The Effect of Genotype, Environment and Agronomic Practices on the Chlorophyll Level in Harvested Canola Seed

A Thesis
Submitted to the Faculty
of
Graduate Studies

by

Kerry A. Ward

In Partial Fulfilment of the
Requirement for the Degree
of
Masters of Science

Department of Plant Science
University of Manitoba

August 1990

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ISBN 0-315-63376-X

Canada

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BY

KERRY A. WARD

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

MASTER OF SCIENCE

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ABSTRACT

Kerry Ward, M.Sc., The University of Manitoba.
<u>The effect of genotype, environment and agronomic practices on the chlorophyll level in harvested canola seed (Brassica sp.)</u>
Major Professor, Dr. R. Scarth.

High levels of chlorophyll in harvested canola seed cause an increase in processing costs, lower returns for producers and poorer quality end products. The effects of genotype, environment and agronomic practices on seed chlorophyll levels were investigated in this study.

When canola seed was frozen for up to one month, either in the pods or after removal, no significant reduction in chlorophyll was observed.

Results from a swathing study indicate that seeds from the side branches contained 1.5 to 2 times as much chlorophyll as seeds from the main stems. Seed that was dried rapidly contained 1.5 to 6 times as much chlorophyll as seed allowed to mature in swaths in the field.

When seeds from each treatment in the swathing study were sudivided according to size, the smallest seeds were found to contain the most chlorophyll. Seed from the treatments with the highest chlorophyll levels also contained the greatest amount of small seed.

Chlorophyll degradation rates were investigated in four cultivars of <u>Brassica napus</u> as the seed ripened. No significant differences in the rate of chlorophyll breakdown

were found between the different cultivars tested. Cultivars that require longer growing seasons to reach maturity were found to initiate seed chlorophyll degradation later in the growing season, increasing the chances that high levels will remain when the seed is harvested. The environment did affect the rate of chlorophyll degradation, as slower breakdown rates did occur in later sown plots. This was assumed to be due to the lower daily mean temperatures which occured later in the growing season.

A number of cultivars of both B. napus and B. campestris grown at sites throughout Manitoba were measured for seed chlorophyll levels at harvest. No significant differences were found among different cultivars of B. campestris but the final chlorophyll levels of B. napus seed were extremely variable, both among triazine tolerant cultivars and those without triazine resistance. The environment also affected the seed chlorophyll level and there was a significant genotype by environment interaction.

Seed samples of a number of cultivars taken from high chlorophyll sites were subdivided according to size and the smallest seeds were found to contain the most chlorophyll. The relationship between the percentage of small seed in any sample and the chlorophyll level was less defined in the "Agroman" material than in the swathing study.

ACKNOWLEDGMENTS

Research is a collaborative effort and any project that is undertaken involves numerous people along the way.

First, I would like to thank my advisor, Dr. Rachael Scarth, for all of her assistance and encouragement. I'd also like to thank Dr. Peter McVetty for his assistance with the statistical analysis of the data, and Dr. Hill and Dr. Bruce MacDonald for serving on my advisory committee.

Thanks to Dr. Jim Daun, Kathy Clear and the rest of the staff of the oilseeds lab at the Canadian Grain Commission for the use of the facilities and for their assistance.

My appreciation also goes out to my summer student Lynn Menard without whom my field work would still be out in the field. Technical assistance from Judith Nugent-Rigby and Lynn Coleman is also appreciated.

Financial support from NSERC and the Department of Plant Science, University of Manitoba is gratefully acknowledged.

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1.0 INTRODUCTION

Canola is the major oilseed crop grown in Canada today. Canola oil accounts for 57% of all deodorized vegetable oil produced in the country and makes up 80% of the salad oil, 50% of the shortening and 40% of the margarine in Canada. Canola production is second only to wheat from a Canadian economic standpoint, and Canada is the world's largest canola exporter.

High levels of chlorophyll in the seed leads to lower grades and a considerable loss of revenue for producers every year. The chlorophyll pigments are extracted into the oil resulting in a green colored product. This green oil is more prone to oxidation, hydrogenation reactions are impaired and processing problems occur when high levels of chlorophyll are present. Limited quantities of chlorophyll can be removed from the oil during refining and processing, but the procedure is costly.

Little is known about what factors contribute to an excess chlorophyll level in the seed. Factors may include the genotype of the plant, agronomic practices such as seeding date, seeding rate and swathing practices and environmental factors including frost, drought and temperature regime.

An investigation of these factors may lead to a clearer understanding of the conditions that contribute to high chlorophyll levels in canola seed at harvest.

LITERATURE REVIEW

2.1 THE CANOLA CROP

2.1.1 History

Oilseed rape has been grown throughout the world for thousands of years. It was cultivated in India as early as 2000 B.C., introduced to Japan from China in 35 B.C. and plantings were undertaken on a large scale in Europe during the 13th century (Canola Council of Canada,1980). Ancient civilizations used rapeseed oil in lamps, soaps, paint and as a cooking oil. During the Second World War rapeseed oil was found to be an ideal lubricant for steam engines since it adheres well to water washed metal. Shortages of oil in Europe as the war progressed led to the initiation of rapeseed production in Canada.

In 1936, Brassica campestris rapeseed was introduced at Shellbrook, Saskatchewan (McLeod,1975). In 1954, the first Canadian Brassica napus rapeseed cultivar, Golden, was licensed for production. This was followed in 1964 by Echo, the earliest licensed cultivar of B. campestris. The first edible oil extracts from rapeseed were produced in Canada in 1956-57 and by 1965 over 400,000 hectares of the Canadian Prairies had been sown to rapeseed (Miller,1988).

2.1.2 Description

The Brassica family includes the turnip, rutabaga, mustards, cabbage, brussel sprouts and rapeseed or canola. Two species of spring canola are grown in Canada-*Brassica campestris*, also known as Polish rapeseed or turnip rape, and *Brassica napus* or Argentine rapeseed. *B. napus* requires 95 to 110 days to reach maturity and the seeds are dark brown to black. *B. campestris* matures 2 to 3 weeks earlier and has seeds which range from yellow to brown to black (Kramer et al,1983). *B. napus* tends to produce slightly higher yields and contain higher levels of oil and protein while *B. campestris* tends to be shorter, more shatter resistant, more tolerant of spring frosts and produces seed with lower chlorophyll and fibre contents (Kramer et al,1983).

Canola is a cool season crop best adapted to the Parkland and Transition zones of the Canadian Prairies on black to grey soil types (Miller,1988).

2.1.3 Development and Breeding

Early rapeseed cultivars contained high levels of eicosanoic and erucic acids in the oil and high levels of glucosinolates in the meal. As rapeseed began to be marketed for human consumption these factors gave cause for concern. High concentrations of erucic acid were found to be associated with fatty deposits in the heart, skeletal muscles and adrenals of rats. The growth of the animals was impaired (Kramer et al,1983). Glucosinolates were a problem in meal fed to poultry and non-ruminant animals. Glucosinolates are hydrolyzed to isothiocyanates and other compounds which interfere with the uptake of iodine by the thyroid gland, contribute to liver disease in poultry, and have a general adverse effect on the growth of the animal (Daun,1987). Lower levels of glucosinolates may also lower the sulfur content of the oil and improve hydrogenation.

In 1968, the first low erucic acid cultivar of B. napus, Oro, was released, followed in 1971 by Span, the first low erucic acid cultivar of B. campestris. In 1974, the first canola cultivar was licensed. Canola is a registered trademark of the Canola Council of Canada reserved for seed of B. napus or B. campestris with oil containing less than 2% erucic acid and meal containing less than 30 micromoles of glucosinolates, including 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate and 2-hydroxy-4-pentenyl glucosinolate, per gram of air dry oil free meal (Canola Council of Canada,1987). The first canola cultivar, Tower, was developed by Dr. Stefansson at the University of Manitoba. This was followed in 1977 by the first B. campestris canola cultivar-Candle. The switch from rapeseed to canola cultivars in Canada was rapid, rising from only 2% of total production in 1974 to 99% by 1984 (Canola Council of Canada,1987).

In 1984, the first triazine tolerant canola cultivar-OAC Triton-was released, followed shortly by Tribute. Recently a cultivar of canola containing low levels of linolenic acid for improved oil stability (Stellar) was registered from the University of Manitoba, and a high erucic acid rapeseed cultivar containing more than 50% erucic acid with low levels of glucosinolates (Hero) was developed to supply industrial oil to the American market (Daun,1987). These cultivars with unusual fatty acid compositions cannot be visually distinguished from standard canola lines so must be handled and marketed separately.

2.1.4 Production

Canola production in Canada is second only to wheat in terms of economic importance. Canola is now grown on 15% of all cultivated land in Canada with the cultivars Westar (*B. napus*) and Tobin (*B. campestris*) predominating throughout the 1980's. Canola oil accounts for 57% of all deodorized vegetable oils produced in Canada including 80% of the salad oil, 50% of Canadian shortening and 40% of the margarine (Miller,1988). In addition, the meal is used for animal feed and occasionally fertilizer, and high erucic acid rapeseed oil has industrial uses.

The major world producers of rapeseed/canola are China, Canada, EEC and India (Canola Council of Canada,1987). Canada is the world's leading exporter of canola with canola exports generating approximately $800 million annually (Canola Council of Canada,1990). Markets include Japan, which purchases half our canola exports, EEC, Mexico, India and recently the USA. In 1985, LEAR oil was given GRAS status in the United States which opened up the market for canola oil in the US. Currently 50% of Canada's canola crop is used domestically and the other half exported (Canola Council of Canada,1987).

2.1.5 Quality

Canola seed contains approximately 40% oil on a dry weight basis and the meal contains 38-40% high quality protein (Downey and Robbelen,1989). Canola yields a high quality oil which is light, stable, colorless, odorless, does not smoke upon heating and drains well from food. It contains no cholesterol, low levels of saturated fatty acids, a high level of oleic acid and intermediate levels of linoleic and linolenic acid. From a health standpoint, this composition is desirable due to the evidence that monounsaturates may lower serum cholesterol and reduce the risk of coronary heart disease (Miller,1988). Linoleic acid has also been implicated in the reduction of serum cholesterol levels. It is an essential fatty acid which should make up 1 to 2% of total calorie intake (Mead et al,1986). Linolenic acid is also an essential fatty acid which cannot be synthesized in the body. It should be taken in as 0.5 to 1% of total calories.

Linolenic acid does, however, tend to decrease the stability of the oil since polyunsaturates are susceptible to peroxidation. High levels of linolenic acid were found to increase the hydrogenation time (Daun,1987). Both linoleic and linolenic acids in canola oil tend to be found primarily at the sn-2 positions of the triglycerides which improves their resistance to oxidation (Mag,1983).

When fed to rats as the primary source of dietary fat, canola oil showed a hypocholesterolemic effect (Rapeseed Association of Canada,1980). Canola oil has been found to be 96.5 % digestible and contains high levels of both alpha-tocopherol and vitamin E (Canola Council of Canada,1987). The fatty acid composition of the oil is monitored each year at the processing plants. The quality and fatty acid composition of the seed are known to depend upon the species, variety, growing area and environmental conditions (Campbell,1984).

2.1.6 Grading

Canola seed must meet rigid quality standards since there are few markets for lower grade seed. Grading standards are set down by the Canadian Grain Commission and are implemented at the grain elevators. Grades are based on the inclusion of foreign material, heated seeds, green seeds, maturity, soundness and overall color (Rapeseed Association of Canada,1980). There are three grades for canola seed-Canada No. 1, Canada No. 2, Canada No. 3, as well as a Sample category for lower quality seed. Top grade canola must contain less than 3% damaged seeds including less than 2% distinctly green seeds and less than 0.1% heated. No. 2 seed is allowed 10% damaged seeds with 6% distinctly green and 0.5% heated, while No. 3 grade allows for 20% damaged seeds including 2% heated (Miller,1988). Prices are set by the Winnipeg Commodity Exchange and are applied to the amount of clean seed of each grade.

The primary degrading factor of canola is green seed. Oil and protein content are not presently included as grading factors. The top quality seed tends to contain the highest level of oil, the lowest amount of chlorophylls and pheophytins, low levels of free fatty acids and low levels of non-hydratable phospholipids. The No. 2 seed, however, yields the meal with the highest protein content (Campbell,1984). When seed is marketed, *B. napus* and *B.*

campestris seed of varying quality is blended to achieve the optimal qualities in the oil.

The loss of income from lower quality seed can be significant. In 1987 discounts were $15/tonne or $0.34/bushel for No. 2 seed (Dean,1987). The price of No. 3 seed was reduced by $70-$95/tonne which was often insufficient to meet production costs. In 1987 in Manitoba, 43% of the canola crop was downgraded to No. 2 and 10% to No. 3 (Dean,1987). It was estimated that 76% of the 1988 crop in Western Canada would grade as No. 1, 20% as No. 2 and 4% as No. 3 or below (Tipples,1988).

2.1.7 Processing

Most processing plants in Western Canada now process only canola since different extraction and processing techniques are required with different oilseeds. During processing, the seed is first fanned and sieved to remove foreign material. The seeds are then flaked or rolled to rupture the seed coat and oil storing cells and cooked to rupture any remaining intact cells and to inactivate myrosinase which hydrolyzes glucosinolates to anti-nutritional compounds. The oil is extracted using a prepress extraction technique followed by solvent extraction. This is followed by refining, degumming and bleaching to remove color compounds, mainly chlorophyll (Mag,1983).

2.1.8 Bleaching

Bleaching can remove chlorophyll levels up to 20 ppm. Levels of 20-30 ppm require additional refining while levels above 50 ppm are unacceptable for edible products (Canola Council of Canada,1987). Bleaching involves the adsorption of the pigments onto acid activated clay. 0.05 to 2% clay is generally used, depending on the initial chlorophyll content of the oil. The process is carried out in a vacuum at 100° to 125°C for 15 to 30 minutes. The higher the initial chlorophyll level the more clay is required and the greater the cost (Canola Council of Canada,1987). A small amount of chlorophyll is also removed during alkali-refining and the refining step increases the efficiency of the bleaching treatment (Mag,1983). Acid activated clays are able to remove chlorophyll by destabilizing the pigments so they are adsorbed to the clay. After bleaching, the processed oil should contain not more than 0.1 ppm chlorophyll (Mag,1983).

Deodorization or hydrogenation follows the bleaching process, both of which are more difficult if chlorophyll levels are high. If the bleaching is inadequate, the remaining derivatives of chlorophyll form green compounds upon hydrogenation, producing a product with unacceptable color. Chlorophyll derivatives are much more difficult to remove following hydrogenation (Mag,1983). With top quality seed, less than 8% of the oil should be lost during refining but up to 21% may be lost if seed is frozen, cracked, damp or green (Rapeseed Association of Canada,1980).

2.2 THE CHLOROPHYLL PROBLEM IN CANOLA

2.2.1 Processing

High levels of chlorophyll in canola oil make refining, bleaching and deodorizing more difficult and costly, shorten the shelf life by promoting rancidity and give an unacceptable green color to the oil and its products (Clear and Daun,1987). Exposure to light in the presence of chlorophyll or pheophytin results in oxidation of the oil (Usuki et al,1984). Pheophytin is derived from chlorophyll during the refining process and is known to be a more powerful prooxidant than the original chlorophyll.

Chlorophyll has been shown to reduce the rate of hydrogenation of canola oil under both selective and non-selective conditions. Under non-selective conditions, the solid fat and trans isomer contents were also reduced. The higher the level of chlorophyll in the oil the slower the rate of hydrogenation since the chlorophyll acts as a catalyst poison. The chlorophyll is believed to physically block the active site of the nickel catalyst, preventing the saturation reaction from proceeding (Abraham and deMan,1986).

2.2.2 Chlorophyll Assessment

The present method used to measure chlorophyll levels in Canadian canola seed involves the visual judgement of at least five strips each containing 100 seeds. These are crushed and the number of distinctly green seeds determined (Canadian Grain Commission,1987). Two problems arise from this method. The first is that the test is subjective in terms of what constitutes "distinctly green". The second is the poor correlation that exists between % green seed and seed chlorophyll, having a correlation coefficient of less than 0.5 (Daun,1982).

There are concerns that the current grading system is unfair to growers who may or may not be paid for the correct grade of seed. It has been shown that a larger proportion of the Canadian crop is exported as No. 1 than is graded as No. 1 at the elevators. This is in part due to the blending which occurs but cannot fully account for the disparity which exists. In 1985, for example, 57% of the Canadian crop was graded as No. 1 at the elevators while 90% was exported as top quality seed (Harris,1988).

Accurate chlorophyll levels are determined by instrument grading. The Canadian General Standards Board recommends up to 25 ppm chlorophyll in top grade crude canola oil or 30 ppm in years when chlorophyll levels are particularly high (Harris,1988). Tests carried out at the Canadian Grain Commission have established that a chlorophyll level of 25 ppm in the oil is equivalent to 22 ppm in the seed, while 30 ppm in the oil adjusts to 24 ppm seed basis (Harris,1988). Thus the cutoff for top grade canola seed is presently considered to be 24 ppm (Clear and Daun,1987).

In 1986, a study was carried out which showed that of a large number of B. napus samples graded No.1 visually, 21% actually had chlorophyll levels above 24 ppm, while 50% of the samples graded as No. 2 contained levels below 24 ppm (Daun,1987). 99% of the B. campestris samples assessed as No. 2 actually had chlorophyll levels below 24 ppm, while 8% of those graded No.1 contained higher levels (Daun,1987).

The disparity between the two species can be explained by background chlorophyll levels. Background chlorophyll is the pigment that contributes to the overall seed color without producing seeds that are distinctly green. B. campestris cultivars tend to have lower background chlorophyll levels than B. napus. Therefore a larger number of distinctly green seeds can be tolerated to yield an oil of the same quality (Harris,1988).

A 1986 Canadian Grain Commission study showed that some samples containing no distinctly green seeds had chlorophyll levels over 24 ppm (Dean,1987). One study involving the cultivar Tobin did find a close correlation between percentage green seed and chlorophyll content, but this appears to be the exception rather than the rule (Cenkowski et al).

In Sweden, chlorophyll content has been included as a grading factor of canola since 1966 (Dahlen,1973). The price is reduced when chlorophyll levels exceed 30 ppm (Larsson and Gottfridsson,1974). Chlorophyll extracted into the oil correlates well with chlorophyll levels in the seed, having a correlation coefficient of 0.95 (Daun,1982). Therefore the Swedish determine chlorophyll content by extraction and spectrophotometric measurements. This method is based on the fact that chlorophyll has characteristic absorption bands in the red portion of the spectrum.

Measurements are made according to the proposed ISO Method (Daun,1989) which involves measuring the absorbance at 670 nm with corrections on either side of the peak. This tends to favour the measurement of chlorophyll a but this is not a problem since chlorophyll b is present at much lower levels. Another concern is the conversion of chlorophyll to pheophytin in the oil which follows a different, but similar, absorption pattern. Heptane/ethanol extracts, however, are known to contain mainly chlorophylls with low levels of pheophytins (Tkachuk et al,1988). Also, the green color of the oil is the primary concern and this is caused solely by the chlorophylls (Yuen and Kelly,1980).

The spectrophotometric technique provides an accurate measure of chlorophyll content but is too time consuming to be used in the Canadian grain handling system at the elevators. Beginning in 1990, the NIR will be introduced to measure chlorophyll. These machines give accurate readings of chlorophyll, protein, oil and moisture contents within minutes (Campbell,1984). Presently the machine costs approximately $12,000 but the price would likely drop with widespread use. NIR measurements have not yet been used for official grading but have been compared to the spectrophotometric method and found to be rapid and accurate (Harris,1988).

The NIR instrument scans ground canola seed at 674 and 696 nm. In performance tests, a number of Dickey-john Instalab 600s were modified to analyze chlorophyll by replacing two standard NIR filters with filters whose central wavelengths were 674 and 696 nm. Extractions were also made and scanned at the same wavelengths using a Cary 17 spectrophotometer. The NIR was calibrated against the spectrophotometer measurements and test samples were run. The NIR readings agreed with those from the spectrophotometer with a correlation coefficient of 0.98 and a standard error of estimate (SSE) of 3.1 ppm

(Tkachuk,1988).

With the introduction of instrument grading, there has been considerable debate over what the cutoff level should be for top quality canola seed. Growers favour an upper limit of 28 ppm to allow them to receive some of the benefits that occur when seed is blended for export. Top grade canola that is exported contains 14 to 28 ppm chlorophyll for an average of 20 ppm. Crushers would like to see a much lower limit, around 18 ppm, since other countries to whom our canola is exported do not have the refining and bleaching facilities to remove chlorophyll from the oil. American refineries, for example, are designed to handle soybean oil which requires no chlorophyll removal (Harris,1988). A cutoff of 24 ppm has been suggested and is presently being used for experimental purposes at the Canadian Grain Commission.

2.2.3 Occurrence

The chlorophyll problem is unique to canola oil (Mag,1983). *B. napus* cultivars generally contain higher levels of chlorophyll than *B. campestris* cultivars. This may be caused either by physiological mechanisms within each species or it may be due to the earlier maturity of *B. campestris*. Westar, Triton and Global have been singled out recently for having higher levels of green seed than other cultivars (Daun,1987) but no evidence exists to support this.

Seed from a wide range of cultivars was analyzed at Saskatoon over a five year period. Seeds from newer cultivars were not found to differ significantly in their chlorophyll content from seeds of older cultivars (Harris,1988). Some concern exists that new cultivars have greater green seed problems but this does not appear to be the case. Chlorophyll levels tend to be higher in the Eastern Prairies due to the greater reliance on B. napus cultivars (Clear and Daun,1987). Somewhat higher chlorophyll levels could be expected overall since the percentage of canola seeded to B. napus cultivars in Canada has increased from 40% in the 1970s to 60% in the 1980's (Daun et al,1983).

