/21	27.0	16.5	22.6	0.0	339.9	-
05/22	30.6	20.1	24.2	0.8	414.1	-
05/23	25.6	14.7	20.4	0.0	356.6	-
05/24	22.3	11.8	16.9	0.0	587.3	-
05/25	27.6	11.1	19.2	0.0	484.3	-
05/26	29.8	14.5	20.7	0.0	465.1	-
05/27	23.4	13.5	18.3	0.0	555.5	-
05/28	25.8	9.1	17.9	0.0	500.0	-
05/29	26.8	13.7	20.2	0.0	558.3	-
05/30	30.2	12.2	21.0	0.0	462.4	-
05/31	25.6	15.5	18.8	20.6	217.0	-
06/01	21.7	16.0	18.3	0.8	344.8	-
06/02	22.1	15.2	18.3	0.0	304.6	-
06/03	27.5	14.8	21.2	0.0	486.3	-
06/04	26.1	15.2	20.3	0.0	513.9	-
06/05	28.0	10.6	20.2	0.0	611.1	-
06/06	24.9	14.6	19.6	3.0	168.3	-
06/07	28.1	18.0	22.9	8.1	414.7	-
06/08	28.7	16.5	21.6	10.2	386.4	-

Table A2: Daily Weather Conditions Recorded During the 1991 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
06/09	23.6	15.9	19.5	0.5	309.5	-
06/10	26.6	11.9	18.6	0.3	558.2	-
06/11	24.6	13.9	19.4	0.0	624.5	-
06/12	31.7	15.2	22.1	0.0	466.4	-
06/13	24.0	14.3	18.5	45.5	197.1	-
06/14	26.5	16.1	21.7	0.0	567.7	-
06/15	24.3	16.8	20.4	0.5	509.9	-
06/16	28.8	14.0	21.6	0.0	651.1	-
06/17	28.2	17.2	23.5	0.0	620.2	-
06/18	23.8	13.3	18.9	0.0	545.4	-
06/19	26.5	10.8	19.6	0.0	623.6	-
06/20	22.4	15.0	18.3	0.0	456.7	-
06/21	24.1	12.1	18.5	0.0	635.7	-
06/22	25.9	9.5	19.0	0.0	648.5	-
06/23	24.7	11.7	18.1	0.0	420.1	-
06/24	29.4	16.4	22.1	0.0	411.4	-
06/25	25.3	18.1	21.0	46.0	249.4	-
06/26	27.3	20.3	23.5	0.8	605.2	-
06/27	21.1	15.3	18.8	0.0	433.8	-
06/28	19.9	12.0	16.5	0.0	178.7	-
06/29	25.1	16.2	20.0	0.0	287.6	-
06/30	19.1	16.2	17.4	10.9	51.8	-
07/01	23.9	16.0	19.2	9.7	289.0	-
07/02	20.5	17.0	18.2	10.4	142.9	-
07/03	20.4	17.9	16.7	1.8	170.6	-
07/04	25.9	15.5	20.0	0.0	477.0	-
07/05	24.1	17.2	19.8	0.8	211.6	-

Table A2: Daily Weather Conditions Recorded During the 1991 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
07/06	26.2	14.6	20.5	0.0	528.5	-
07/07	21.8	13.8	18.0	0.0	361.7	-
07/08	24.8	11.0	18.5	0.0	632.2	-
07/09	26.2	15.6	20.3	7.1	512.2	-
07/10	28.9	13.1	21.9	0.0	567.7	-
07/11	21.5	18.0	19.8	40.4	73.9	-
07/12	22.4	17.5	19.5	13.2	264.2	-
07/13	25.6	15.1	20.3	0.0	559.3	-
07/14	29.4	13.9	22.4	0.0	570.4	-
07/15	33.8	20.6	26.5	0.0	521.6	-
07/16	33.0	21.7	27.1	0.0	493.7	-
07/17	31.2	20.6	25.2	2.3	308.1	-
07/18	27.5	18.8	23.0	19.6	370.8	-
07/19	28.7	15.1	22.3	0.0	579.2	-
07/20	27.9	16.9	22.1	3.8	517.1	-
07/21	29.3	13.9	22.6	0.5	518.9	-
07/22	24.9	15.9	21.0	0.0	519.8	-
07/23	26.9	13.1	19.7	0.0	540.2	-
07/24	21.6	14.1	17.5	0.5	477.3	12.50
07/25	24.2	11.7	18.1	0.0	472.9	25.56
07/26	26.4	10.3	19.3	0.0	520.4	39.89
07/27	27.7	11.1	20.3	0.0	574.6	55.14
07/28	28.7	12.1	21.3	0.0	558.1	71.44
07/29	26.3	14.8	19.9	0.0	312.8	86.32
07/30	30.4	15.4	21.9	2.3	543.4	103.23
07/31	26.6	12.2	19.8	0.0	571.6	118.04
08/01	24.1	11.3	18.4	0.0	474.2	131.42

Table A2: Daily Weather Conditions Recorded During the 1991 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
08/02	19.9	14.6	16.7	2.0	176.0	143.12
08/03	24.1	13.5	18.4	2.3	401.4	156.47
08/04	27.0	11.3	19.0	0.0	472.1	170.46
08/05	24.0	12.4	18.2	0.0	204.1	183.62
08/06	22.4	15.5	18.1	8.4	161.7	196.74
08/07	27.7	14.3	21.3	0.0	551.1	213.04
08/08	31.0	12.4	21.8	0.0	472.8	229.80
08/09	31.4	13.6	22.8	0.0	518.8	247.58
08/10	32.2	14.4	23.7	0.0	513.4	266.32
08/11	33.5	15.1	24.2	0.0	422.1	285.49
08/12	34.6	16.6	25.5	0.0	502.0	306.03
08/13	33.2	16.8	25.2	0.0	475.9	326.27
08/14	33.8	17.1	25.3	0.0	430.7	346.60
08/15	28.3	18.2	23.3	0.0	397.0	364.94
08/16	28.9	15.8	22.3	0.0	399.9	382.23
08/17	22.5	13.2	18.9	0.0	399.6	396.12
08/18	24.6	9.9	17.5	0.0	487.2	408.60
08/19	29.1	12.8	20.9	0.0	471.9	424.52
08/20	32.5	17.5	24.6	0.0	416.5	444.14
08/21	26.6	15.9	21.9	0.0	481.9	461.03
08/22	25.0	11.4	17.6	0.0	232.4	473.60
08/23	25.4	11.7	18.5	0.0	478.8	487.05
08/24	32.2	13.7	23.6	0.0	381.3	505.67
08/25	34.9	16.6	25.7	0.3	361.6	526.38
08/26	27.6	17.7	21.9	0.0	261.6	543.30
08/27	34.2	16.7	24.4	0.0	358.9	562.71
08/28	37.2	22.3	29.6	0.0	402.5	587.26

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
05/12	12.4	0.9	7.8	0.0	355.6	-
05/13	14.8	-0.8	7.4	0.0	412.1	-
05/14	19.4	7.3	12.7	2.8	420.2	-
05/15	19.6	4.9	12.6	0.0	538.6	-
05/16	12.8	8.1	10.1	4.1	66.3	-
05/17	19.6	3.0	11.7	0.0	575.6	-
05/18	26.2	9.2	18.4	0.0	553.6	-
05/19	35.5	16.3	25.2	0.0	563.5	-
05/20	33.2	17.2	26.5	0.0	561.6	-
05/21	26.4	6.2	19.7	0.8	544.0	-
05/22	7.6	1.9	4.4	2.3	230.0	-
05/23	10.9	0.1	5.3	0.0	573.7	-
05/24	11.3	1.7	6.8	0.0	267.7	-
05/25	18.6	0.4	10.4	0.0	470.5	-
05/26	18.6	6.9	12.7	0.0	618.7	-
05/27	24.2	3.4	15.2	0.0	623.7	-
05/28	25.0	9.3	18.6	0.0	626.0	-
05/29	30.6	13.0	21.1	0.0	564.0	-
05/30	26.7	13.1	18.4	2.3	406.4	-
05/31	29.0	12.4	20.2	0.0	434.5	-
06/01	29.2	13.7	21.9	0.0	426.1	-
06/02	27.0	17.3	22.4	0.0	367.6	-
06/03	29.9	13.3	20.2	22.6	349.2	-
06/04	22.5	11.7	14.8	23.4	285.2	-
06/05	13.7	6.1	9.8	0.5	170.9	-
06/06	10.9	6.2	8.2	0.0	205.4	-
06/07	14.7	5.2	9.8	0.3	328.4	-