The average chlorophyll contents for each species in Canada from 1980-1986 were as follows: (Dean,1987)

Table 1: Chlorophyll levels in canola from 1980-1986

Year	B. napus	B. campestris
1980	26 ppm	12 ppm
1981	23 ppm	7 ppm
1982	25 ppm	11 ppm
1983	16 ppm	9 ppm
1984	18 ppm	6 ppm
1985	18 ppm	9 ppm
1986	12 ppm	7 ppm

Average chlorophyll levels of different cultivars within each species did not vary greatly from one another (Daun,1987).

In Western Canada, the canola crop averaged 20 ppm from 1976-1984 (Clear and Daun,1987). In 1988, the chlorophyll level for top grade canola averaged 12 ppm, up from 10 ppm in 1987 but identical to the 1980-1987 average (Tipples,1988). In 1987, exported canola averaged 18 ppm and in 1988, 20 ppm. Chlorophyll levels fluctuate from year to year but there is no apparent long term increase.

2.3 FACTORS AFFECTING CHLOROPHYLL LEVELS

Environmental factors have a large effect on the chlorophyll content of canola seed. Traits such as uniform seed size, larger seeds, early maturity, improved shatter resistance, determinant flowering and higher levels of chlorophyllase all have potential for reducing the problem (Daun,1987). Species, cultivars, weather conditions, soil type and agronomic practices are all believed to contribute to the green seed problem.

2.3.1 Agronomic Practices

A positive correlation was found between lower chlorophyll levels in the seed and early planting (Daun et al,1983). It is recommended that canola be seeded during the first two weeks of May to allow the crop to mature before a frost. If it is necessary to seed later, *B. campestris* should be planted. High seeding rates also reduce chlorophyll significantly by preventing branching which leads to uneven maturity. A seeding rate of 5.6 to 7.9 kg/ha is recommended (Clear and Daun,1987).

Swathing is recommended to reduce shattering losses, prevent frost damage and speed the ripening process. *B. napus* requires a growing period of approximately 95 days prior to swathing, *B. campestris* 7 to 14 days less. The field should be swathed when it appears brownish green, seeds are at 35 to 40% moisture, firm, and 25 to 35% of the seeds on the main stem have turned from green to brown (Canola Council of Canada,1980). At this stage, the swath should mature within 10 to 15 days (Daun et al,1983).

Swathing at zero color change under cool, moist conditions did not increase the levels of green seed (Dean,1987). This is because under cool, moist conditions ripening proceeds in the swath. However, early swathing increases the chances of rapid dry down in the swath which does lead to elevated chlorophyll levels. Rain on the swath has been found beneficial in this regard. Also, if swathing is carried out too early yield is reduced and smaller seeds are produced, which contain lower levels of oil and protein and higher levels of free fatty acids. In addition, more non-viable and shrivelled seed is formed (Canola Council of Canada,1980). Oil produced from immature seeds also received lower flavor scores (Saskatchewan Canola Growers,1987).

Beginning at 40% moisture, seeds are known to lose water at a rate of 2 to 3% per day so the dry down process proceeds rapidly (Dean,1987). A standing crop of Tobin was swathed and sampled at four day intervals between 52 and 10% moisture. Drying rates were rapid initially and during periods of low relative humidity (Cenkowski et al). The dry weight of the seed and the oil content increased quickly between 52 and 30% moisture. Plots which had been swathed ripened two days earlier than those left standing (Cenkowski et al).

To optimize both crop quality and yield, swathing during the last two weeks of August is recommended. Rolled swaths have a greater tendency to fix chlorophyll so swaths should be rolled only when severe wind losses are likely to occur.

Desiccants are sometimes applied to heavy crops which have a tendency to lodge. The use of desiccants, however, tends to increase shattering losses by promoting rapid dry down. Reglone is presently registered for use on canola. It results in complete desiccation within three days giving the seed little time to mature and increasing shattering. A newer desiccant, Ignite (Hoe-39866), is slower acting so should result in less shattering and give the crop a better chance to mature. Ignite is presently being tested and is not yet registered for use on canola (Harris,1988). The best quality oil comes from crops which have been swathed; at present chemical drying is less satisfactory (Saskatchewan Canola Growers,1987).

In the spring, a well prepared seedbed is recommended. Seeding should be done into moisture and the seedbed packed to conserve water. Adequate weed control must also be carried out. Other conditions including uneven germination, amount and type of fertilizer applied, growing period, row spacing and yield also had minor effects on seed chlorophyll levels. Higher levels of nitrogen fertilizer were associated with slight increases in chlorophyll. Higher yields are inversely correlated with seed chlorophyll levels

(Clear and Daun,1987). Unevenness in seed maturity when the crop is swathed contributes to the green seed problem. Uneven maturity is affected by sporadic germination, usually caused by insufficient moisture at the time of seeding, and by secondary growth (Dean,1987). Proper management throughout the year allows both optimal yield and crop quality.

2.3.2 Storage

It is recommended that canola seed be stored below 10.5% moisture (Canola Council of Canada,1987). No significant reduction of chlorophyll has been observed in storage in Canada since stored seed is too dry for physiological activity.

The degree to which seed chlorophyll decreases after harvest depends upon the moisture content of the seeds. A minimum of 25% moisture is required for chlorophyll breakdown after harvest with higher levels correlated with greater chlorophyll degradation. In seeds containing over 30% moisture, only half the chlorophyll remained after three days under ventilated storage conditions. Seeds with only 15% moisture, however, contained 80 to 90% of their original chlorophyll after three days in storage (Larsson and Gottfridsson,1974).

No close relationship between moisture and chlorophyll content has been detected, although the chlorophyll content of the seed is known to be a superior measure of maturity than the moisture content (Saskatchewan Canola Growers,1987). Moist stored seeds exhibited greater chlorophyll breakdown at storage temperatures above 10°C. The higher the moisture content the greater the temperature effect. Raising the temperature had no effect on the rate of chlorophyll degradation at low moisture levels (Larsson and Gottfridsson,1974).

Storing canola seed at high temperature and moisture content is, however, not practical since these conditions decrease germinability, increase the free fatty acid content of the seed and allow fungi to accumulate and bin heating to occur (Larsson and Gottfridsson,1974). When stored below 10% moisture there is little or no chlorophyll breakdown occurring in the seed. Canola seed was observed for four months under normal storage conditions and no significant changes occurred in either moisture, chlorophyll content or percentage green seed (Dean,1987).

2.3.3 Frost

The occurrence of frost is known to raise chlorophyll levels since freezing stops the maturation process and fixes the chlorophyll in the seed. The effect of frost on the quality of canola was investigated following the early frosts of 1982. Top quality seed averaged 9 ppm chlorophyll while frost damaged seed averaged 75 ppm (Saskatchewan Canola Growers,1987). As a result of this, 38% of the 1982 canola crop graded No. 3 or lower, in comparison to an average of 4% (Daun et al,1985). The downgrading resulted from general visual damage and a severe green seed problem. Half the crop had not reached full maturity at the time of the frost. This resulted in a crop with low oil and protein contents and high levels of chlorophyll and free fatty acids (Daun et al,1985).

Frost damaged seeds are immature. Metabolism stops prior to the deposition of oil and protein and the degradation of chlorophyll. No significant correlation was found between oil and green seed or protein and green seed (Daun et al,1985). This is explained by the fact that oil and protein are laid down prior to chlorophyll degradation. A correlation was found between chlorophyll and the level of free fatty acids. Frost damaged seeds were also found to contain more saturated fatty acids, particularly palmitic acid. A decrease in germination was also observed as green seed levels rose (Daun et al,1985).

A recent study examined the effect of freezing on the degreening of Westar canola embryos (Johnson-Flanagan,1988). Both the temperature and the moisture content of the seed were important. At high moisture levels and a temperature of $-7.5^{\circ}C$, freezing resulted in a disruption of the chloroplasts. At $-5^{\circ}C$, the enzymes involved in pigment catabolism were inhibited. Chlorophyllase, the enzyme which degrades chlorophyll to chlorophyllide and phytol, was not inhibited but chlorophyll degradation was. Decreasing seed moisture was associated with improved frost tolerance. Freezing above 70% moisture also resulted in reduced seed set (Johnson-Flanagan,1988).

2.3.4 Drought

Higher chlorophyll levels can also result from moisture stress which prevents maturation, fixing the chlorophyll level in the seed. Drought conditions may facilitate rapid maturity of a standing crop but hot, dry weather in the swath tends to raise chlorophyll levels (Harris,1988). The chlorophyll content declines rapidly as the moisture content of the seed falls from 60 to 40% (Daun et al,1985). If the seed desiccates too quickly, water required for respiration is unavailable so metabolic processes within the seed stop. Rain falling on a swath reactivates the seed's physiology allowing chlorophyll degradation to proceed (Harris,1988). Therefore if the weather is hot and dry and there is no risk of frost, the crop is best left standing. However, if conditions are cool and moist it is best to swath to facilitate dry down.

2.3.5 Visual Damage

Flea beetle damage has been found to increase the green seed problem, likely by cutting off the water supply to the seed. Mouldy seeds near the damaged areas contain above average levels of chlorophyll (Dean,1987).

Visually damaged seed contains higher chlorophyll levels. In a 1982 study, visually sound seed was found to contain 38 ppm chlorophyll compared to 57 ppm for visually damaged seed (Daun et al,1985). Cracked seed and large seed fragments also contained more chlorophyll than intact seeds. Seeds that had germinated contained higher pigment levels and weed seeds, which may be present as contaminants, also tend to be high in chlorophyll (Saskatchewan Canola Growers,1987).

2.4 SUMMARY

The most common reason for canola seed to be downgraded is an unacceptably high level of chlorophyll. Chlorophyll pigments cause numerous problems at all stages of processing, resulting is reduced product quality and lost revenue. The present method used to estimate chlorophyll levels for grading purposes is often inaccurate. The introduction of instrument grading is expected to eliminate this and ensure that farmers are paid for the correct grade of seed.

A number of factors are suspected to contribute to the chlorophyll level in the seed including genotype, agronomic practices and environmental effects. A better understanding of the factors which contribute to the green seed problem is necessary if the chlorophyll problem is to be eliminated.

MATERIALS AND METHODS

3.1 FREEZING STUDY

Plants of four cultivars - Westar, Regent, Tribute and Global - were grown in the field in a RCB design with six replicates of each cultivar. Three seeding dates ,beginning May 10th, were used, approximately two weeks apart. The recommended seeding rate of 6 kg/ha was used for Regent with adjustments made for equivalent densities of the other cultivars which had different seed weights. Each plot contained six rows 5m long with 0.3 meter row spacing.

The plants were monitored according to the Harper and Berkenkamp growth scale (Harper and Berkenkamp,1974). At growth stage 5.4 when seeds were partially ripe, plants from each of three replicates were sampled in each seeding date. The cultivar Regent was sampled for the first seeding date while Westar was sampled for the second and third.

Approximately thirty plants were sampled from each plot. The pods were removed from the main stems and mixed together to give a uniform sample. This was then divided in half. One half was further subdivided into four treatments - fresh podded, frozen two days podded, frozen one week podded and frozen one month (30-34 days) podded. The pods were then frozen for the appropriate length of time. Seeds were removed from the pods immediately in the other half of the treatments. The seeds were then frozen for the same time intervals as those in the pods - fresh, two days, one week and one month (30-34 days).

Following the freezing treatment, each sample was freeze-dried for approximately 48 hours to remove the moisture. Seeds were then removed from the podded material. All samples were then analyzed for chlorophyll content.

Chlorophyll measurements were carried out by extraction and measuring the absorbance on a Spectronic 1001 spectrophotometer according to the proposed ISO Method ISO/TC 34/SC 2 N385E (Daun,1989). One gram samples of the freeze-dried seed were weighed out and placed in stainless steel test tubes with ball bearings and 30 mL of 3:1 isooctane/ethanol. Samples were shaken for one hour, filtered and 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. Three extractions and measurements were made on each sample and the results averaged.

An analysis of variance was performed on the data in order to determine whether the chlorophyll content of the seed was altered by freezing and whether freezing seeds in the pods altered the chlorophyll levels relative to seeds frozen after removal.

3.2 SWATHING STUDY

There were three objectives of the swathing study:

1) to determine whether rapid drying of the seed contributes to the chlorophyll problem;
2) to determine whether the branches contain significantly higher chlorophyll levels than the main stems; and
3) to determine the effect of late seeding on the chlorophyll content of the seed at harvest.

The swathing study was conducted over two years using four cultivars of *Brassica napus* - Westar, Regent, Tribute and Global. Plots were laid out in a RCB design at the University of Manitoba (The Point) location.

In the first year of the study, two sowings were planted, May 17th and June 7th, with three replicates of each cultivar each time. Eight row plots, three meters long were seeded with 0.3 meter row spacing between rows. The seeding rate was the recommended 6 kg/ha.

Growth stages of the plants were monitored throughout the growing season according to the Harper and Berkenkamp scale (Harper and Berkenkamp,1974). The growth stage key is presented in Table A1 of the Appendix and growth stage data for each cultivar is in Table A2.

Swaths were cut when at least 50% of the plants in the plot had reached growth stage 5.3. Two one meter swaths were cut from each plot and the main stems were separated from the branches. One swath was tied together and left in the field to mature while the second was placed in a burlap bag in a drying room maintained at a minimum temperature of 25°C.

When the swaths were completely dry, the seeds were removed from the pods and analyzed for chlorophyll content by extraction and absorbance on a Spectronic 1001 spectrophotometer, according to the proposed ISO Method ISO/TC 34/SC 2 N385E (Daun,1989). Two gram samples of the seed were weighed out and placed in stainless steel test tubes with 30 mL of 3:1 heptane/ethanol. Samples were shaken for one hour, filtered and the absorption of the extracts measured. Two extractions and measurements were made on each sample and the results averaged.

In the second year of the study, the two seeding dates were May 10th and June 9th. There were six replicates of each cultivar and the plot design was four rows 5 meters long and 0.3 meters apart. The seeding rate was 6 kg/ha for Regent with adjustments made for equivalent densities of the other cultivars which had different seed weights.

The sampling procedure was identical to the first year, except the entire inner two rows of each plot were swathed.

Chlorophyll analysis was carried out in the same manner except isooctane/ethanol was substituted for heptane/ethanol.

In the second year of the study, to further investigate the differences in maturity between seeds harvested at the same time under the same conditions, the seed samples collected for each treatment of each plot in the swathing study were separated according to size.

Two seeding dates were included in the study. Each involved six replicates of four cultivars and each plot was subdivided into main stems and branches which were either swathed in the field or dried quickly in the drying room.

Sieves were used to separate the seed into small, medium and large size classes as follows:

large	5.0-5.5	
medium	4.5-5.0	(0.4th mm)
small	4.0-4.5	

Seeds larger and smaller than the specified size range were discarded along with any foreign material.

Chlorophyll contents were determined for each size class in order to assess the contribution of each size class to seed chlorophyll levels.

Chlorophyll was measured by extraction and absorbance according to the proposed ISO Method (Daun,1989). One measurement was made on each sample with checks of known chlorophyll content included periodically.

The proportion of seed in each size class was also determined.

Statistical analysis was carried out on the U of M mainframe computer using the SAS program (Helwig and Council,1979). Appropriate ANOVAs and means separation tests were run on the data. All graphing was done using Sigmaplot.

3.3 CHLOROPHYLL DEGRADATION RATES IN FOUR CULTIVARS OF *BRASSICA NAPUS*

There were three objectives of the study:

1) to determine whether there is a difference in the rate of chlorophyll breakdown between cultivars;
2) to determine whether there is a difference in the time of chlorophyll degradation between cultivars; and
3) to determine whether there is a difference in either the rate or time of chlorophyll degradation between early and late planting dates.

This study was carried out over two years with early and late seeding dates each year. Planting dates were May 17th and June 7th the first year and May 10th and May 24th the second year. Plot design was identical to that used in the swathing study.

The four cultivars that were included were Westar, which is an early maturing cultivar, Global, which is a late maturing cultivar, Regent, which has been widely grown on the Canadian prairies and Tribute, which is a triazine tolerant cultivar.

Emergence dates (50%) for each plot were recorded and the growth stages of the plants were monitored throughout the growing season. Sampling began when 50% of the plot reached growth stage 5.3. Each sample consisted of taking 5 to 10 plants, removing the main stems and placing them in a plastic bag in a cooler. In the lab, the seeds were removed from the pods, weighed and frozen until analysis. Samples were taken at approximately weekly intervals depending on how quickly plants were ripening. Sampling continued until the plants were completely senescent.

In the second year of the study, each plant was given a color coded tag when it began to flower so the exact date of flowering was known. When sampling was conducted, plants with the same flowering date were chosen, within each cultivar.

Prior to analysis, each sample was freeze-dried for 24 to 48 hours and the moisture content of the harvested seed was determined. Chlorophyll measurement was carried out by extraction and absorption according to the ISO Method as outlined in the swathing study. Two measurements were made on each sample and the results averaged.

The majority of the statistical analysis was carried out on the U of M mainframe computer using the SAS program (Helwig and Council,1979). Graphs of chlorophyll levels versus days after sampling were generated for each seeding date of each year. The data was then transformed to a logarithmic scale to linearize it for easier analysis and interpretation. Regression analysis was performed to determine the slope of each line. Pairwise t-tests were then carried out to determine homogeneity of regression coefficients.

A second set of graphs was generated which plotted the logarithm of the chlorophyll level against growing degree days. Growing degree days are a measure of accumulated heat units. They were calculated by taking the daily mean temperature minus 5°C (five degrees is assumed to be the minimum temperature required for chlorophyll degradation to occur) and summing over the entire sampling period (Morrison et al,1989). Regression analysis was performed and homogeneity of regression coefficients was tested.

3.4 "AGROMAN" TRIALS

Canola seed of both B. napus and B. campestris cultivars was obtained from the "Agroman" Trials in which all registered cultivars are grown at a number of locations throughout the province over seven zones based on the average number of frost free days and soil types. These are outlined in Figure A9 in the Appendix.

In the first year of the study, the following ten locations and fourteen cultivars were available:

<u>Locations</u>

Melita (zone 1)
Waskada (zone 1)
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)

<u>Cultivars</u>

B. napus	*B. campestris*
Regent	Tobin
Westar	Colt
Global	Horizon
Topas	
Stellar	
Alto	
Legend	
Delta	
OAC Triton (triazine tolerant)	
OAC Triumph (triazine tolerant)	
Tribute (triazine tolerant)	

In the second year, the following eleven locations and twenty-two cultivars were available:

Location
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)

Cultivars

B. napus	*B. campestris*
Regent	Tobin
Westar	Colt
Global	Horizon
Topas	ACS Parkland
Stellar	
Alto	
Legend	
Celebra	
Vanguard	
Delta	
Profit	
Hero	
Hyola 40	
OAC Triton (triazine tolerant)	
OAC Triumph (triazine tolerant)	
Tribute (triazine tolerant)	
ACS-N4-TT (triazine tolerant)	
SV 8525953 (triazine tolerant)	

Four replicates of each cultivar were sown at each location each year. In the first year of the study, the data was reduced to three replicates due to numerous missing plots.

After harvest, the plants were hung in jute bags to dry prior to threshing. The seeds were passed through sieves to remove foreign material and chlorophyll measurements were made on each sample using the NIR. Near infrared reflectance spectrophotometers can be modified to analyze chlorophyll by replacing the standard infrared filters with filters whose central wavelengths are 674, 696 and 2100 nm (Tkachuk,1988). Dickey-John Instalab 600 machines were used. The canola seeds were ground in a coffee grinder for thirty seconds, loaded into sample cups, leveled and the reflectance of the sample measured. This reading is then converted into a chlorophyll concentration using a calibration equation.

The six locations showing the highest chlorophyll levels were selected for further study of five cultivars - Westar, Regent, Global, Tribute and Triton. The locations chosen in the first year were Bagot, Mariapolis, Melita, Roblin, Shoal Lake and Waskada. In the second year, The Pas, Melita, Mariapolis, Teulon, Shoal Lake and Beausejour were selected.

The bulk seed was separated into small, medium and large size classes as follows:

large	>5.0	
medium	4.5-5.0	(0.4th mm)
small	<4.5	

The proportion of seed in each size class was determined and chlorophyll was measured by extraction and absorbance with one measurement made on each sample.

Thousand seed weights were also determined for each size class of each cultivar at each location in the first year of the study.

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. Sigmaplot was used to generate graphs.

RESULTS AND DISCUSSION

4.1 FREEZING STUDY

An analysis of variance was run on the data on each individual sowing date. At the 5% level of significance, there were no differences between seeds frozen in the pods and those frozen after removal. There were also no differences in chlorophyll content between fresh seeds and those frozen for two days, one week or one month (Table 2).

The average chlorophyll values for seeds frozen in the pods compared to those separated from the pods prior to freezing are presented in Figure 1 and the average chlorophyll values for each freezing treatment are presented in Figure 2.

No attempt was made to combine the data over sowing dates since different cultivars were sampled each time and each sowing date was not harvested at precisely the same physiological growth stage.