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
06/08	23.6	4.0	15.1	0.0	586.5	-
06/09	26.7	12.6	19.7	0.0	470.7	-
06/10	31.6	14.3	23.6	0.0	587.7	-
06/11	34.2	15.5	24.7	0.0	567.0	-
06/12	23.9	14.2	18.5	0.0	324.5	-
06/13	25.5	14.9	19.1	0.0	292.9	-
06/14	21.7	10.5	16.4	0.0	547.0	-
06/15	25.4	9.1	18.3	0.0	570.9	-
06/16	20.3	14.2	16.9	8.4	39.5	-
06/17	25.6	17.1	20.8	24.6	11.1	-
06/18	23.2	9.1	12.1	0.0	158.9	-
06/19	17.7	9.2	13.0	0.0	490.2	-
06/20	22.3	5.7	14.7	0.0	643.0	-
06/21	17.4	9.3	13.4	7.6	149.7	-
06/22	21.3	11.7	15.1	8.1	227.6	-
06/23	19.4	13.4	16.4	1.0	345.0	-
06/24	19.7	12.2	14.9	19.8	297.1	-
06/25	16.7	8.9	13.1	0.0	396.6	-
06/26	22.4	7.4	15.7	0.0	552.1	-
06/27	29.2	13.4	18.6	1.5	382.8	-
06/28	16.9	8.4	14.8	0.0	386.4	-
06/29	20.3	5.3	13.5	0.0	631.5	-
06/30	17.4	7.2	13.2	0.0	341.5	-
07/01	15.2	12.7	13.8	11.7	62.1	-
07/02	16.1	13.5	14.6	9.7	30.1	-
07/03	15.6	12.5	14.0	26.4	103.2	-
07/04	18.9	11.8	14.9	0.0	426.9	-

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
07/05	23.4	9.9	17.2	0.0	529.1	-
07/06	19.8	12.4	16.1	0.8	168.6	-
07/07	27.7	15.1	21.1	0.3	474.6	-
07/08	23.7	16.1	19.8	3.3	366.3	-
07/09	21.4	14.2	17.5	0.0	329.7	-
07/10	21.8	10.4	16.0	0.0	478.2	-
07/11	23.4	9.5	16.3	1.8	489.2	-
07/12	24.8	12.2	18.5	0.0	554.2	-
07/13	27.3	13.5	20.3	0.0	573.3	-
07/14	26.0	15.7	20.7	29.7	442.3	-
07/15	23.7	14.8	19.6	0.0	416.8	-
07/16	24.4	12.6	18.1	0.0	459.7	-
07/17	23.1	12.5	17.3	0.0	411.6	-
07/18	24.2	13.0	18.2	0.0	397.1	-
07/19	19.2	13.5	16.8	10.2	270.6	-
07/20	21.0	10.6	15.6	0.0	499.3	-
07/21	24.5	10.3	17.5	0.0	528.6	-
07/22	25.6	9.3	18.6	0.0	557.6	-
07/23	26.7	11.4	20.3	0.0	541.4	-
07/24	20.3	14.3	17.9	0.0	165.0	-
07/25	24.0	13.9	19.8	6.9	288.1	-
07/26	21.3	11.9	15.4	0.0	431.3	-
07/27	20.7	10.2	15.9	10.9	167.1	-
07/28	21.0	11.2	15.6	0.5	496.2	-
07/29	24.3	9.7	17.1	0.0	565.1	-
07/30	25.7	11.4	18.8	0.0	511.6	-
07/31	27.4	13.8	19.8	0.8	326.3	-

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
08/01	24.1	14.9	19.5	0.0	559.0	-
08/02	22.9	13.4	17.8	0.0	485.1	-
08/03	22.6	10.7	16.0	0.0	386.7	-
08/04	24.9	11.0	18.8	0.0	507.4	-
08/05	25.1	12.0	18.9	0.0	493.6	-
08/06	27.6	13.6	20.7	0.0	414.7	-
08/07	26.8	15.9	21.4	0.0	338.3	-
08/08	32.6	16.8	24.7	1.5	504.2	-
08/09	28.5	15.5	23.2	13.7	404.3	-
08/10	24.3	13.4	18.0	0.0	461.5	-
08/11	16.1	11.2	14.3	4.6	217.1	9.25
08/12	21.5	8.3	14.8	0.0	389.0	19.00
08/13	26.1	10.3	18.2	0.0	508.5	32.18
08/14	28.7	11.2	20.4	0.0	494.1	47.61
08/15	26.6	14.0	20.5	0.0	486.3	63.13
08/16	28.9	15.5	22.0	0.0	443.3	80.09
08/17	24.3	14.1	18.7	9.9	459.9	93.79
08/18	27.8	10.6	19.1	0.0	499.6	107.92
08/19	27.6	11.0	20.2	0.0	376.8	123.12
08/20	25.9	14.0	19.7	5.3	236.5	137.84
08/21	23.6	11.6	16.8	0.0	394.1	149.48
08/22	26.5	13.9	19.4	7.1	28.5	163.83
08/23	17.1	10.8	13.8	7.4	19.8	172.65
08/24	13.1	10.1	11.3	0.3	72.0	178.97
08/25	17.4	8.4	12.8	0.5	413.2	186.61
08/26	21.2	5.0	13.7	0.0	394.0	195.32
08/27	21.4	9.3	15.4	na	na	205.72

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
08/28	24.2	12.1	18.2	na	na	218.92
08/29	16.8	11.4	14.1	30.7	46.6	228.02
08/30	14.7	11.2	13.3	2.8	78.0	236.36
08/31	15.8	9.5	12.2	0.0	152.0	243.59
09/01	19.3	6.1	13.5	0.0	259.0	252.12
09/02	20.9	10.5	16.3	5.6	194.7	263.38
09/03	19.6	5.9	13.2	0.0	349.5	271.54
09/04	23.1	12.3	17.3	0.0	351.2	283.80
09/05	24.3	7.2	14.9	14.2	71.5	293.67
09/06	13.9	5.0	9.2	0.8	119.7	297.82
09/07	13.4	5.4	9.6	0.0	172.4	302.39
09/08	19.1	1.8	10.3	0.0	330.5	307.64
09/09	12.6	9.9	11.0	35.3	18.7	313.66
09/10	17.5	6.4	11.6	0.0	325.2	320.29
09/11	24.1	8.0	14.7	0.0	321.0	330.03
09/12	22.4	7.0	15.5	1.0	235.6	340.50
09/13	22.1	11.0	17.8	3.8	265.2	353.29
09/14	17.2	6.9	11.9	0.0	371.5	360.21
09/15	22.1	6.5	13.0	2.8	182.3	368.18
09/16	13.6	5.3	9.9	0.0	192.0	373.11
09/17	12.6	1.9	7.2	20.3	18.5	375.30
09/18	11.1	2.6	7.6	0.3	301.6	377.92
09/19	19.8	1.5	11.1	0.0	253.0	384.00
09/20	24.5	9.0	16.2	0.0	212.4	395.23
09/21	14.6	3.3	9.9	0.0	126.9	400.08
09/22	12.4	-1.9	5.5	0.0	334.1	400.59
09/23	21.1	4.2	12.8	0.0	301.5	408.35

Table A3: Daily Weather Conditions Recorded During the 1992 Field Study (cont.)

Date (mm/dd)	High (°C)	Low (°C)	Mean (°C)	Precip. (mm)	Solar (cal/cm^2)	GDD
09/24	26.0	14.3	20.0	0.0	243.1	423.37
09/25	22.8	13.3	17.4	0.8	225.7	435.79
09/26	14.4	5.6	10.3	0.0	204.5	441.10
09/27	8.1	0.0	3.5	4.1	9.0	441.10
09/28	12.0	-1.6	5.1	0.0	301.4	441.19
09/29	19.9	1.5	9.8	0.0	257.3	445.98
09/30	25.1	2.8	12.4	0.0	285.6	453.38
10/01	32.4	7.7	18.4	0.0	269.6	466.76
10/02	21.7	8.7	15.3	0.0	276.4	477.08
10/03	25.1	5.1	14.4	0.0	268.3	486.47
10/04	22.7	6.1	13.8	0.0	267.5	495.30
10/05	20.2	7.9	12.8	0.0	198.5	503.08

Table A4: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Westar) from the Early Seeding Date of 1991.

Pigment		Days from Start of Sampling					
		1	6	14	20	27	35
PHO A	Low	1.5	1.4	0.3	0.7	-	-
	High	2.6	2.0	0.9	3.5	-	
METHYL A	Low	0	0.6	0.3	0.2	1.8	0
	High	9.4	7.0	3.2	0.9	4.7	2.1
CHL B	Low	16.0	15.6	9.4	23.6	49.2	0
	High	26.6	29.7	22.5	35.3	63.0	26.3
CHL A	Low	4.8	14.8	12.0	51.4	32.3	73.7
	High	32.8	32.5	40.8	64.5	49.0	100.0
PHY B	Low	6.1	3.3	3.5	0	-	-
	High	10.5	11.5	20.4	7.9	-	-
PHY A	Low	34.4	30.0	29.6	4.5	0	-
	High	55.8	47.4	51.5	14.9	2.2	-
PYRO A	Low	0.9	1.9	2.2	0	-	-
	High	1.6	2.7	2.9	trace	-	-

Table A5: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Westar) from the Late Seeding Date of 1991.