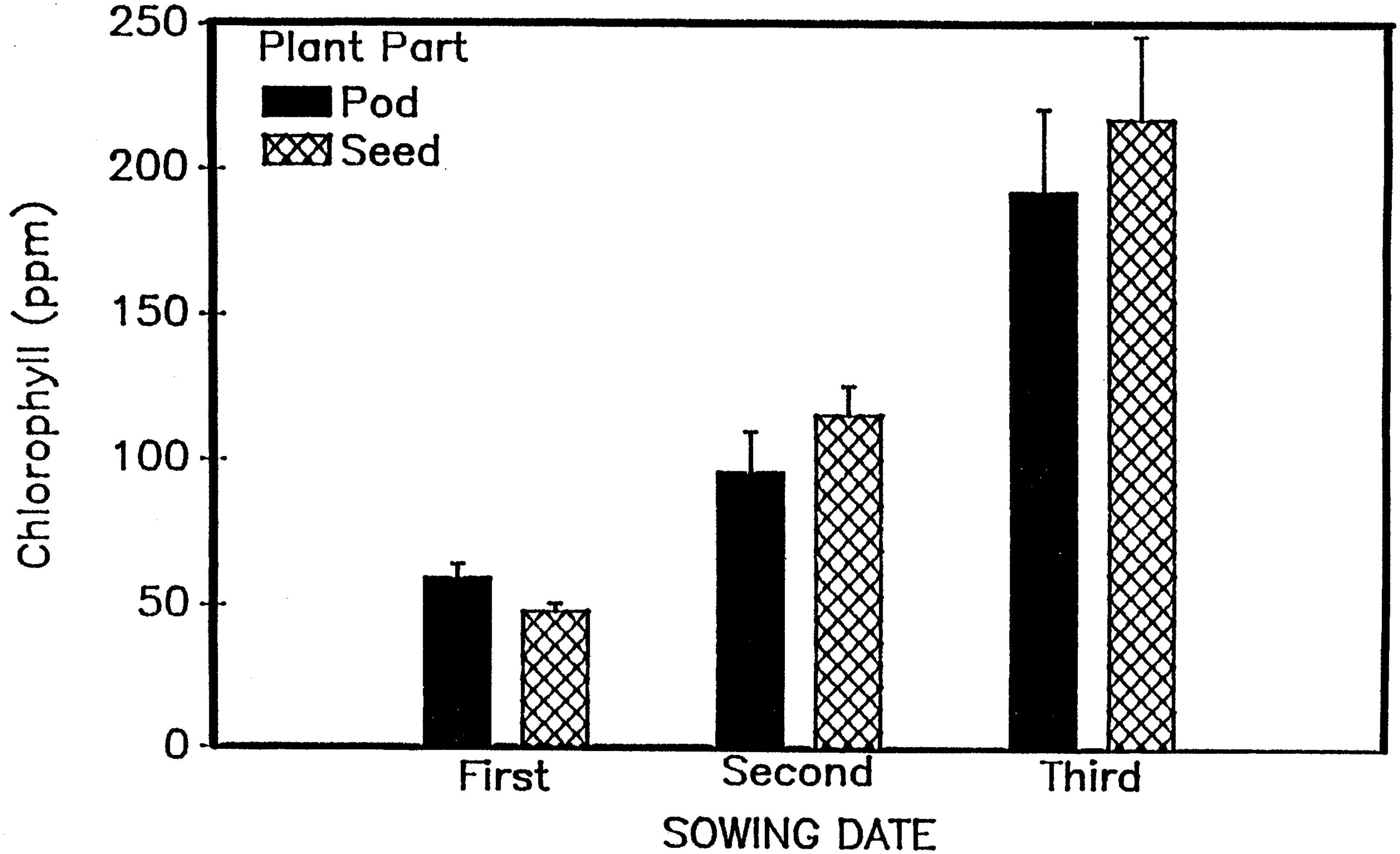

Fig. 1: Effect of freezing canola seeds in the pods or after removal

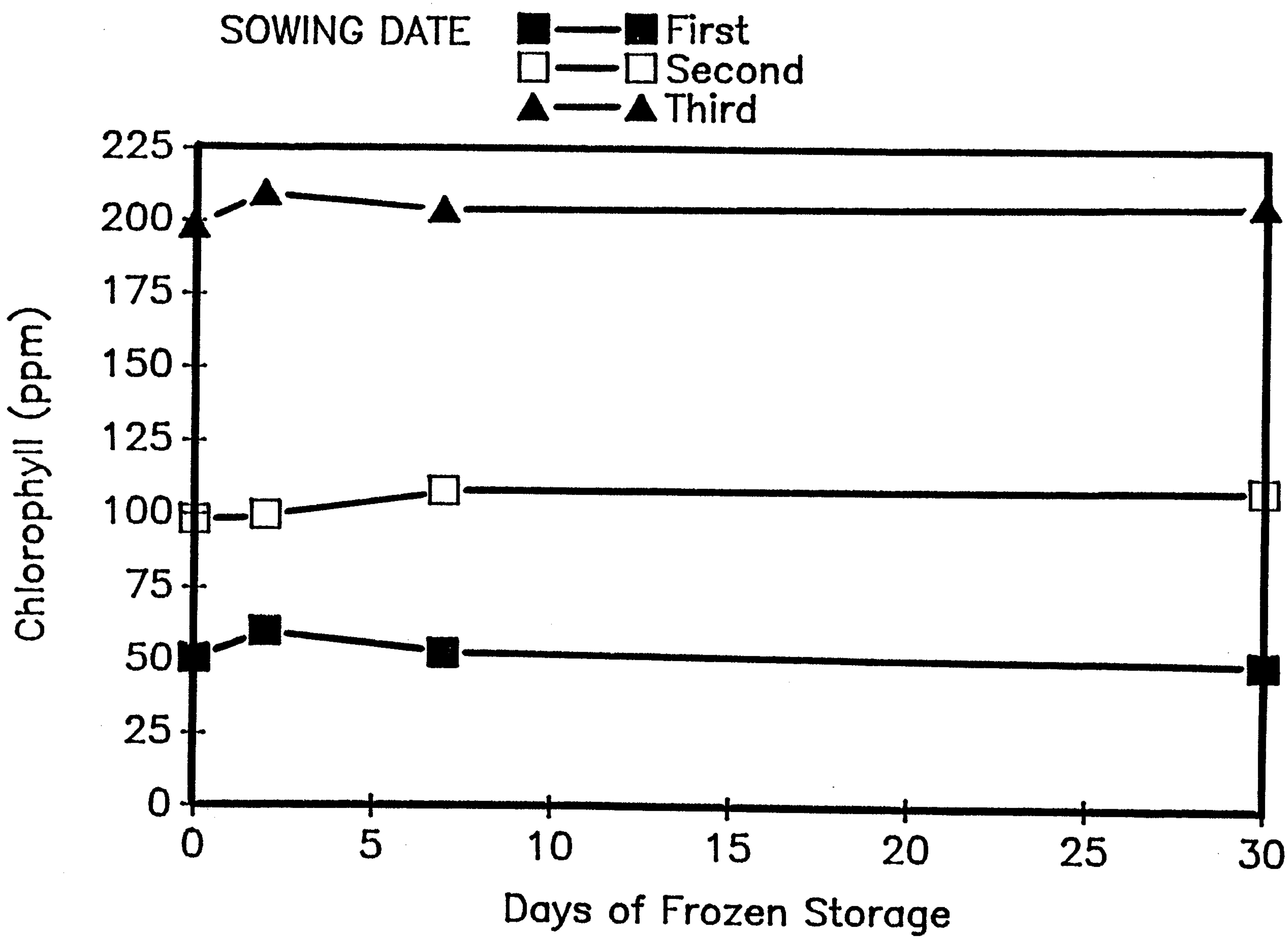

Fig. 2: Effect of frozen storage on the chlorophyll content of canola seed

Table 2: Anova Results for Frozen Canola Seed

SOWING: First
Cultivar: Regent

Source	DF	Anova MS	F-Value	PF>F
Pod vs Seed	1	785.47	3.90	0.0629
Freezing Trts	3	131.28	0.65	0.5911

SOWING: Second
Cultivar: Westar

Source	DF	Anova MS	F-Value	PR>F
Pod vs Seed	1	2332.48	1.15	0.2971
Freezing Trts	3	150.16	0.07	0.9732

SOWING: Third
Cultivar: Westar

Source	DF	Anova MS	F-Value	PR>F
Pod vs Seed	1	3672.90	0.32	0.5776
Freezing Trts	3	112.95	0.01	0.9983

The results of this study indicate that seed for chlorophyll analysis can be frozen and stored in the freezer for one month prior to analysis without a significant change in chlorophyll levels.

There was no significant difference in chlorophyll content when the seeds were removed from the pods prior to freezing compared to being frozen in the pods. The ability to freeze the seed while still in the pods saves considerable time during the sampling period. The seeds can also be removed much more rapidly after the pods have been freeze-dried.

The ability to freeze canola seed prior to measuring the chlorophyll content would save considerable time when sampling from the field and allows chlorophyll measurement to be delayed until field work is complete. Seed samples may be stored for considerable periods of time without concern for chlorophyll degradation.

4.2.1 SWATHING STUDY

ANOVAs were run on each sowing date of each year and the results are presented in Table 3.

Table 3: ANOVA Results from Each Sowing of the Swathing Study (MS=main stems)

1988

Seeding Date: Early

Source	DF	Mean S	F-Value	PR>F
Swath vs Dry	1	99148.94	33.88	0.0001
MS vs Branches	1	12060.75	4.12	0.0490
Replicates	2	986.38	0.34	0.7159

Seeding Date: Late

Source	DF	Mean S	F-Value	PR>F
Swath vs Dry	1	6754.67	6.29	0.0180
MS vs Branches	1	13032.09	12.13	0.0016
Replicates	2	161.70	0.15	0.8609

1989

Seeding Date: Early

Source	DF	Mean S	F-Value	PR>F
Swath vs Dry	1	57433.06	74.81	0.0001
MS vs Branches	1	9961.34	12.97	0.0005
Replicates	5	344.18	0.45	0.8134

Seeding Date: Late

Source	DF	Mean S	F-Value	PR>F
Swath vs Dry	1	355558.73	132.74	0.0001
MS vs Branches	1	54321.14	20.28	0.0001
Replicates	5	1087.72	0.40	0.8441

In both sowings of both years, there was a significant difference between both the two drying treatments and between the main stems and branches at the 5% level of significance.

The 1989 data was then combined over sowing dates and an ANOVA was performed on the combined data (Table 4).

Table 4: ANOVA Results for 1989 Swathing Study Combined Over Sowing Dates (MS=main stems)

Source	DF	Mean S	F-Value	PR>F
Swath vs Dry	1	349397.35	124.14	0.0001
MS vs Branches	1	55403.03	19.68	0.0001
Sowing Dates	1	129755.60	181.62	0.0001

Both drying treatments and plant parts indicated significant differences at the 1% level. Results from the early and late seeding dates were also significantly different at the 1% level of significance.

The 1988 data was not combined over sowings since the late maturing cultivar Global was lost to insect damage in the late sowing. This resulted in an unbalanced design and statistics performed on the combined data would be biased since Global tends to have the highest seed chlorophyll levels of the four cultivars included in the study.

The comparison of the two drying treatments - field drying of the swath compared to a rapid dry down in the drying room - clearly indicated that rapid drying results in elevated seed chlorophyll levels (Figure 3). Chlorophyll levels from the drying treatment ranged from 1.5 to 6 times higher than those from the swaths dried down out in the field.

Rapid dry down of the seed resulted in unacceptably high seed chlorophyll contents (above 24 ppm) in all sowing dates. Because of the rapid desiccation, moisture is unavailable for respiration, so metabolism within the seed stops. The seed remains physiologically immature and the degradation of chlorophyll pigments ceases.

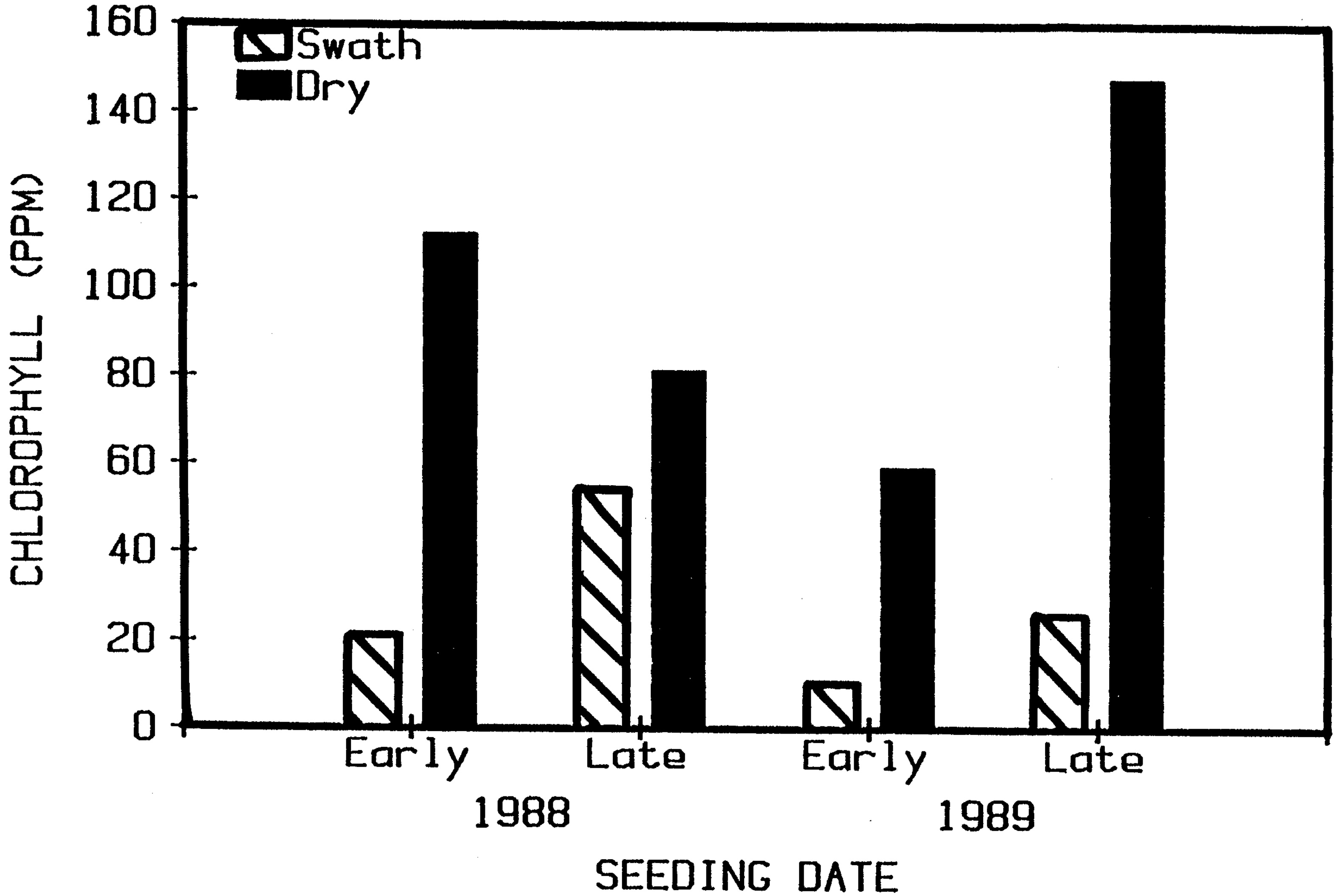

Fig. 3: Seed chlorophyll levels from swathed plants compared to rapid drying

Rapid drying of the swath will occur if swathing is performed during hot dry weather or if chemical desiccants are applied to the crop. Both of these situations have the potential to cause elevated seed chlorophyll levels and should be avoided.

Even under the hot dry weather conditions of 1988 and 1989 (temperature data in Appendix Tables A3 and A4) the swaths which dried down in the field yielded seed with acceptable chlorophyll levels while those dried down more rapidly in the drying room did not. Rapid artificial drying should therefore be avoided if possible.

The second objective of this study was to compare chlorophyll levels of seed harvested from the branches and the main stems to assess the degree to which the side branches contribute to the green seed problem. In all cases, the average chlorophyll content of seeds from the branches of each seeding date of each year contained chlorophyll levels 1.5 to 2 times as high as seed from the main stems (Figure 4).

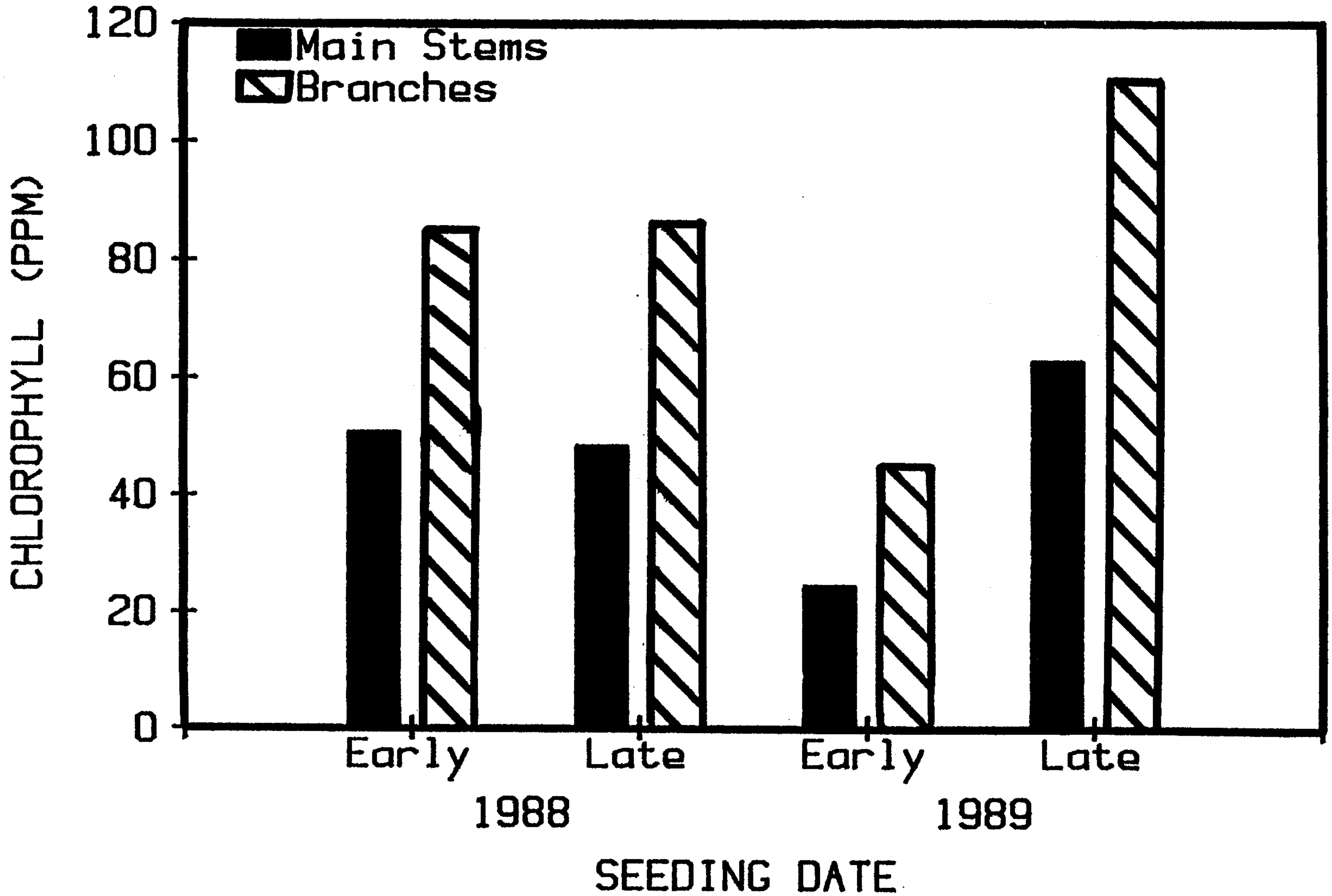

Fig. 4: Comparison of seed chlorophyll levels from main stems and branches

This result can be explained by the indeterminate nature of growth in canola. The terminal bud gives rise to the main stem and axillary buds produce branches, which in turn produce new axillary buds. The oldest pods are located at the base of the main stem and new pods form towards the tips of the branches (Scarisbrick and Daniels,1986). Axillary buds on the branches flower later, set seed later and consequently the seeds ripen later than those on the main stems. Seeds formed on the branches are less mature than seeds on the main stem and can therefore contribute significantly to the green seed problem. The greater the degree of branching, the higher the probability that the seed will not have time to mature prior to harvest, resulting in immature seeds with high chlorophyll contents. Therefore in order to reduce seed chlorophyll content, it is important to minimize branching. This can be achieved by using a sufficiently high seeding rate, which results in greater competition for space by each plant. This modifies the form of the plant with fewer branches being produced.

Early and late seeding dates were included in each year of the study. In the first year of the study, the seeding dates were May 17th and June 7th, a difference of twenty days. In the second year, the seeding dates were twenty-nine days apart - May 10th and June 9th. The 1988 data cannot be validly compared between planting dates since the late maturing cultivar Global was lost to insect damage in the late seeding. From the 1989 data however, the trend towards higher chlorophyll levels with later planting is readily apparent (Table 5). In all cases, the chlorophyll levels from the late seeding date were approximately 2.5 times higher than those from the early seeding date.

Table 5: Average Chlorophyll Levels (ppm) for Two Sowing Dates in the Swathing Study

		1988		1989	
		Early	Late	Early	Late
Main Stems	Swath	19.9	49.0	7.5	17.1
	Dry	81.3	47.3	41.1	108.4
Branches	Swath	22.1	57.9	12.6	34.2
	Dry	142.5	114.5	76.8	186.4

This illustrates the importance of early planting so the crop has adequate time to mature before harvest or a frost. The late seeding date contains higher seed chlorophyll levels because it contains a greater percentage of immature seed in which the chlorophyll did not have a chance to degrade. In addition to creating a chlorophyll problem, immature seeds are also known to contain lower levels of oil and protein and higher levels of free fatty acids (Daun et al,1985).