Pigment		Days from Start of Sampling			
		17	22	31	36
PHO A	Low High	1.9 5.2	1.4 4.3	- -	- -
METHYL A	Low High	0.4 13.9	0 2.2	- -	0 1.7
CHL B	Low High	14.0 32.3	28.4 30.7	26.7 45.0	18.0 31.0
CHL A	Low High	8.0 33.5	48.8 56.8	55.0 72.2	63.7 80.9
PHY B	Low High	3.2 13.1	- -	- -	0 trace
PHY A	Low High	26.8 49.5	11.2 17.4	0 1.3	0 5.6
PYRO A	Low High	1.4 2.0	- -	- -	- -

Table A6: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Stellar) from the Early Seeding Date of 1991.

Pigment		Days from Start of Sampling					
		2*	8	14	20	27	35
PHO A	Low High	2.0	1.2 2.0	0.5 1.4	1.5 5.2	0 3.2	- -
METHYL A	Low High	0.8	0.7 7.6	0 5.3	0.2 1.2	0 23.3	0 2.5
CHL B	Low High	32.5	18.1 28.6	12.1 23.5	26.1 32.4	29.4 66.7	23.6 32.0
CHL A	Low High	31.8	18.0 36.6	19.2 46.0	36.9 61.7	33.3 43.7	68.0 72.5
PHY B	Low High	2.0	3.3 11.0	3.7 15.5	- -	- -	- -
PHY A	Low High	29.8	27.5 41.3	25.3 44.0	10.3 23.7	0 5.7	- -
PYRO A	Low High	1.0	2.1 2.7	1.1 3.1	0 0.6	- -	- -

* only one replicate available

Table A7: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Stellar) from the Late Seeding Date of 1991.

Pigment		Days from Start of Sampling			
		17	22	31	36
PHO A	Low	0.3	2.1	0	0
	High	8.6	7.9	5.1	2.5
METHYL A	Low	0.8	0	0	0
	High	43.1	1.6	7.5	1.3
CHL B	Low	8.7	28.6	22.9	22.9
	High	33.8	37.6	24.8	26.6
CHL A	Low	5.4	37.5	35.4	63.9
	High	30.6	53.2	74.7	75.0
PHY B	Low	4.6	0	0	-
	High	12.5	0.4	12.4	-
PHY A	Low	25.5	16.1	2.4	2.0
	High	28.9	18.8	16.3	5.7
PYRO A	Low	1.6	-	-	-
	High	2.2	-	-	-

Table A8: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Delta) from the Early Seeding Date of 1991.

Pigment		Days from Start of Sampling					
		3	9	16	21	29	34*
PHO A	Low	3.2	0.9	2.9	-	-	-
	High	8.7	2.1	3.7	-	-	-
METHYL A	Low	0.2	1.1	-	0	-	0
	High	4.6	3.2	-	0.8	-	trace
CHL B	Low	29.0	20.0	24.0	31.5	-	54.6
	High	34.7	22.2	27.0	65.9	-	57.4
CHL A	Low	11.5	29.3	40.9	34.1	-	42.6
	High	32.0	35.5	47.7	60.7	-	45.5
PHY B	Low	1.3	6.8	0.6	-	-	-
	High	5.7	10.1	3.3	-	-	-
PHY A	Low	22.4	31.0	20.4	0	-	-
	High	44.1	34.1	27.7	12.5	-	-
PYRO A	Low	0.8	2.0	0.6	-	-	-
	High	1.3	2.3	0.9	-	-	-

* averaged over two replicates where pigments were detectable

Table A9: Ranges Observed Over 4 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Delta) from the Late Seeding Date of 1991.