In summary, the results of this study indicate that seed from the branches contains significantly higher levels of chlorophyll at harvest than seed from the main stems. Rapid drying results in higher seed chlorophyll levels at harvest, as does late seeding. This emphasizes the importance of planting early enough to give the crop adequate time to mature.

4.2.2 SIZED SEED

The average chlorophyll content of the seed in each size class is presented in Tables 6 and 7 and depicted graphically in Figures 5-12.

Table 6: Average Chlorophyll (CHL) Contents (ppm) for Each Size Class in the Early Sowing

(MS=main stems, BR=branches)
(STE=standard error of estimate)

	SMALL		MEDIUM		LARGE	
	CHL	STE	CHL	STE	CHL	STE
Cult:Westar						
MS SWATH	12.4	1.6	5.2	1.0	3.7	0.4
MS DRY	26.9	3.0	15.2	1.9	11.7	1.4
BR SWATH	8.0	0.9	4.2	0.4	4.0	0.5
BR DRY	42.0	5.0	27.0	3.4	23.7	3.1
Cult:Regent						
MS SWATH	7.9	1.2	6.1	0.8	4.3	0.6
MS DRY	41.3	4.3	21.4	2.4	14.3	1.4
BR SWATH	14.6	2.4	10.9	1.5	8.3	1.1
BR DRY	57.7	4.3	48.1	2.9	28.5	1.6
Cult:Tribute						
MS SWATH	18.0	3.0	9.4	0.9	8.2	1.6
MS DRY	62.6	9.8	34.3	5.3	16.5	3.0
BR SWATH	23.6	3.7	13.3	1.2	10.4	1.0
BR DRY	122.6	24.6	84.9	16.2	62.6	3.0
Cult:Global						
MS SWATH	25.0	1.9	19.8	1.6	14.7	1.8
MS DRY	110.6	8.9	79.7	8.8	70.8	8.2
BR SWATH	25.4	2.3	18.3	1.7	17.4	1.7
BR DRY	176.1	18.7	153.1	12.0	134.5	10.9

Table 7: Average Chlorophyll (CHL) Contents (ppm) for Each Size Class in the Late Sowing

(MS=main stems, BR=branches)
(STE=standard error of estimate)

		SMALL		MEDIUM		LARGE	
		CHL	STE	CHL	STE	CHL	STE
Cult:Westar							
MS	SWATH	31.5	1.5	16.9	1.8	11.4	0.4
MS	DRY	274.0	24.0	184.4	24.4	120.1	17.5
BR	SWATH	39.8	2.4	24.1	2.3	18.4	2.0
BR	DRY	359.2	10.5	289.2	19.8	186.5	18.1
Cult:Regent							
MS	SWATH	32.0	2.7	17.7	0.7	11.7	0.5
MS	DRY	112.5	9.4	68.3	3.9	46.4	2.4
BR	SWATH	47.4	3.5	28.3	1.9	16.8	1.5
BR	DRY	142.5	11.2	89.0	8.3	51.6	7.9
Cult:Tribute							
MS	SWATH	33.0	4.8	14.0	1.5	9.1	1.5
MS	DRY	109.4	10.5	59.6	6.8	31.0	7.5
BR	SWATH	57.3	6.3	25.8	3.9	11.7	0.9
BR	DRY	159.1	12.0	100.4	10.7	48.7	5.7
Cult:Global							
MS	SWATH	44.2	2.8	19.1	1.4	10.2	0.3
MS	DRY	182.4	11.2	145.7	9.4	98.3	11.2
BR	SWATH	60.8	1.9	29.2	2.2	18.5	2.4
BR	DRY	213.0	19.3	150.6	10.3	101.2	10.3

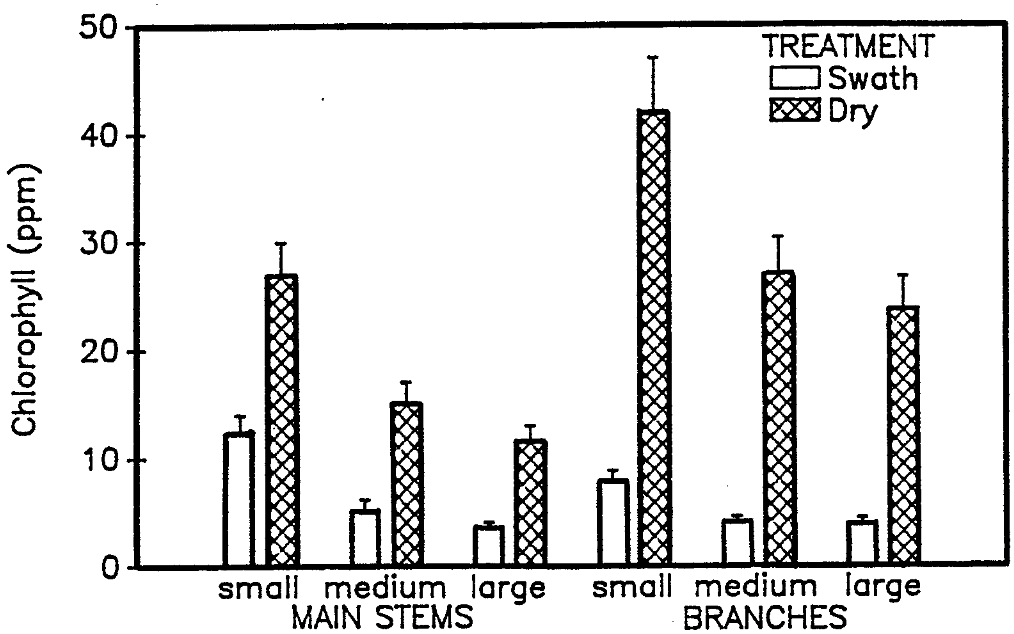

Fig. 5: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Westar Early Sowing

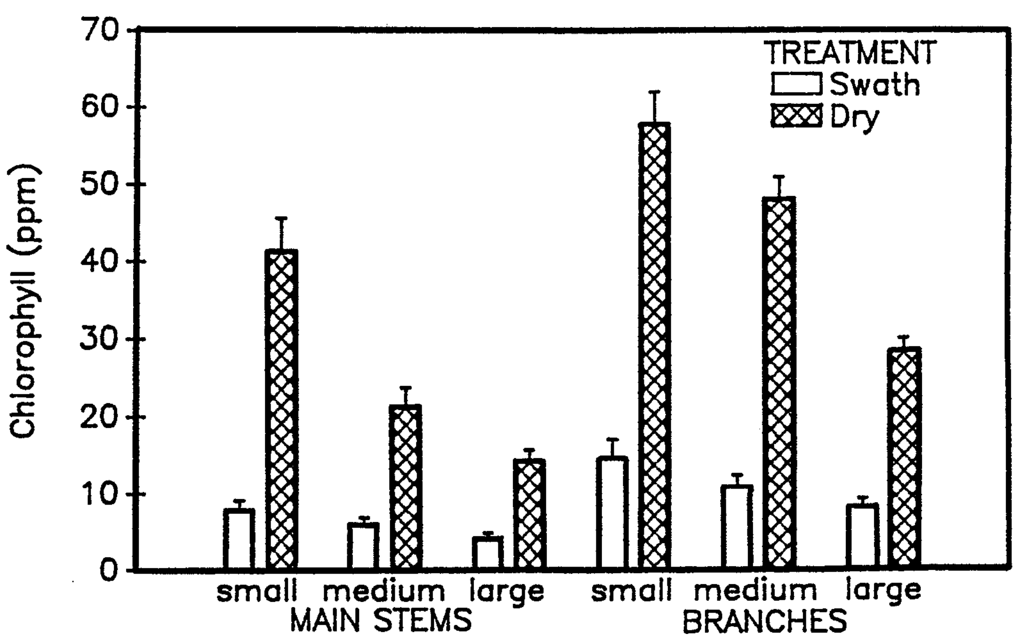

Fig. 6: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Regent Early Sowing

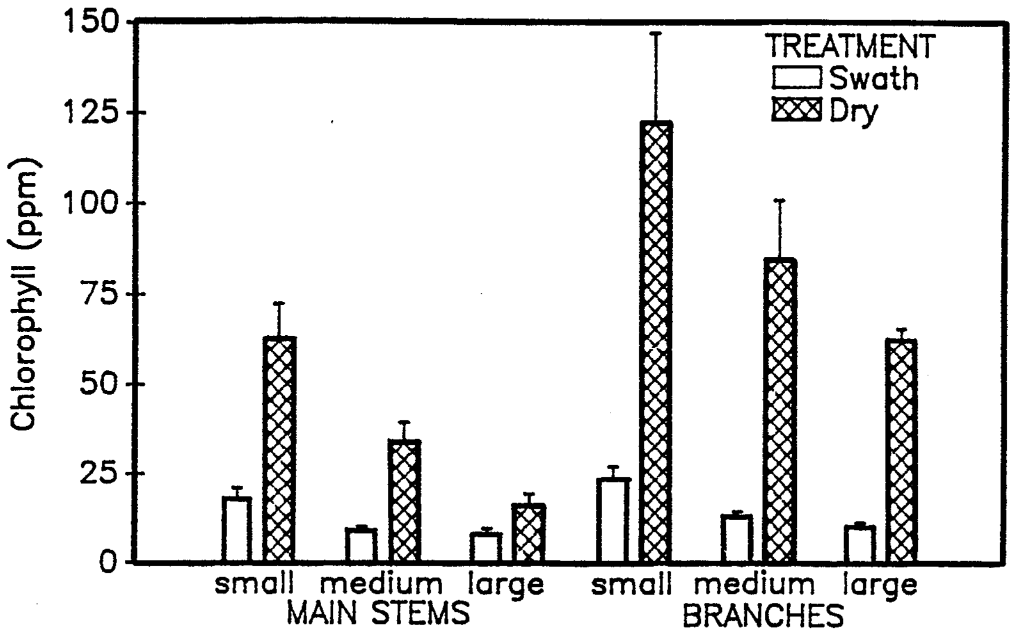

Fig. 7: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Tribute Early Sowing

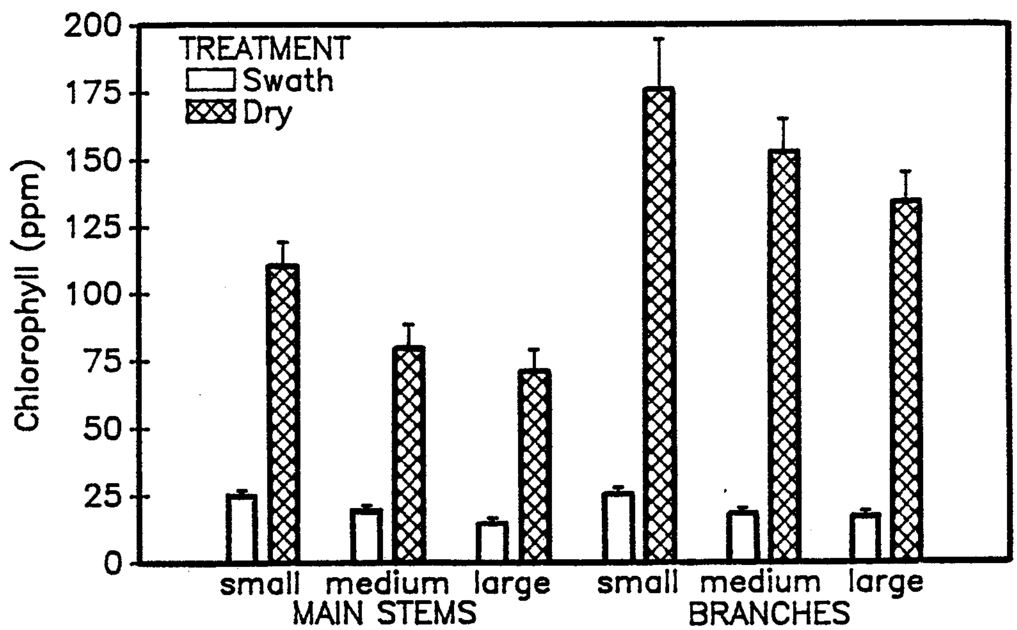

Fig. 8: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Global Early Sowing

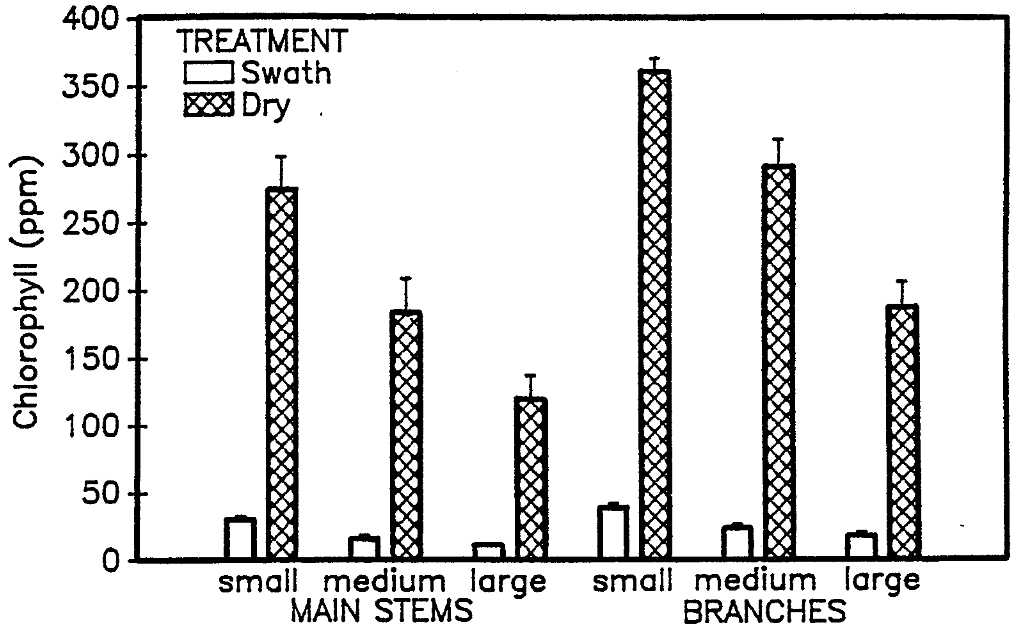

Fig. 9: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Westar Late Sowing

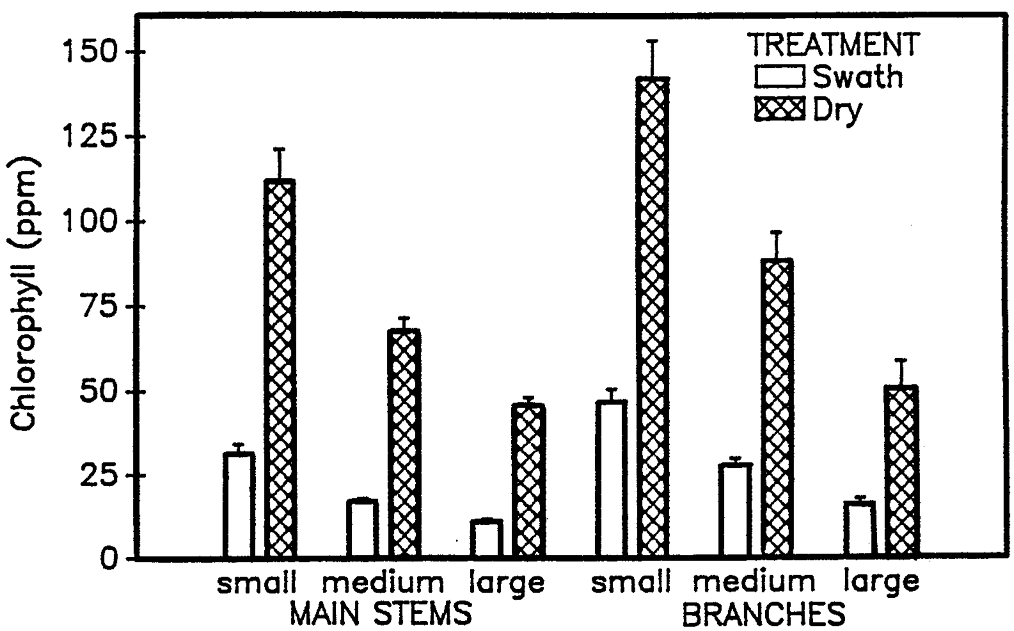

Fig. 10: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Regent Late Sowing

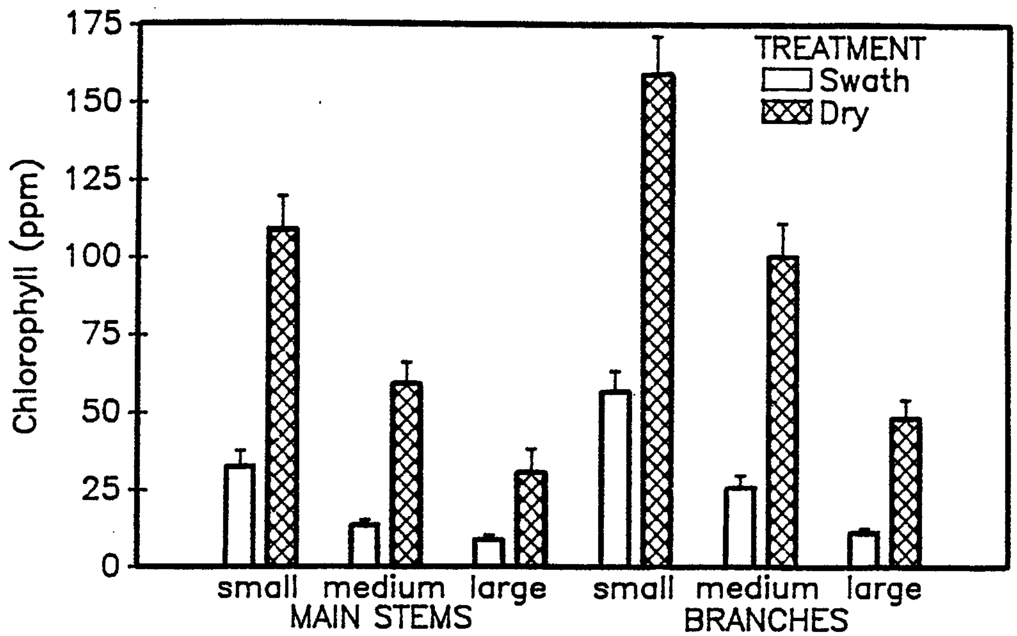

Fig. 11: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Tribute Late Sowing

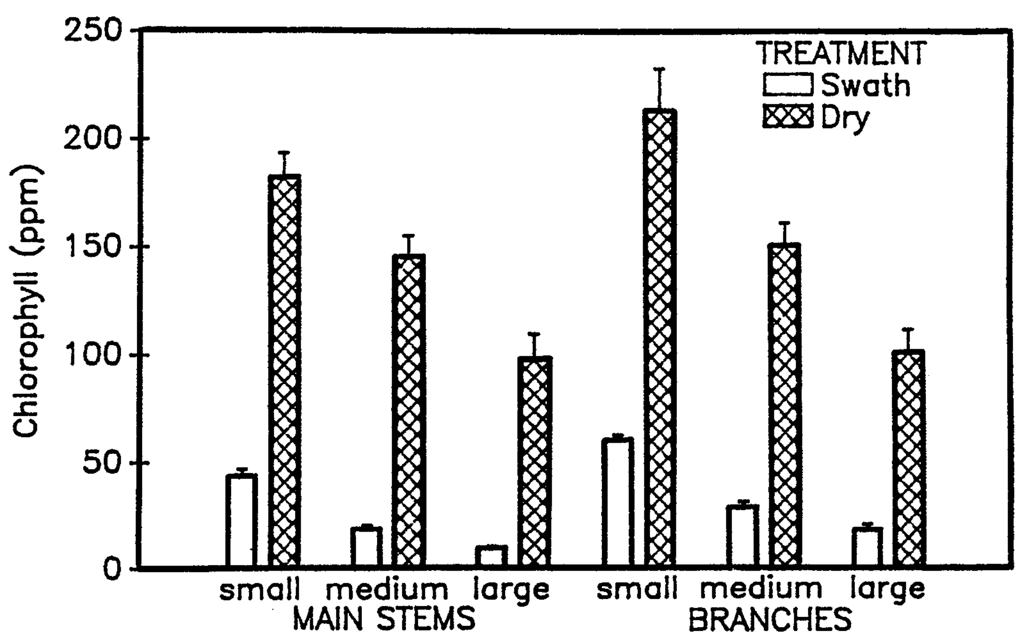

Fig. 12: Comparison of seed chlorophyll levels in small, medium and large seed from the swathing study - Global Late Sowing

It is clear that for all treatments the chlorophyll content is higher in the smaller size class. This can be explained in terms of seed maturity, as the largest seeds are those that form first and have the longest time period over which to mature and ripen, while the smaller seed forms later and is therefore more immature.

This finding highlights the problem of uneven maturity in a crop with an indeterminate flowering habit. To achieve the lowest chlorophyll levels possible, the percentage of small seed should be minimized. This can be achieved by minimizing branching, using a high seeding rate and thorough cleaning of the seed.

The higher levels of chlorophyll in the seeds from the branches compared to those from the main stems and the higher levels from the rapid dry down compared to field swathing are also readily apparent. These results, which have already been discussed for the bulk seed samples, also apply to each size class. The trend toward higher chlorophyll levels in the later sowing is also apparent.