Pigment		Days from Start of Sampling			
		16	21	29	34
PHO A	Low High	2.7 4.4	3.9 6.6	0.5 1.5	- -
METHYL A	Low High	0.4 10.7	0.5 1.9	0 0.4	- -
CHL B	Low High	20.2 30.7	34.4 37.7	19.7 26.6	28.7 57.7
CHL A	Low High	21.2 26.7	30.2 37.4	58.1 70.2	42.3 66.9
PHY B	Low High	5.1 7.3	0 1.2	- -	- -
PHY A	Low High	33.2 35.3	20.1 25.2	8.5 13.9	0 4.4
PYRO A	Low High	1.0 1.5	0.3 1.1	- -	- -

Table A10: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Westar) from the Early Seeding Date of 1992.

Pigment		Days from Start of Sampling					
		1	7	14	22	31	37
PHO A	Low	0	-	-	-	-	-
	High	1.3	-	-	-	-	-
METHYL A	Low	-	-	-	-	-	1.9
	High	-	-	-	-	-	4.8
CHL B	Low	25.3	23.4	15.9	17.1	9.1	21.2
	High	30.4	28.9	28.6	19.0	17.7	27.2
CHL A	Low	60.6	70.1	67.4	81.0	82.4	70.9
	High	68.6	76.6	84.1	82.9	90.9	74.0
PHY A	Low	6.1	trace	0	-	-	-
	High	7.8	2.2	4.0	-	-	-

Table A11: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Westar) from the Late Seeding Date of 1992.

Pigment		Days from Start of Sampling						
		15	21	29	35	42	49	56
PHO A	Low	-	-	0	-	-	-	-
	High	-	-	1.6	-	-	-	-
METHYL A	Low	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-
CHL B	Low	25.1	21.3	26.2	35.7	20.6	14.8	9.4
	High	28.2	26.2	40.5	39.1	25.4	18.7	20.4
CHL A	Low	63.3	70.5	49.8	57.1	74.7	81.3	79.6
	High	69.4	72.0	71.7	61.7	78.1	85.2	90.6
PHY A	Low	5.3	1.8	2.0	2.6	0	-	-
	High	11.4	6.8	8.1	5.2	1.3	-	-

Table A12: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Stellar) from the Early Seeding Date of 1992.

Pigment		Days from Start of Sampling					
		1	7	14	22	31	37
PHO A	Low	0	-	-	-	-	-
	High	1.2	-	-	-	-	-
METHYL A	Low	0	-	-	-	-	0
	High	0.3	-	-	-	-	2.4
CHL B	Low	27.7	26.0	26.1	19.7	20.5	14.7
	High	33.5	27.5	34.7	27.3	30.5	24.3
CHL A	Low	58.6	68.6	60.2	70.7	67.1	74.0
	High	67.8	72.2	70.3	80.3	79.5	82.9
PHY A	Low	4.4	1.9	3.6	1.4	0	-
	High	6.4	4.0	6.5	2.1	2.4	-

Table A13: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Stellar) from the Late Seeding Date of 1992.

Pigment		Days from Start of Sampling						
		15	21	29	35	42	49	56
PHO A	Low	-	-	0	0.4	-	0	-
	High	-	-	2.1	2.1	-	0.4	-
METHYL A	Low	-	-	-	0	-	-	-
	High	-	-	-	0.4	-	-	-
CHL B	Low	27.9	22.7	30.2	36.4	35.4	24.2	20.0
	High	28.5	27.5	40.2	39.2	41.6	28.0	21.9
CHL A	Low	57.9	64.9	47.8	50.3	56.4	69.1	78.1
	High	63.2	72.2	65.3	54.0	61.5	75.4	80.0
PHY A	Low	8.6	5.2	3.1	6.2	2.0	0.4	-
	High	13.6	9.0	9.9	11.8	3.8	2.6	-

Table A14: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Delta) from the Early Seeding Date of 1992.

Pigment		Days from Start of Sampling					
		1	7	14	22	31	37
PHO A	Low	-	-	-	-	-	0
	High	-	-	-	-	-	5.3
METHYL A	Low	-	-	-	-	-	0
	High	-	-	-	-	-	3.3
CHL B	Low	27.3	22.0	20.0	18.5	19.9	0
	High	30.9	25.5	25.7	22.7	21.8	26.0
CHL A	Low	63.3	72.7	71.3	77.3	78.2	68.7
	High	68.3	75.6	79.4	81.5	80.1	100.0
PHY A	Low	4.4	1.9	0.7	-	-	0
	High	6.0	2.6	3.0	-	-	2.4

Table A15: Ranges Observed Over 3 Replicates for the Relative Proportion (%) of Each Chlorophyll Pigment Detected in Ripening Canola Seed (cv. Delta) from the Late Seeding Date of 1992.