The percentage of seed falling into each size class was also determined for each treatment (Tables 8 and 9).

Table 8: Percentage of Seed Falling Into Each Size Class in Each Treatment of the Swathing Study in the Early Sowing

(MS=main stems, BR=branches)
(STE=standard error of estimate)

		SMALL		MEDIUM		LARGE	
		%	STE	%	STE	%	STE
Cult:Westar							
MS	SWATH	6.67	0.64	44.98	1.97	48.35	2.61
MS	DRY	9.25	1.23	47.68	1.47	43.07	2.45
BR	SWATH	13.43	1.09	52.75	1.06	33.82	1.80
BR	DRY	13.98	0.96	52.33	0.88	33.70	1.53
Cult:Regent							
MS	SWATH	16.95	0.67	52.98	0.79	30.07	1.30
MS	DRY	20.07	1.42	54.88	0.72	25.07	1.42
BR	SWATH	27.00	1.07	55.13	0.64	17.87	0.71
BR	DRY	30.65	1.13	56.02	0.66	13.33	1.14
Cult:Tribute							
MS	SWATH	13.87	1.66	50.88	1.67	35.25	3.11
MS	DRY	17.70	1.51	56.53	1.21	25.75	2.14
BR	SWATH	21.73	1.80	54.35	2.53	20.75	0.91
BR	DRY	25.30	1.76	58.22	0.93	16.48	2.10
Cult:Global							
MS	SWATH	21.02	1.40	47.93	1.37	31.03	2.68
MS	DRY	19.23	1.54	47.20	0.74	33.57	1.74
BR	SWATH	29.52	2.43	49.90	1.12	20.55	1.85
BR	DRY	28.37	1.02	52.57	0.78	19.07	0.73

Table 9: Percentage of Seed Falling Into Each Size Class in Each Treatment of the Swathing Study in the Late Sowing

(MS=main stems, BR=branches)
(STE=standard error of estimate)

		SMALL		MEDIUM		LARGE	
		%	STE	%	STE	%	STE
Cult:Westar							
MS	SWATH	20.00	1.11	52.77	1.34	27.27	2.21
MS	DRY	24.05	1.42	54.17	0.69	21.78	2.02
BR	SWATH	29.56	1.73	53.47	1.13	16.98	2.22
BR	DRY	34.18	1.88	53.67	1.12	12.17	1.71
Cult:Regent							
MS	SWATH	21.52	0.48	53.17	0.74	25.33	1.06
MS	DRY	23.18	1.07	54.10	0.86	22.72	1.62
BR	SWATH	31.07	1.16	57.55	1.42	11.40	0.56
BR	DRY	32.35	1.66	57.42	0.96	10.22	1.12
Cult:Tribute							
MS	SWATH	15.57	0.84	44.08	1.34	40.32	2.11
MS	DRY	18.38	0.76	47.93	1.06	33.70	1.34
BR	SWATH	22.70	1.27	48.67	0.80	28.63	1.85
BR	DRY	23.85	0.67	49.68	0.80	26.48	1.32
Cult:Global							
MS	SWATH	16.22	0.52	48.32	1.34	35.47	1.81
MS	DRY	15.90	0.50	50.22	1.12	33.87	1.61
BR	SWATH	25.95	0.67	53.20	1.06	20.87	1.41
BR	DRY	27.82	0.92	54.37	1.12	17.82	0.87

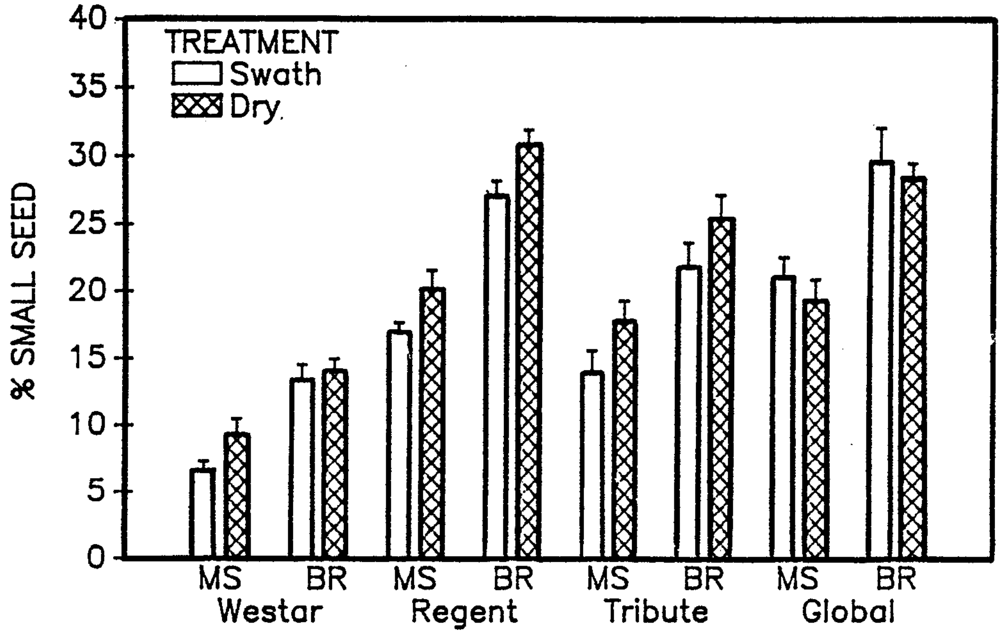

Fig. 13: Percentage small seed in each treatment of the swathing study - Early Sowing
(MS-main stems, BR-branches)

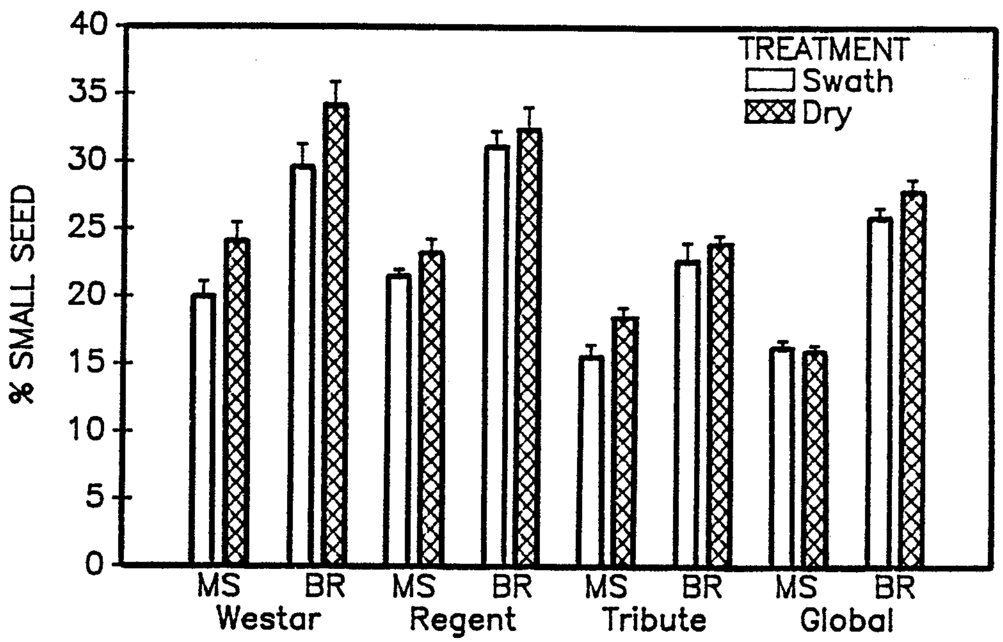

Fig. 14: Percentage small seed in each treatment of the swathing study - Late Sowing
(MS-main stems, BR-branches)

There was a greater percentage of large seed in treatments with lower chlorophyll contents and there was a higher proportion of small seed in the treatments with high chlorophyll contents (Figures 13 and 14). Specifically, the proportion of large seed was greatest in the early seeding date, seed from the main stems and seed dried in swaths in the field. Conversely, the largest proportion of small seed was present in the late seeding date, seed from the branches and seed dried down rapidly in the drying room.

In summary, the conclusions reached for each objective are:

1) rapid drying of the seed does result in elevated seed chlorophyll levels and should be avoided;
2) extensive branching results in the formation of increased quantities of small seeds containing high chlorophyll levels; and
3) late seeding prevents the seed from reaching maturity prior to harvest, therefore high seed chlorophyll levels remain.

4.3 CHLOROPHYLL DEGRADATION RATES IN FOUR CULTIVARS OF *BRASSICA NAPUS*

Each sample was analyzed for its chlorophyll content and moisture level. The correlation between moisture and chlorophyll levels has been described as poor in the literature (Loof,1972). In the first year of the study, the correlation coefficient between moisture and chlorophyll levels was 0.83 while in the second year of the study it was 0.76. While this correlation is fairly good it is not high enough to use the moisture content to predict chlorophyll levels.

The chlorophyll levels for each sample were plotted against the number of days after the start of sampling (Figures A1-A4 in the Appendix).

In order to linearize the date and simplify the analysis it was converted to the logarithmic form to generate graphs of log chlorophyll versus days after sampling for each seeding date of each year. These graphs are presented in the Appendix in Figures A5-A8. A regression analysis was then performed (Tables 10 and 11) and the best straight line fitted to each cultivar (Figures 15-18).

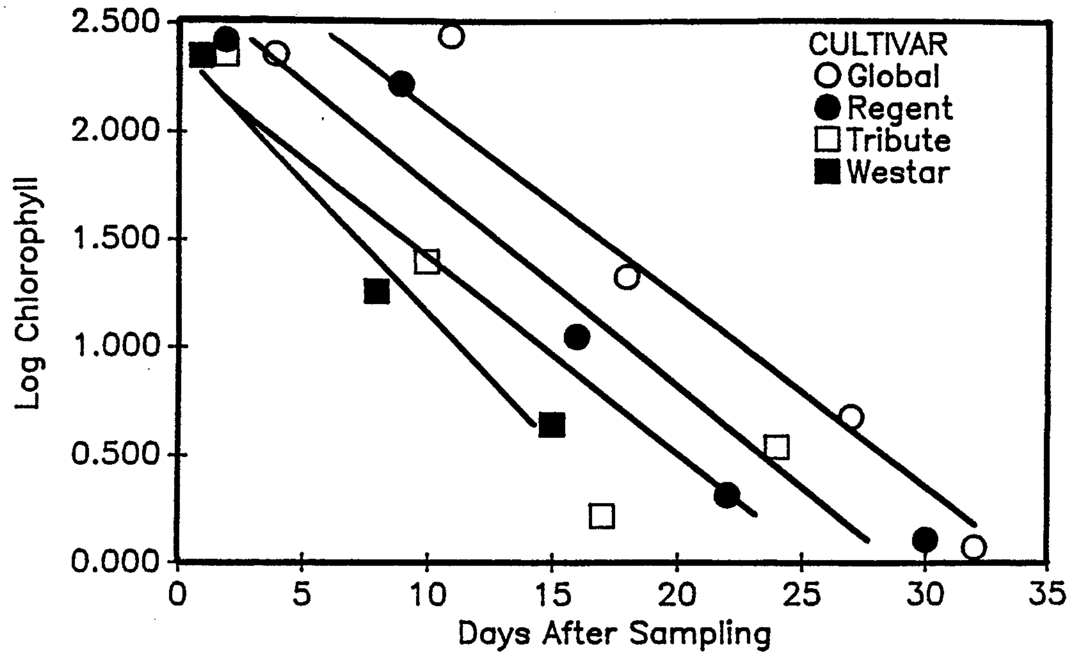

Fig. 15: Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1988. Regression of Logchl vs Time.

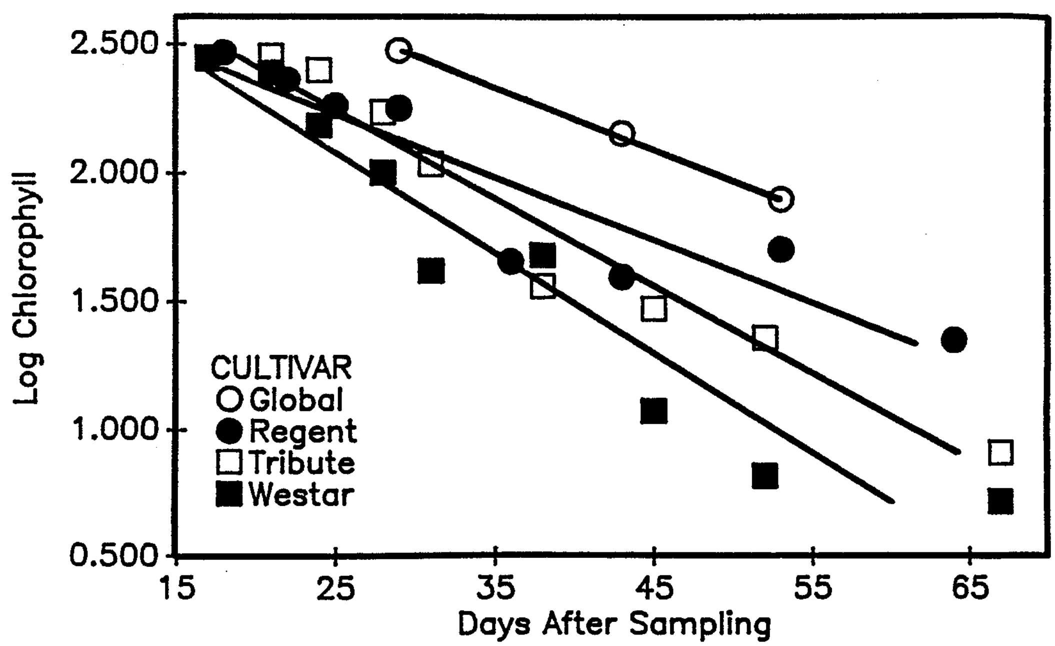

Fig. 16: Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1988. Regression of Logchl vs Time.

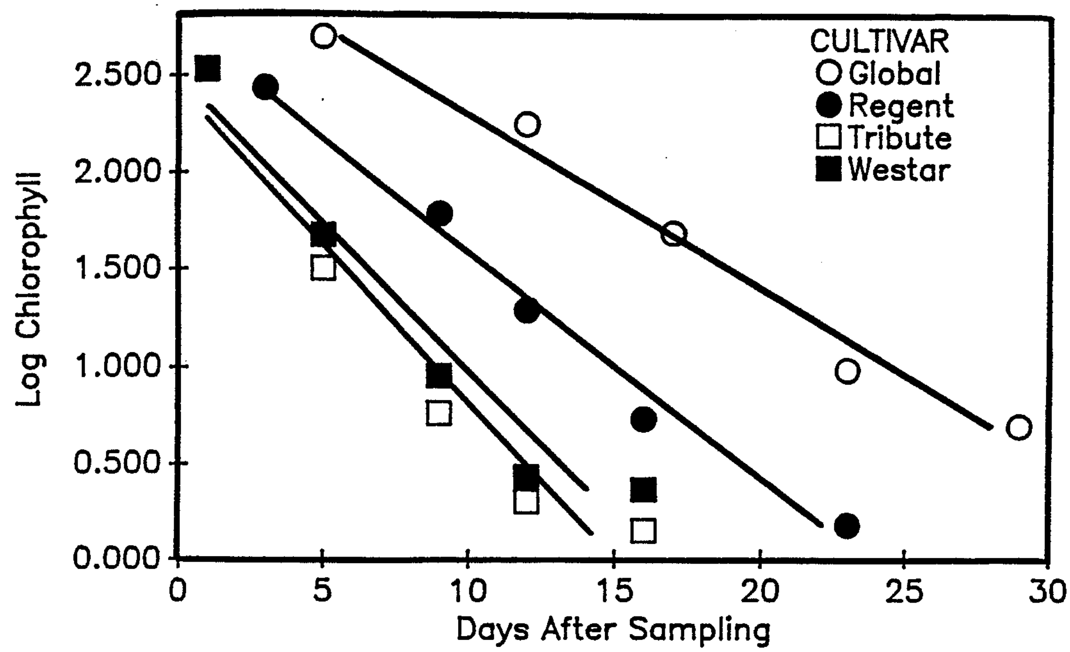

Fig. 17: Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1989. Regression of Logchl vs Time.

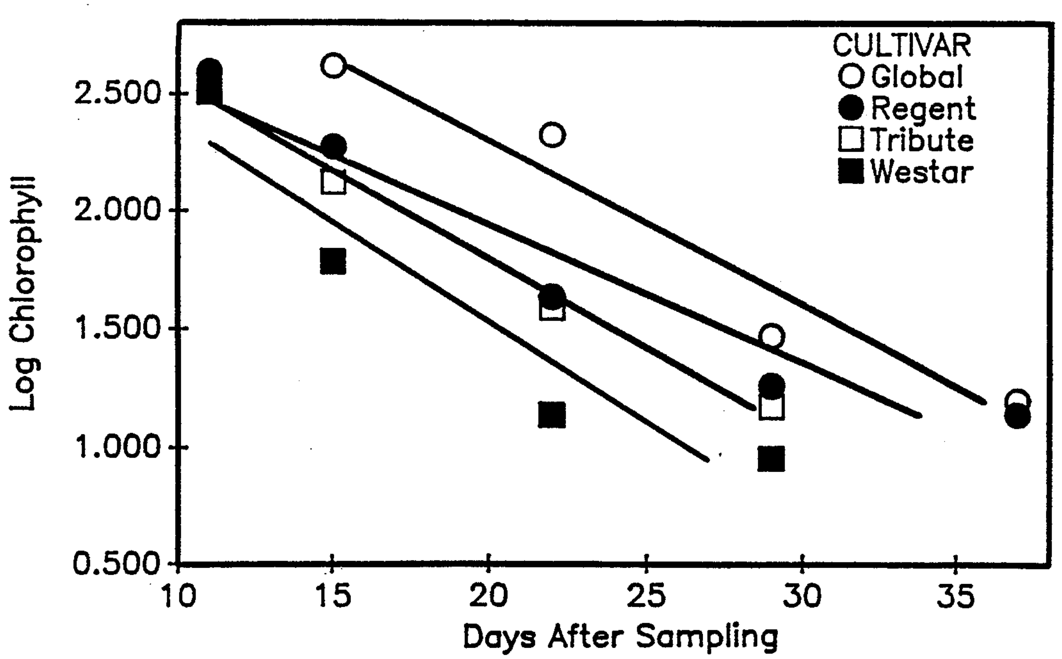

Fig. 18: Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1989. Regression of Logchl vs Time.

Table 10: Regression Analysis Results for Chlorophyll Degradation Rates in Four Cultivars of *B. napus* in 1988

CULTIVAR	EARLY SOWING	LATE SOWING
Global	r^2 = 0.94 ms error=0.0931 slope= -0.0879	r^2 = 1.0 ms error=0.0002 slope= -0.0240
Regent	r^2 = 0.93 ms error=0.1056 slope= -0.0941	r^2 = 0.86 ms error=0.0304 slope= -0.0244
Tribute	r^2 = 0.81 ms error=0.2578 slope= -0.0916	r^2 = 0.96 ms error=0.0155 slope= -0.0340
Westar	r^2=0.98 ms error=0.0368 slope= -0.1217	r^2 = 0.92 ms error=0.0376 slope= -0.0389

Table 11: Regression Analysis Results for Chlorophyll Degradation Rates in Four Cultivars of *B.napus* in 1989

CULTIVAR	EARLY SOWING	LATE SOWING
Global	r^2 = 0.98 ms error=0.0176 slope= -0.0892	r^2 = 0.94 ms error=0.0382 slope= -0.0700
Regent	r^2 = 0.97 ms error=0.0157 slope= -0.1166	r^2 = 0.93 ms error=0.0384 slope= -0.0582
Tribute	r^2 = 0.93 ms error=0.0851 slope= -0.1621	r^2 = 0.99 ms error=0.0066 slope= -0.0749
Westar	r^2 = 0.94 ms error=0.0707 slope= -0.1510	r^2 = 0.89 ms error=0.0794 slope= -0.0839

The slope of each line represents the rate of chlorophyll breakdown in that cultivar. A number of conclusions can be made. The first is that the slopes of the different lines on the same graph are very similar. This means that the four cultivars tested all had similar rates of seed chlorophyll degradation when grown in the same environment. There is therefore little scope for selection to improve the green seed problem using any of the cultivars tested as parental material.

Paired t-tests were performed to test for homogeneity of the regression coefficients to determine whether or not the slopes were statistically different from one another in each seeding date and year (Table 12). In most cases there were no significant differences between slopes, with one exception in each year's data. In 1988, Regent and Westar from the later seeding date had slopes that were significantly different at the 5% level. In 1989, the slopes of Global and Tribute from the early seeding were significantly different at the 5% level. Neither of these was significant at the 1% level.

Table 12: Paired T-Test Results Comparing Slopes in Four Cultivars of *B. napus*

1988

CULTIVAR COMPARISON	S. DATE	DF	|T|	5% SIG.	1% SIG.
Global x Regent	Early	6	0.31	--	--
Global x Tribute	Early	5	0.12	--	--
Global x Westar	Early	4	1.09	--	--
Regent x Tribute	Early	5	0.08	--	--
Regent x Westar	Early	4	0.83	--	--
Tribute x Westar	Early	3	0.59	--	--
Global x Regent	Late	7	0.04	--	--
Global x Tribute	Late	8	1.38	--	--
Global x Westar	Late	8	1.32	--	--
Regent x Tribute	Late	13	2.02	--	--
Regent x Westar	Late	13	2.46	**	--
Tribute x Westar	Late	14	0.98	--	--

1989

CULTIVAR COMPARISON	S. DATE	DF	|T|	5% SIG.	1% SIG.
Global x Regent	Early	6	1.08	--	--
Global x Tribute	Early	6	3.19	**	--
Global x Westar	Early	6	1.74	--	--
Regent x Tribute	Early	6	1.87	--	--
Regent x Westar	Early	6	1.53	--	--
Tribute x Westar	Early	6	0.33	--	--
Global x Regent	Late	5	0.77	--	--
Global x Tribute	Late	4	0.35	--	--
Global x Westar	Late	4	0.60	--	--
Regent x Tribute	Late	5	1.20	--	--
Regent x Westar	Late	5	1.26	--	--
Tribute x Westar	Late	4	0.42	--	--

The second finding is that the slopes are different between the early and late seeding dates in each year. Paired t-tests were performed to test whether or not the slopes from the early seeding dates were significantly different from those from the later seeding date (Table 13).