Pigment		Days from Start of Sampling						
		15	21	29	35	42	49	56
PHO A	Low	0	-	0	0	-	-	-
	High	0.6	-	2.3	3.8	-	-	-
METHYL A	Low	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-
CHL B	Low	25.6	19.4	20.0	36.4	20.6	18.7	21.4
	High	29.5	24.0	39.3	38.4	30.5	20.5	23.4
CHL A	Low	57.5	67.7	50.8	51.8	67.7	79.5	76.6
	High	68.3	75.9	78.7	60.0	79.4	81.4	79.6
PHY A	Low	6.1	4.1	1.4	3.5	0	-	-
	High	12.4	8.3	7.6	6.0	1.9	-	-

CROP VARIETY ZONE MAP FOR MANITOBA

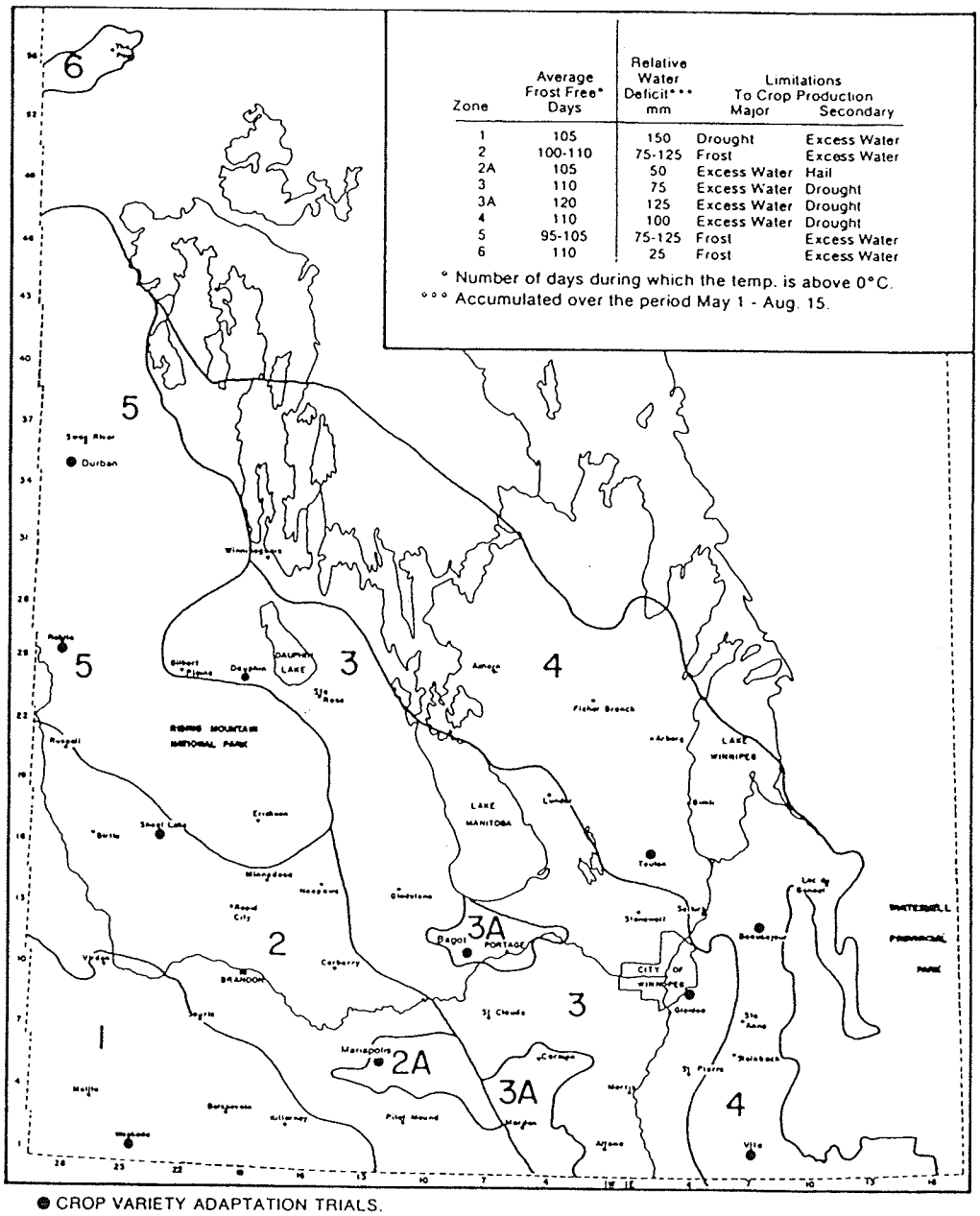


Figure A1: Crop zones for the provincial "Agroman" field trials (Manitoba Agriculture, 1991)