Table 13: Paired T-Test Results from Different Planting Dates

YEAR	EARLY VS LATE	DF	|T|	5% SIGNIFICANCE
1988	Global	4	3.30	**
1988	Regent	9	5.75	**
1988	Tribute	9	7.35	**
1988	Westar	8	4.13	**
1989	Global	5	1.47	--
1989	Regent	6	4.33	**
1989	Tribute	5	3.35	**
1989	Westar	5	2.20	--

In most cases the slopes were significantly different. The rate of chlorophyll breakdown becomes slower when the crop is planted later as indicated by the steeper slopes in the earlier planting dates. This appears to be an environmental effect, likely due to warmer temperatures during the period when the early seeded crop was ripening. Other environmental variables such as moisture may also be involved. In order to benefit from the more rapid breakdown, the crop should be seeded as early as possible.

To further examine the effect of the environment on the rate of chlorophyll degradation, the two years were compared to see whether chlorophyll breakdown rates differed. Paired t-tests were conducted to compare the same treatment from the two years. For example, early Westar 1988 was compared to early Westar 1989 (Table 14).

Table 14: Paired T-Test Results Comparing Chlorophyll Degradation Rates in 1988 and 1989

S. DATE	CULTIVAR	COMPARISON	DF	¦T¦	5% SIGNIFICANCE
Early	Global	1988 vs 1989	6	0.08	--
Early	Regent	1988 vs 1989	6	1.13	--
Early	Tribute	1988 vs 1989	5	2.66	**
Early	Westar	1988 vs 1989	4	0.89	--
Late	Global	1988 vs 1989	3	3.39	**
Late	Regent	1988 vs 1989	9	3.51	**
Late	Tribute	1988 vs 1989	9	4.75	**
Late	Westar	1988 vs 1989	9	2.73	**

Some comparisons indicated differences between years while others did not. For the most part, the early seeding dates showed similar chlorophyll degradation rates while the late sowings differed between years. Differences between years can be attributed to environmental influences.

In order to discover whether the slower chlorophyll breakdown rates observed with late seeding could be explained by temperature differences, the logarithm of the chlorophyll level was plotted against growing degree days (GDD). GDD are a measure of the heat units accumulated during the ripening period. They were calculated by taking the average daily temperature minus 5°C, which is assumed to be the minimum temperature needed for chlorophyll degradation, and summing this over the period of seed ripening from growth stage 5.3 to growth stage 5.5 (Morrison et al,1989). By using GDD rather than days after physiological maturity, comparisons can be made between seeding dates with the same number of accumulated heat units.

A regression analysis was performed and the best straight line fitted to each cultivar (Figures 19-22). A summary of the regression analysis is presented in Tables 15 and 16.

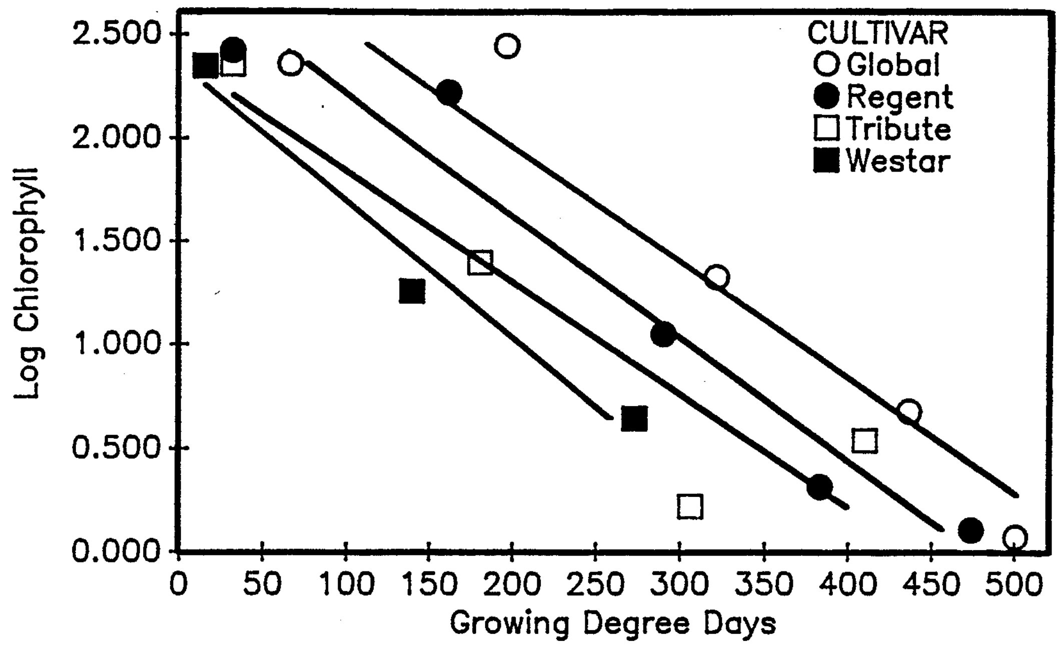

Fig. 19: Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1988. Regression of Logchl vs GDD.

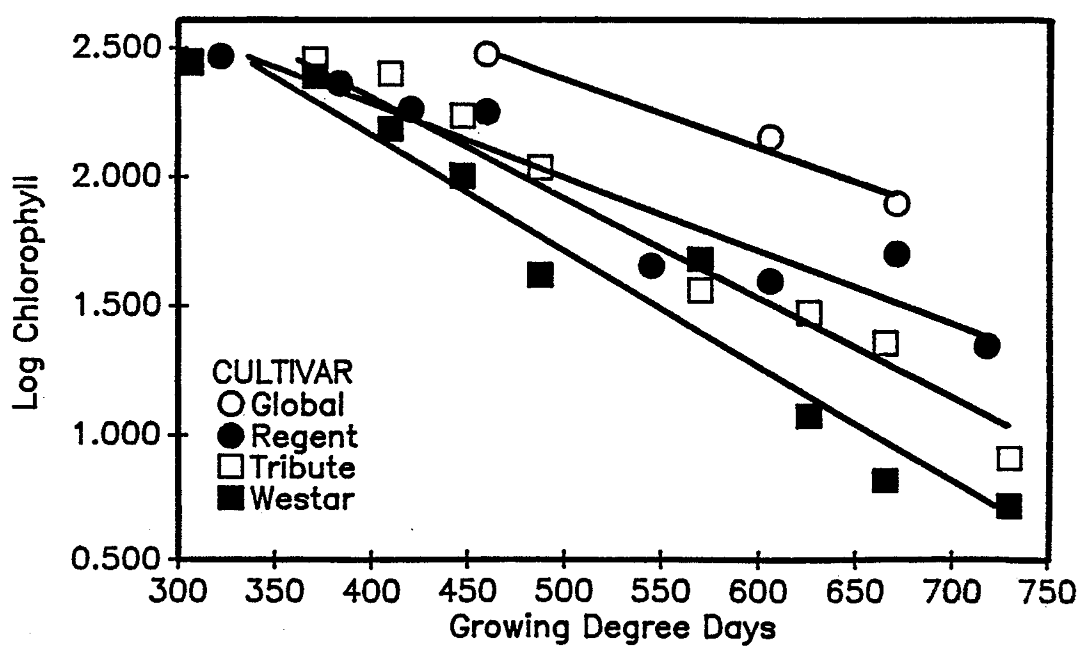

Fig. 20: Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1988. Regression of Logchl vs GDD.

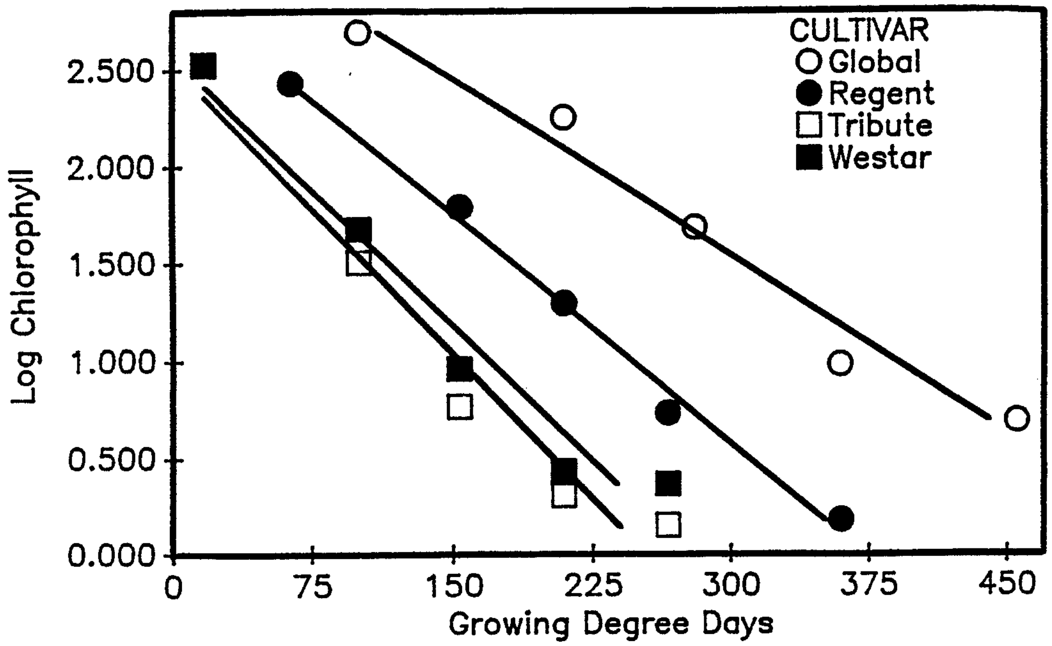

Fig. 21: Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1989. Regression of Logchl vs GDD.

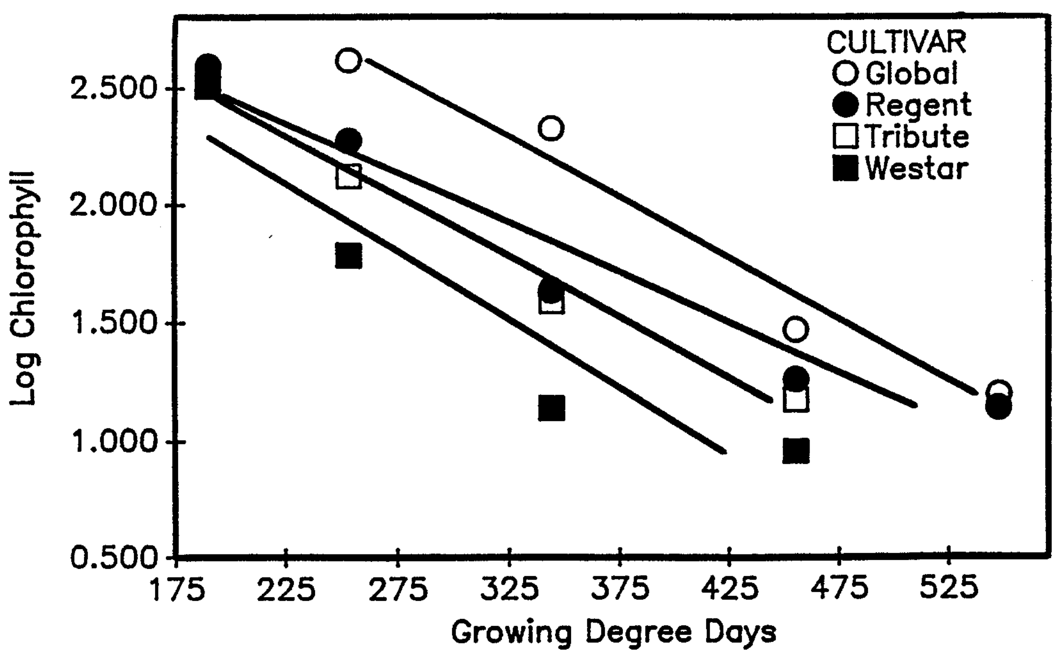

Fig. 22: Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1989. Regression of Logchl vs GDD.

Table 15: Regression Analysis Results for 1988: Log Chlorophyll versus Growing Degree Days

CULTIVAR	EARLY SOWING	LATE SOWING
Global	r^2 = 0.91 ms error=0.1287 slope= -0.005614	r^2 = 0.98 ms error = 0.003826 slope= -0.002638
Regent	r^2 = 0.95 ms error=0.07965 slope= -0.005914	r^2 = 0.91 ms error=0.01928 slope= -0.002839
Tribute	r^2 = 0.85 ms error=0.2089 slope= -0.005415	r^2 = 0.96 ms error=0.01619 slope= -0.003852
Westar	r^2 = 0.97 ms error=0.04727 slope= -0.006594	r^2 = 0.95 ms error=0.02390 slope= -0.004475

Table 16: Regression Analysis Results for 1989: Log Chlorophyll versus Growing Degree Days

CULTIVAR	EARLY SOWING	LATE SOWING
Global	r^2 = 0.97 ms error=0.0263 slope= -0.006075	r^2 = 0.97 ms error=0.02242 slope= -0.005192
Regent	r^2 = 0.99 ms error=0.007167 slope= -0.007848	r^2 = 0.94 ms error=0.03102 slope= -0.004228
Tribute	r^2 = 0.95 ms error=0.05864 slope= -0.009594	r^2 = 0.98 ms error=0.008894 slope= -0.005136
Westar	r^2 = 0.95 ms error=0.0511 slope= -0.009261	r^2 = 0.88 ms error=0.08667 slope= -0.005730

To test whether these slopes were significantly different from one another, paired t-tests were carried out on the different cultivars within each seeding date of each year (Table 17).

Table 17: Paired T-Test Results Comparing Slopes of Log Chlorophyll Versus Growing Degree Days

1988

CULTIVAR COMPARISON	S. DATE	DF	|T|	5% SIG.	1% SIG.
Global x Regent	Early	6	0.23	--	--
Global x Tribute	Early	5	0.11	--	--
Global x Westar	Early	4	0.48	--	--
Regent x Tribute	Early	5	0.30	--	--
Regent x Westar	Early	4	0.41	--	--
Tribute x Westar	Early	3	0.46	--	--
Global x Regent	Late	7	0.22	--	--
Global x Tribute	Late	8	1.44	--	--
Global x Westar	Late	8	1.80	--	--
Regent x Tribute	Late	13	2.11	--	--
Regent x Westar	Late	13	3.06	**	**
Tribute x Westar	Late	14	1.27	--	--

1989

CULTIVAR COMPARISON	S. DATE	DF	|T|	5% SIG.	1% SIG.
Global x Regent	Early	6	2.37	--	--
Global x Tribute	Early	6	2.69	**	--
Global x Westar	Early	6	2.55	**	--
Regent x Tribute	Early	6	1.41	--	--
Regent x Westar	Early	6	1.21	--	--
Tribute x Westar	Early	6	0.19	--	--
Global x Regent	Late	5	1.03	--	--
Global x Tribute	Late	4	0.07	--	--
Global x Westar	Late	4	0.34	--	--
Regent x Tribute	Late	5	1.01	--	--
Regent x Westar	Late	5	1.07	--	--
Tribute x Westar	Late	4	0.38	--	--

This analysis supports the conclusions drawn from the plot of log chlorophyll versus days after sampling. A few differences between cultivars appear at the 5% level of significance but none at the 1% level. It can be concluded that the four cultivars all have the same rate of chlorophyll degradation when grown in the same environment.

The conversion to GDD becomes useful when comparing chlorophyll degradation rates between seeding dates. Paired t-tests were performed to compare the slopes of log chlorophyll versus growing degree days between the early and late seeding dates of each year (Table 18).

Table 18: Paired T-Test Results Between Early and Late Seeding Dates

YEAR	EARLY VS LATE	DF	$\|T\|$	5% SIGNIFICANCE
1988	Global	4	1.34	--
1988	Regent	9	3.98	**
1988	Tribute	9	1.49	--
1988	Westar	8	2.15	--
1989	Global	5	0.97	--
1989	Regent	6	4.65	**
1989	Tribute	5	3.14	**
1989	Westar	5	1.92	--

When these results are compared to those in Table 13, it is apparent that many of the differences between slopes have been eliminated by converting to GDD, confirming the influence of temperature on the rate of chlorophyll breakdown. However, some differences between seeding dates still exist, indicating that temperature, although important, is not the only factor influencing the rate of chlorophyll degradation.

Chlorophyll breakdown rates were also compared between years using GDD. Homogeneity of regression coefficients was tested using pair-wise t-tests and the results are presented in Table 19.

Table 19: Paired T-Test Results Comparing Chlorophyll Degradation Rates Between Years

S. DATE	CULTIVAR	COMPARISON	DF	|T|	5% SIG.
Early	Global	1988 vs 1989	6	0.36	--
Early	Regent	1988 vs 1989	6	1.75	--
Early	Tribute	1988 vs 1989	5	1.93	--
Early	Westar	1988 vs 1989	4	1.58	--
Late	Global	1988 vs 1989	3	2.53	--
Late	Regent	1988 vs 1989	9	2.10	--
Late	Tribute	1988 vs 1989	9	1.91	--
Late	Westar	1988 vs 1989	9	1.16	--

When these results are compared to those in Table 14, it is evident that the conversion from days after sampling to GDD eliminated all of the differences between the two years at the 5% level of significance. Different rates of chlorophyll breakdown in different years are, therefore, in large part caused by different temperatures during seed ripening.

Using GDD, the different cultivars tested within the same seeding date all had the same rate of chlorophyll breakdown in both years of the study. The rate of chlorophyll degradation within these four cultivars is therefore influenced strongly by temperature.

Although chlorophyll degradation occurs at the same rate in each cultivar it does not begin at the same time. Cultivars which require longer growing seasons to mature also initiate chlorophyll breakdown later, increasing the probability that unacceptable seed chlorophyll levels will remain at harvest. Westar and Tribute initiated seed chlorophyll degradation at approximately the same time with Regent following 4-7 days later and Global a week or more later. Therefore, given that chlorophyll degradation rates are the same, a cultivar which requires a longer growing season to mature, has a greater likelihood of unacceptably high seed chlorophyll levels remaining at harvest, in comparison to an earlier maturing cultivar.

To summarize this section, the conclusions reached for each objective are :

1) the four cultivars of *B. napus* tested - Westar, Tribute, Regent and Global - had essentially the same rate of seed chlorophyll breakdown in the same environment;
2) the time at which chlorophyll degradation is initiated in any given cultivar is related to the relative maturity of the cultivar; and
3) differences in chlorophyll degradation rates between early and late seeding dates and between different years are reduced by converting to GDD.

4.4 "AGROMAN" TRIALS

4.4.1 Genotypic and Environmental Effects on Seed Chlorophyll Levels at Harvest

Chlorophyll levels in the seed were measured for all registered canola cultivars of both B. napus and B. campestris grown at a number of sites in Manitoba over a two year period. Climatic zones are based on the average number of frost-free days and on soil types (see Appendix Figure A9).

The four objectives of this study were to determine the following:

1) whether variation in seed chlorophyll levels can be attributed to genotype i.e. cultivar differences;
2) whether the location of the trial has an effect on the chlorophyll content of the harvested seed;
3) whether chlorophyll levels are affected by year to year variation; and
4) whether there is an interaction between genotype and environment.

The "Agroman" data was divided according to species into two groups of data, one for B. napus and one for B. campestris. The GLM procedure was first carried out on each year's data at each individual location.

Among the *B. campestris* cultivars tested, none showed significant differences in final seed chlorophyll levels in 1988 at any location in the trials. In 1989 when an additional cultivar (Parkland) was added to the study, there were differences among cultivars at two locations.

The *B. napus* cultivars tested, however, showed significant differences between cultivars at all locations in both years. All locations indicated cultivar differences at the 5% level of significance and most at the 1% level.

The GLM analysis by location also indicated that the error variances were highly heterogeneous among locations. Homogeneity of experimental error variances is a prerequisite for the GLM analysis of experiments combined over locations. A GLM analysis was run for each species in each year combined over all locations and the results were then modified according to Cochran and Cox (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, the tabular F-value is distributed approximately with 1 and p-1 degrees of freedom where p is the number of locations. This is a more conservative test of locations which takes into account the maximum distortion in F which could occur.

Heterogeneity among the experimental error variances invalidates the F-test of the interaction mean square against the pooled error mean square. 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 with the greatest error variance (Cochran and Cox,1957).

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

Modified GLM results for each species in each year combined over locations are presented in Tables 20 and 21. The data was analyzed as a split-plot design with locations as a main effect.