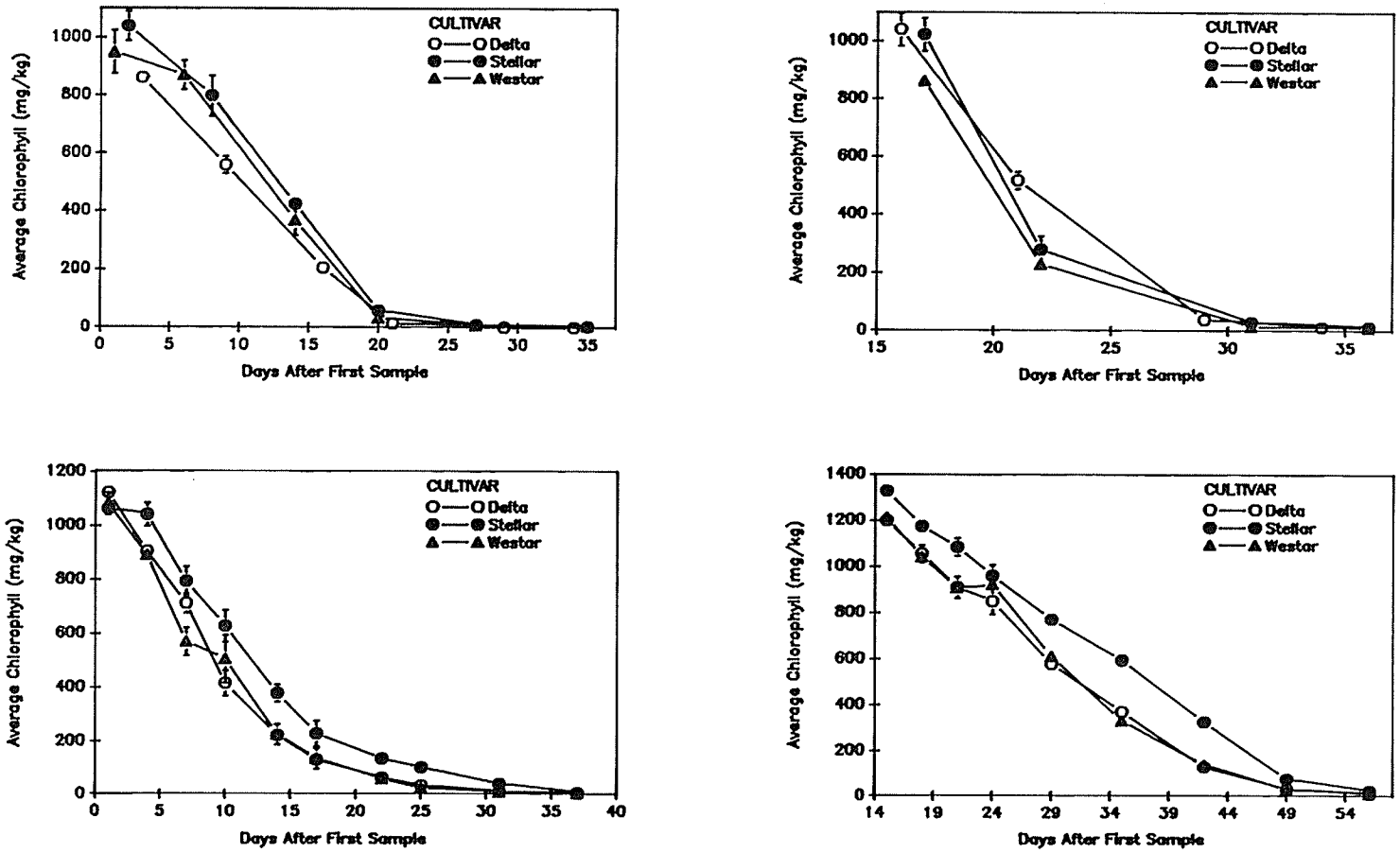


Figure A2: Rates of chlorophyll breakdown (mg kg^{-1}) day^{-1} in three cultivars of ripening B. napus seed (Stellar, Delta and Westar) grown in early and late seeding dates in 1991 and 1992.