Table 20: Modified GLM Results for B. campestris Cultivars Combined Over Locations

1988

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Locations	9	593.78	8.43	8.43	(1.9)	*
Cultivars	2	15.11	0.55	0.47	(2,40)	-
Loc*Cult	18	32.38	1.19	1.19	(2,4)	-
Error a (Rep*Loc)	30	46.98				
Error b	40	27.26				

1989

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Locations	9	800.20	90.22	90.22	(1,9)	**
Cultivars	3	37.67	9.96	3.12	(3,84)	*
Loc*Cult	27	12.05	3.19	3.19	(3,9)	-
Error a (Rep*Loc)	30	8.87				
Error b	84	104.65				

Table 21: Modified GLM Results for B. napus Cultivars Combined Over Locations

1988

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Locations	9	3080.77	24.68	24.68	(1,9)	**
Cultivars	10	1958.98	17.13	5.73	(10,200)	**
Loc*Cult	90	341.65	2.99	2.99	(10,20)	*
Error a (Rep*Loc)	20	124.82				
Error b	200	114.37				

1989

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Locations	10	10021.00	60.80	60.80	(1,10)	**
Cultivars	17	1277.99	30.71	11.09	(17,545)	**
Loc*Cult	170	115.27	2.77	2.77	(17,46)	--
Error a (Rep*Loc)	33	164.82				
Error b	545	41.61				

Within the B. campestris cultivars, modified GLM results indicated significant differences between locations in both years of the study at the 5% level of significance. In 1988 when three cultivars were tested, no significant differences between the cultivars were found. In 1989 when a fourth cultivar (Parkland) was added, differences were found between cultivars at the 5% level of significance. This should be interpreted with caution however, since only two of the ten individual locations indicated cultivar differences and at most locations all B. campestris cultivars contained acceptably low levels of chlorophyll (<24 ppm). No interactions between location and cultivar were indicated in either year.

Among the B. napus cultivars tested, locations were found to be significantly different in both years of the study at the 1% level of significance. Cultivars were also significantly different at the 1% level. A significant interaction between location and cultivar was indicated at the 5% level in 1988 but not in 1989.

A new data set was then created which included only cultivars and locations that were tested in both years of the study. Within the B. campestris species, this included three cultivars - Tobin, Colt and Horizon and nine locations. Within the B. napus species, eleven cultivars and nine locations were included.

A GLM was run on this set of data combined over years and it was modified according to Cochran and Cox (Cochran and Cox,1957) as before (Tables 22 and 23).

Table 22: Modified GLM Results for B. campestris Combined Over Years

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Year	1	225.55	6.06	6.06	(1,9)	*
Location	8	1021.32	27.46	27.46	(1,9)	**
Loc*Year	7	300.35	8.08	8.08	(1,9)	*
Error a (Loc*Rep(Year))	42	37.19				
Cultivar	2	1.33	0.09	0.05	(2,81)	--
Cult*Year	2	57.16	3.85	2.05	(2,81)	--
Cult*Loc	16	24.66	1.66	1.66	(2,4)	--
Cult*Loc*Year	14	27.94	1.88	1.88	(2,4)	--
Error b	81	14.85				

Table 23: Modified GLM Results for B. napus Combined Over Years

Source	DF	MS	F-Value	Mod. F	New DF	Sig.
Year	1	11299.31	107.19	107.19	(1,9)	**
Location	8	4050.51	38.42	38.42	(1,9)	**
Loc*Year	8	2244.03	21.29	21.29	(1,9)	**
Error a (Loc*Rep(Year))	45	105.41				
Cultivar	10	2180.68	32.77	9.01	(10,442)	**
Cult*Year	10	440.84	6.63	2.18	(10,442)	*
Cult*Loc	80	241.70	3.64	3.64	(10,20)	**
Cult*Loc*Year	80	202.61	3.04	3.04	(10,20)	*
Error b	442	66.54				

Within the B. campestris cultivars, there were no significant differences indicated at the 5% level and consequently there were no significant interactions involving cultivars. Locations were significantly different at the 1% level and years at the 5% level. There were also significant differences between locations by years at the 5% level. This indicates that locations performed differently in each of the two years so location effects should be interpreted within each year separately.

The B. napus cultivars showed significant differences at the 1% level. Cultivars also showed significant interactions with years at the 5% level and with locations at the 1% level. The interaction of location by cultivar by year was also significant at the 5% level. This indicates that cultivars performed differently in comparisons between years and between locations. The ranking of each cultivar, with respect to seed chlorophyll content, changes from one location to the next so ideally each cultivar's performance should be assessed at each location of interest, rather than assessing average performance over locations and specific recommendations made for each location.

Years, locations and the interaction between the two were also significant at the 1% level indicating that the effect of location should be examined individually in each year of the study. Results interpreted in this manner are, however, of very limited use.

These significant effects can be categorized as either genotypic, environmental or genotype by environment (GxE) effects. Variation among cultivars is a genotypic effect while locations, years and the interaction of location by year are environmental effects. There was also a significant GxE interaction as indicated by the significance of cultivar by location, cultivar by year and cultivar by location by year.

The final chlorophyll levels reached in harvested canola seed are therefore dependent on both the genotype of the plant and environmental conditions.

4.4.1.1 Genotypic Effect

B. napus cultivars had considerably higher seed chlorophyll levels at harvest than *B. campestris* cultivars. At all locations in both years of the study, the *B. campestris* cultivars ranked below all or most of the *B. napus* cultivars on the basis of seed chlorophyll level.

The *B. campestris* cultivars were combined over locations and Duncan's means separation tests were carried out to determine cultivar differences in each year of the study (Table 24).

Table 24: Duncan's Means Separation Test Results for the B. campestris Cultivars

1988

Cultivar	Mean Chl (ppm)	Group
Horizon	11.3	A
Colt	10.4	A
Tobin	10.0	A

1989

Cultivar	Mean Chl (ppm)	Group
Tobin	9.0	A
Colt	8.2	A B
Parkland	7.9	A B
Horizon	7.1	B

There was no indication of any major differences in chlorophyll content in harvested seeds of different cultivars of B. campestris and all cultivars achieved acceptably low chlorophyll levels (<10 ppm) on average.

B. napus cultivars, however, showed significant differences in final seed chlorophyll levels. The 1989 GLM indicated no significant location by cultivar interaction occurred so cultivar performance could be assessed over locations. In 1988, there was a significant location by cultivar interaction at the 5% level but not at the 1% level indicating that the ranking of some cultivars did change between locations. For the sake of simplicity however, Duncan's means separation tests were performed, at the 5% level of significance, on the cultivars combined over locations in each year of the study (Tables 25 and 26).

Table 25: Duncan's Means Separation Test Results for the *B. napus* Cultivars in 1988

Cultivar	Mean Chl (ppm)	Group
Stellar	44.9	A
Triumph (TT)*	42.2	A B
Triton (TT)	37.1	A B C
Topas	36.2	A B C
Legend	33.1	B C D
Global	32.1	B C D
Alto	30.1	C D
Westar	29.8	C D
Regent	29.7	C D
Tribute (TT)	23.1	D E
Delta	16.4	E

* (TT) indicates a triazine tolerant cultivar

Table 26: Duncan's Means Separations Test Results for the *B. napus* Cultivars in 1989

Cultivar	Mean Chl (ppm)	Group
Stellar	35.7	A
Global	31.2	A B
Regent	29.5	B C D
Topas	26.0	C D E
Celebra	24.7	D E
Profit	24.6	D E
Triumph (TT)*	24.0	D E
Westar	24.0	D E
ACSN4TT (TT)	23.7	D E
SV8525953 (TT)	23.4	D E
Legend	23.2	D E
Triton (TT)	22.9	D E
Vanguard	21.8	D E
Alto	20.6	D E F
Tribute (TT)	19.3	E F
Hero	16.5	F G
Hyola 40	16.3	F G
Delta	13.9	G

* (TT) indicates a triazine tolerant cultivar

Comparisons between the *B. campestris* and *B. napus* cultivar means show that the *B. campestris* cultivars all had significantly lower seed chlorophyll levels than any of the *B. napus* cultivars. This may be due to a more rapid rate of chlorophyll degradation in *B. campestris* cultivars or to the earlier maturity of the species, which allows seed ripening to occur under conditions favorable to chlorophyll breakdown.

Secondly, there has been some concern that triazine tolerant cultivars might have inherently higher seed chlorophyll levels than other cultivars. The results of this analysis showed triazine tolerant cultivars to have the same range of seed chlorophyll contents as non-triazine tolerant cultivars. Triumph was ranked the highest of the five cultivars tested for seed chlorophyll content over all tests and Tribute the lowest. The two newer cultivars which were registered in 1990 - ACSN4TT (AC Tristar) and SV8525953 had intermediate levels of seed chlorophyll.

The non triazine tolerant cultivars of *B. napus* also showed a wide range of chlorophyll levels. Stellar was ranked consistently high for seed chlorophyll levels at harvest. Global also tended to be relatively high. Three of the newer cultivars to be developed - Hero, Hyola 40 and in particular Delta - ranked consistently low in terms of chlorophyll content.

In order to determine whether low chlorophyll levels were associated with early maturity, the correlation between average chlorophyll content at harvest and days to maturity was determined for each cultivar. The maturation time for each cultivar is given in the Appendix (Table A5).

Maturation time was taken from the 1990 Field Crop Variety Recommendations for Manitoba with information on additional cultivars provided from Co-op trials. In 1988, the correlation coefficient was 0.79, in 1989 it was 0.76 and combined over both years the correlation between chlorophyll and maturation time was 0.80. These values are quite high and support previous results which indicated that cultivars which mature earlier also undergo seed chlorophyll degradation earlier in the growing season so less remains at harvest.

The two most notable exceptions to this are Stellar and Delta. Stellar requires only 94 days to reach maturity in Manitoba. This is in comparison to 92 days for Westar and 100 days for Global. However the seed chlorophyll content of Stellar is consistently higher than Global at harvest. Delta, on the other hand, requires 93 days to reach maturity, midway between Westar and Regent. However the seed chlorophyll content of Delta at harvest is considerably lower than either Westar or Regent. The number of days that a cultivar requires to reach maturity is therefore not the only factor contributing to the final seed chlorophyll level.

Previous research has indicated that there are no significant differences in chlorophyll content between different cultivars of the same species - either *B. napus* or *B. campestris* (Daun,1987). This study supports this conclusion for *B. campestris*, although it should be noted that only four cultivars were tested. The lack of variation in chlorophyll content may be due to the relatively early maturity of these cultivars allowing them all to achieve acceptable chlorophyll levels by harvest time.

However, there were significant differences in seed chlorophyll contents at harvest among different cultivars of *B. napus* in the "Agroman" trials. This does not necessarily contradict earlier research since in most cases the cultivars which had the extremes in chlorophyll levels are newly registered cultivars, for example Stellar, having very high chlorophyll levels, and Delta, Hero and Hyola 40 having low chlorophyll contents. There were also significant differences between the chlorophyll contents of the older cultivars, although the differences were not so extreme.

In conclusion, genotype does have a significant effect on the final chlorophyll content of canola seed at harvest, as *B. napus* cultivars have consistently higher seed chlorophyll levels than *B. campestris* cultivars. Seed chlorophyll contents vary significantly among different cultivars of *B. napus*, both the normal and the triazine tolerant cultivars.

4.4.1.2 Environmental Effects

The influence of environment in the "Agroman" trials consisted of three variables - locations, years and the interaction between location and year.

Within the *B. campestris* cultivars, locations were significantly different at the 1% level, years at the 5% level and the interaction of location by year was significant at the 5% level.

Duncan's means separation tests were performed to compare the different locations in each year of the study (Tables 27 and 28).

Table 27: Duncan's Means Separation Test Results Comparing Locations Within the *B. campestris* Species in 1988

Location	Mean Chl (ppm)	Group
Melita	23.1	A
Bagot	17.1	A B
Beausejour	16.6	A B
Swan River	14.8	A B
Shoal Lake	14.0	A B C
Teulon	12.8	B C
Mariapolis	4.9	C D
Waskada	2.2	D
Roblin	0.8	D
Dauphin	0.0	D

Table 28: Duncan's Means Separation Test Results Comparing Locations Within the B. campestris Species in 1989

Location	Mean Chl (ppm)	Group
Teulon	21.9	A
Shoal Lake	16.3	B
Beausejour	13.7	C
Melita	12.2	C
Winnipeg	7.5	D
The Pas	5.5	D
Mariapolis	1.7	E
Swan River	0.7	E
Roblin	0.7	E
Dauphin	0.6	E

Within the B. napus cultivars, years, locations and the interaction of the two were all significant at the 1% level. Duncan's means separation tests were done to compare the different locations based on chlorophyll levels within the B. napus cultivars (Tables 29 and 30).

Table 29: Duncan's Means Separation Test Results Comparing Locations Within the B. napus Species in 1988

Location	Mean Chl (ppm)	Group
Shoal Lake	49.2	A
Waskada	44.6	A B
Bagot	41.8	B
Melita	32.5	C
Teulon	29.0	C D
Mariapolis	28.0	C D
Roblin	27.8	C D
Beausejour	26.0	C D E
Swan River	23.2	E
Dauphin	20.4	E

Table 30: Duncan's Means Separation Test Results Comparing Locations Within the B. napus Species in 1989

Location	Mean Chl (ppm)	Group
The Pas	50.7	A
Teulon	32.4	B
Melita	28.9	C
Beausejour	27.9	C
Mariapolis	27.5	C
Shoal Lake	23.5	D
Bagot	19.2	E
Winnipeg	17.2	F
Swan River	11.9	G
Roblin	11.8	G
Dauphin	8.8	H

The ranking of chlorophyll levels at most locations changed from 1988 to 1989. Dauphin had significantly lower seed chlorophyll levels than all other locations tested in both years of the study. Dauphin was followed by Swan River and Roblin, both of which are also in the central western region of the province. From the data available it is impossible to tell exactly which aspects of the environment are affecting seed chlorophyll levels but a few observations can be made.

The seed harvested from Dauphin, Swan River and Roblin had lower chlorophyll levels than seed from areas such as Waskada and Melita located in the drier southwest corner of the province. The drought that affected this area may have been a contributing factor to the high chlorophyll levels.

The Pas, located north of all other sites, not surprisingly produced seed with the highest chlorophyll levels in the trials averaged over all B. napus cultivars. Lower temperatures in this area during the ripening period may explain this. It can also be noted that seed from The Pas does not have the highest chlorophyll levels when averaged over the B. campestris cultivars. This suggests that the shorter growing season may be the primary problem contributing to the high seed chlorophyll levels in the B. napus cultivars grown at northern locations.

Location differences must be interpreted with caution since each site in the "Agroman" trials was seeded and harvested on different dates and management was not necessarily uniform. Each site differs in a wide range of variables including temperature, length of growing season, precipitation and soil type. Therefore it is impossible to determine which environmental variables are affecting seed chlorophyll breakdown. The microclimate of the plots may be more important than weather conditions in the general area.

There was also a significant difference in the average chlorophyll level of the seed harvested in 1988 and 1989. Drier conditions in 1988 may have contributed to the higher chlorophyll levels. Also it should be noted that there was a significant location by year interaction confirming that the environment influences chlorophyll levels in harvested canola seed.

4.4.1.3 Genotype By Environment Interaction

When the data was combined over years, no significant interactions between genotype and environment occurred among the *B. campestris* cultivars, reflecting the small amount of variation between the individual cultivars. Among the *B. napus* cultivars, however, significant genotype by environment interactions were found. The cultivar by location interaction was significant at the 1% level, while the cultivar by year interaction was significant at the 5% level. The three way interaction of location by cultivar by year also was significant at the 5% level. Thus none of the variables examined influence seed chlorophyll content independently.

For example, the location by cultivar interaction, indicates that not only did the absolute value of the chlorophyll content of each cultivar change from one location to the next but also the relative ranking of the cultivars changed. This can be seen by comparing seed from The Pas, which had high chlorophyll levels for all cultivars to seed grown at Dauphin which had much lower levels. The relative ranking of each cultivar also changed at each location. A few of the cultivars were quite stable - for example, Stellar consistently had the highest seed chlorophyll levels and Delta the lowest among the *B. napus* cultivars tested. However other cultivars such as Westar and Regent changed in rank.

Similarly, interactions of cultivar by year and cultivar by location by year indicated that cultivars performed differently at different sites and in different years. These interactions between genotype and environment influence seed chlorophyll levels at harvest.

4.4.1.4 Summary

Both the genotype of the plant and the environment in which it is grown contributed significantly to chlorophyll levels in *B. napus* seed at harvest. Under a range of environments, *B. napus* cultivars had significantly higher chlorophyll contents than *B. campestris* cultivars. There was little variability among the different cultivars of *B. campestris* tested but significant differences did exist among the *B. napus* cultivars, both the normal canola and the triazine tolerant cultivars.

Location and year also affected chlorophyll levels and each variable interacts with the genotype of the plant. Although certain cultivars and locations that produce seed with high and low chlorophyll levels can be identified, it is clear that each cultivar must be tested at each location over a number of years to adequately assess its performance.

When selecting a cultivar to grow at a particular site it is important to choose not only a cultivar which performs well in general, but also one that is adapted to the growing area. Cultivars must therefore be tested over a wide range of sites over a number of years and their performance in any given area determined.

4.4.2 THE EFFECT OF DIFFERENT SEED SIZES FROM THE "AGROMAN" TRIALS

In each year of the study, the six locations showing the highest seed chlorophyll levels were chosen for further study. The bulk seed samples from each plot were subdivided into small, medium and large seed samples using sieves as described previously for the swathing study. Five cultivars of *B. napus* were included in this study - Westar, Global, Regent, Tribute and Triton - and four replicates of each were available at each location.

The purpose of the study was to determine whether the small seeds contain the most chlorophyll and whether the cultivars with high seed chlorophyll contents at certain locations contain greater quantities of small seed.

The average seed chlorophyll content of each subsample showed clearly that as seed size decreases, the chlorophyll level in the seed increases. These results were consistent across all cultivars and locations tested.

Graphs comparing the chlorophyll levels of each size class of seed for each cultivar are presented in Figures 23-34 for each location tested.

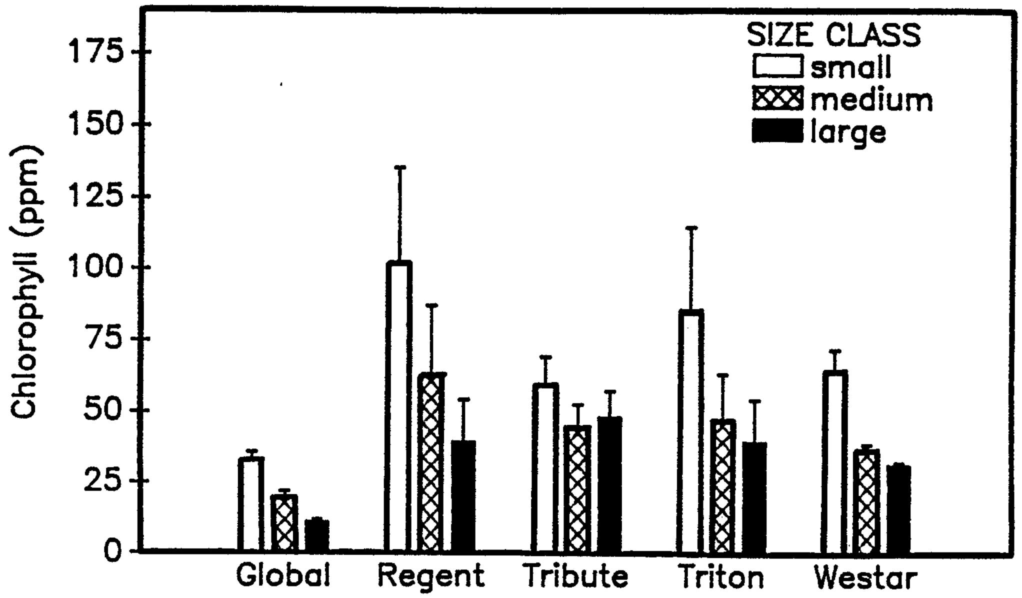

Fig. 23: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Bagot.

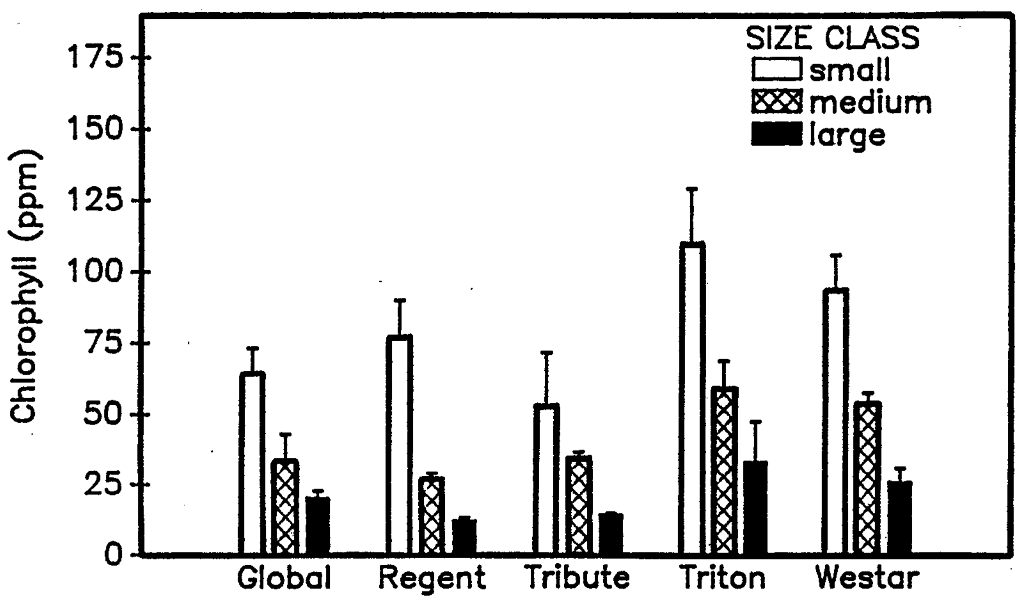

Fig. 24: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Mariapolis.

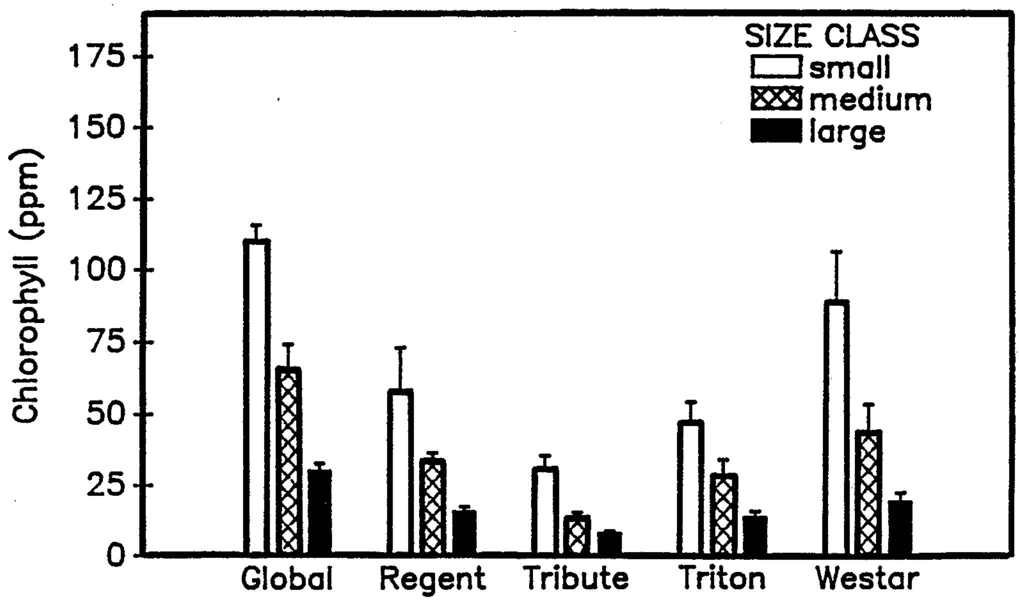

Fig. 25: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Melita.

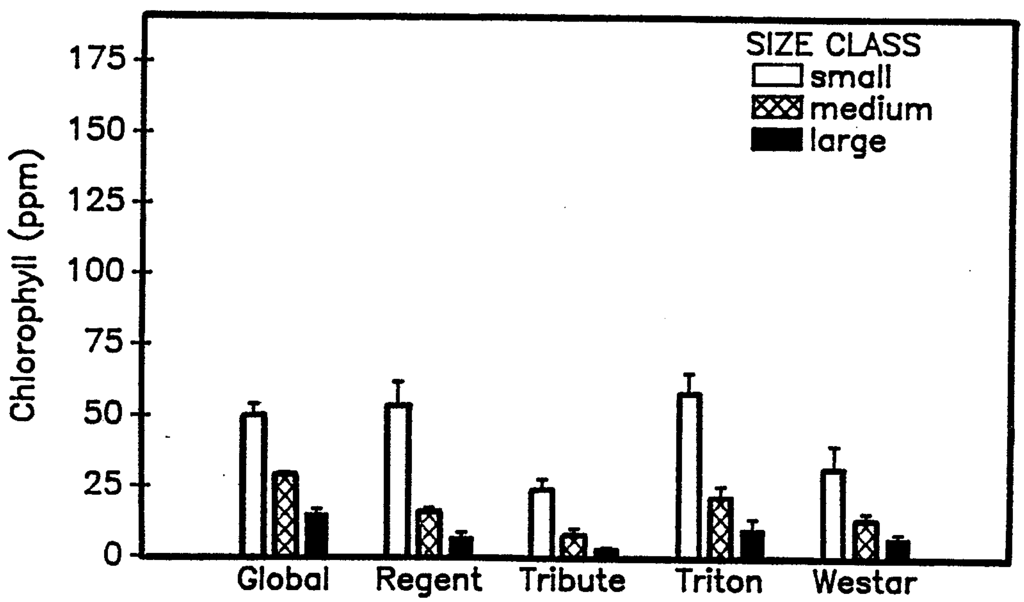

Fig. 26: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Roblin.

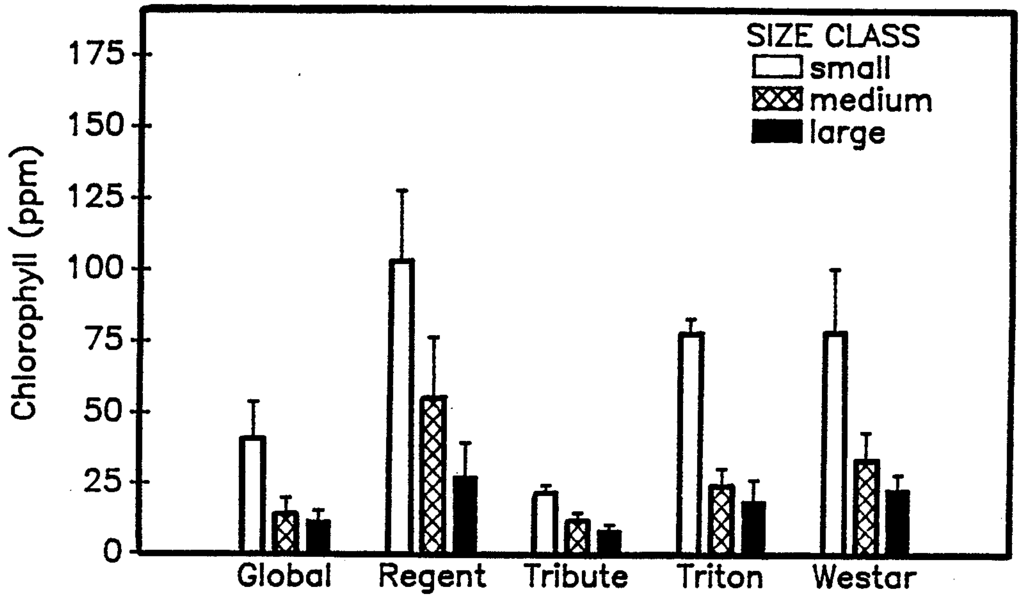

Fig. 27: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Shoal Lake.

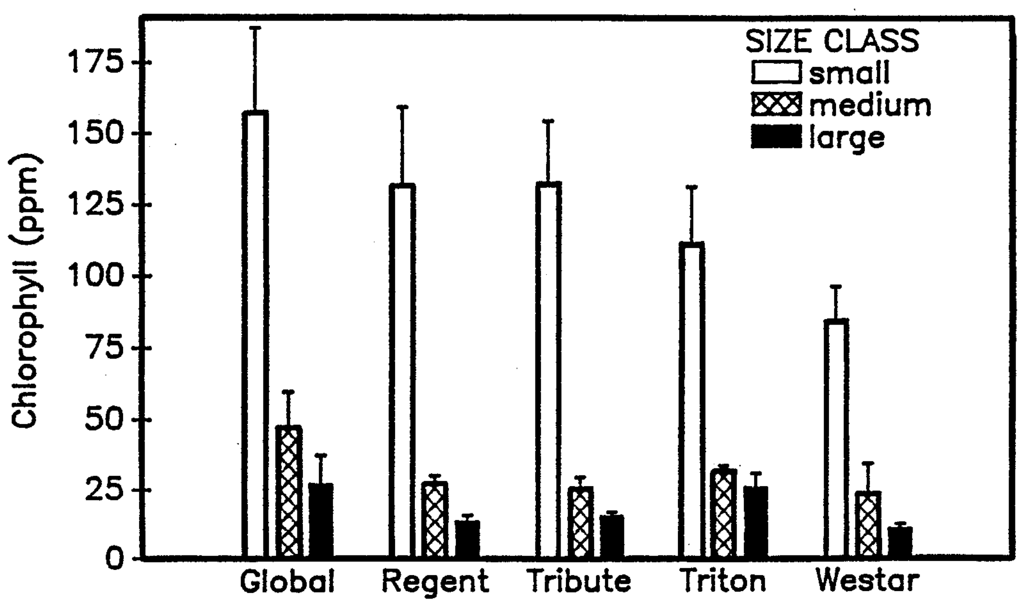

Fig. 28: Comparison of chlorophyll levels in small, medium and large seed from the 1988 "Agroman" trials - Waskada.

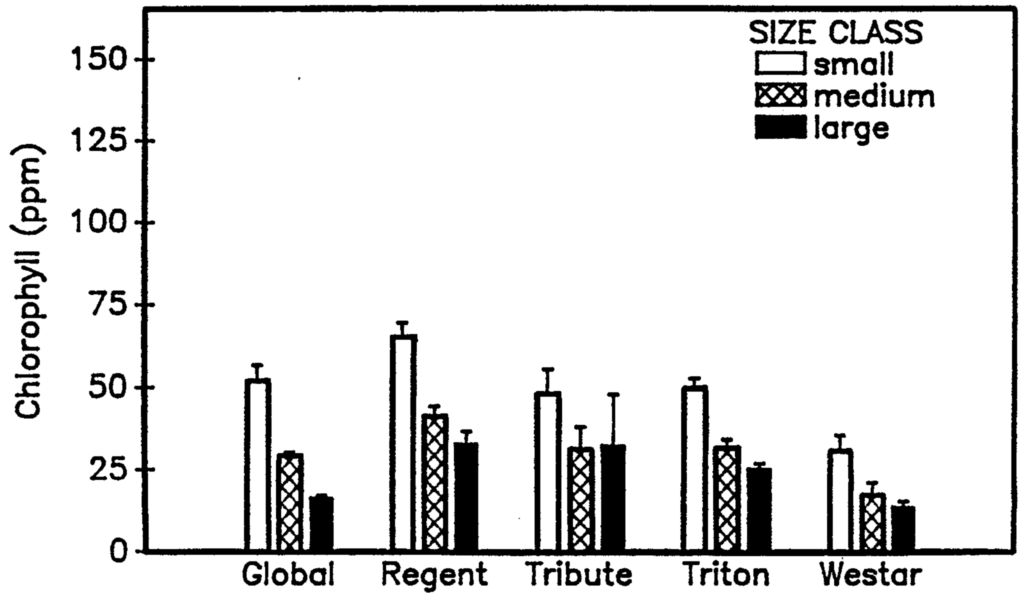

Fig. 29: Comparison of chlorophyll levels in small, medium and large seed from the 1989 "Agroman" trials - Beausejour.

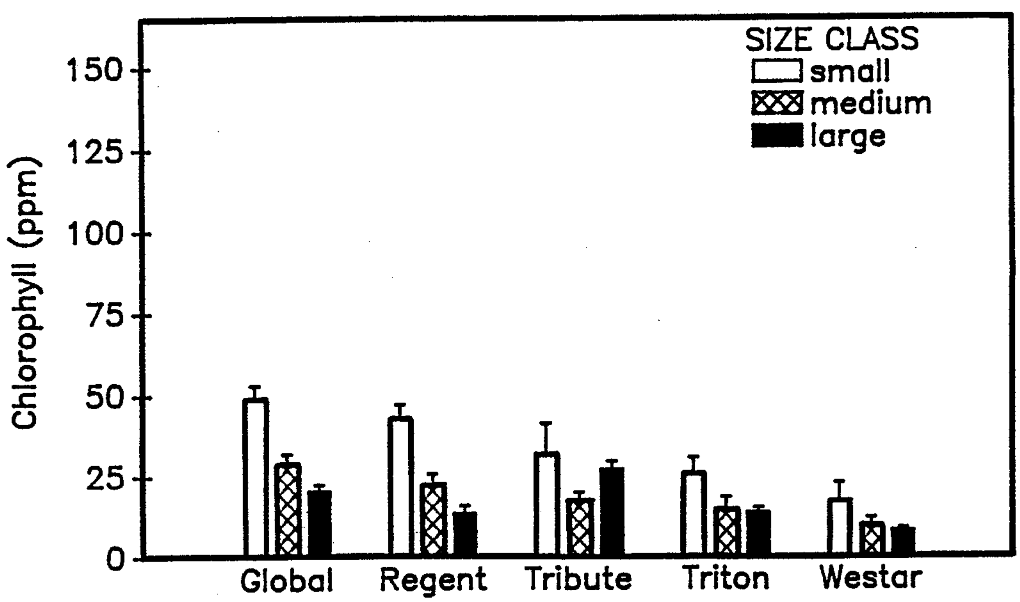

Fig. 30: Comparison of chlorophyll levels in small, medium and large seed from the 1989 "Agroman" trials - Mariapolis.

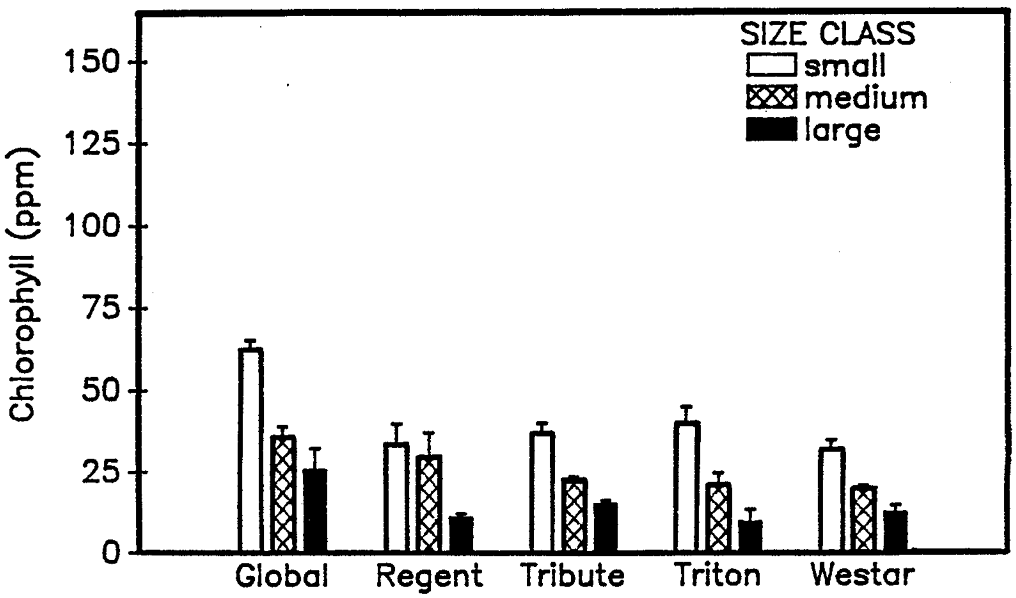

Fig. 31: Comparison of chlorophyll levels in small, medium and large seed from the 1989 "Agroman" trials - Melita.

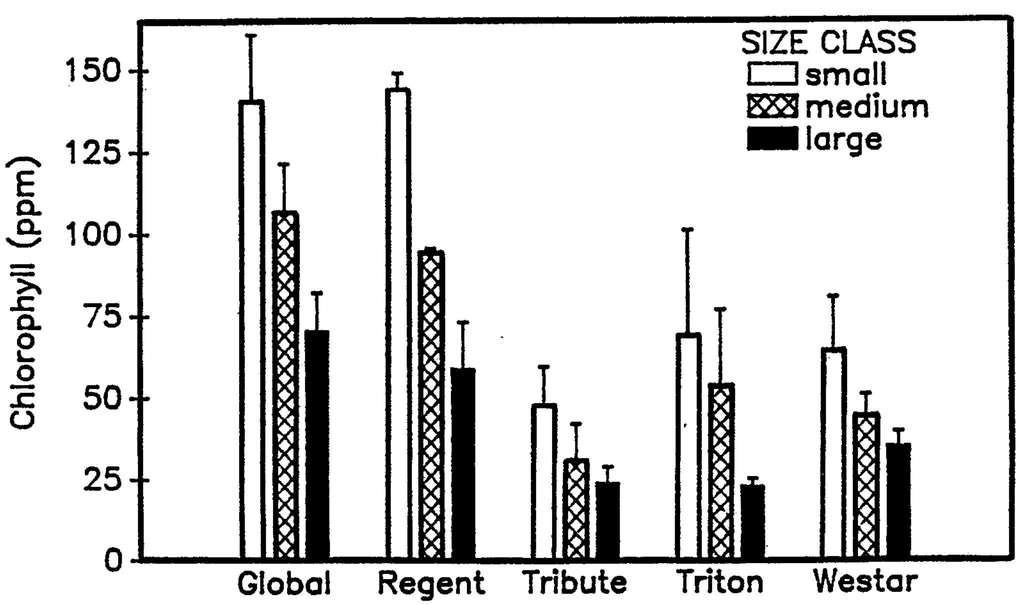

Fig. 32: Comparison of chlorophyll levels in small, medium and large seed from the 1989 "Agroman" trials - The Pas.

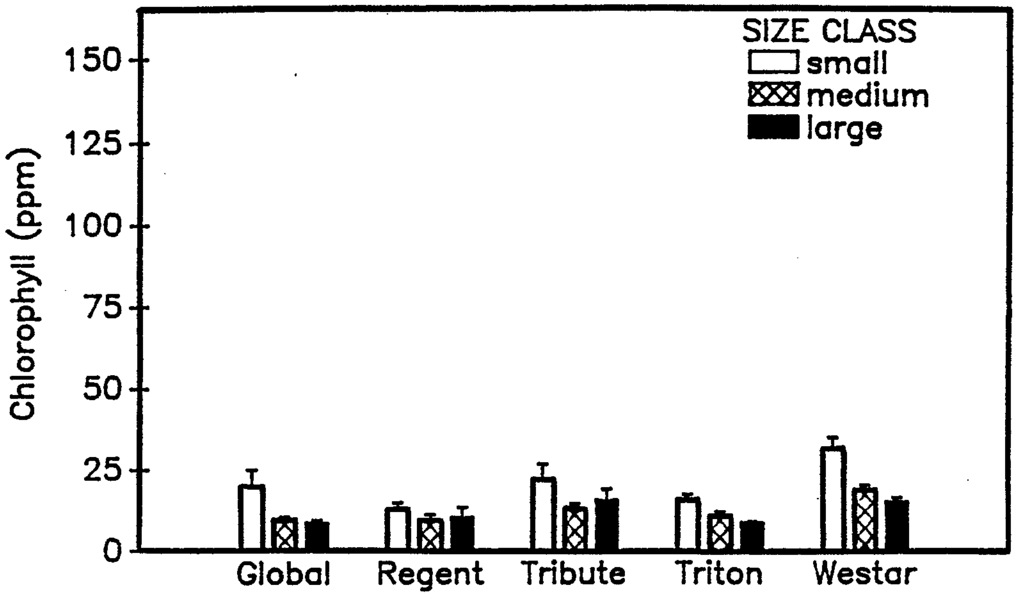

Fig. 33: Comparison of chlorophyll levels in small, medium and large seed in the 1989 "Agroman" trials - Shoal Lake.

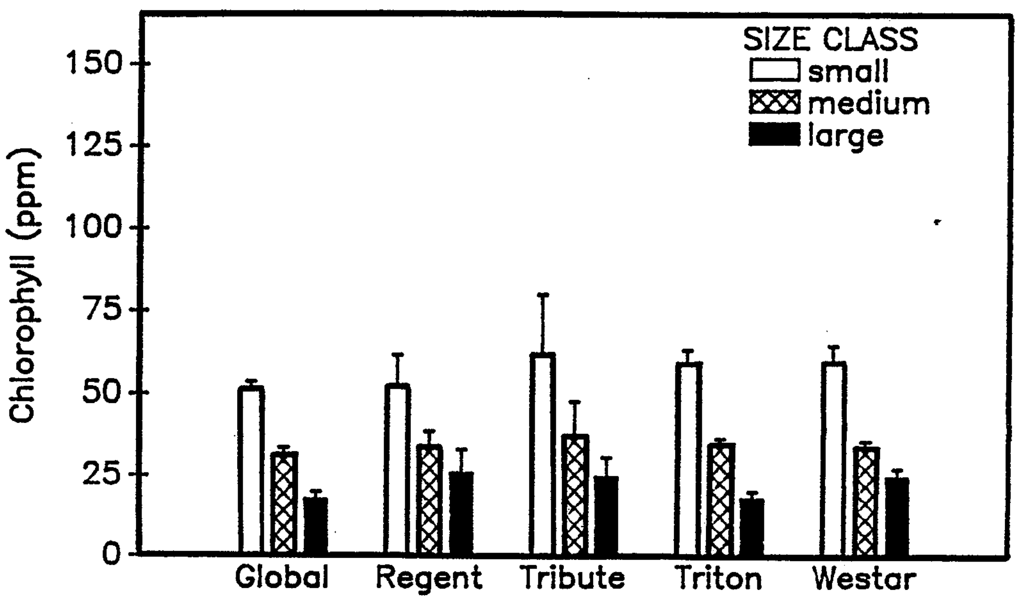

Fig. 34: Comparison of chlorophyll levels in small, medium and large seed in the 1989 "Agroman" trials - Teulon.

These results are consistent with results obtained from the swathing study. The small seeds are those which form later during the growing season and therefore have had less time for chlorophyll degradation to occur before harvest.

The percentage of seed falling into each size class was measured for each cultivar at each location. It was hypothesized that the cultivars and locations containing the highest levels of seed chlorophyll would also contain the largest percentage of small seed. However, no significant correlation between the chlorophyll content and the percentage of small seed present was found in either year of the study.

The results of the swathing study presented earlier indicated that the high chlorophyll treatments contained the most small seed. In the "Agroman" trials, however, there was not a great deal of variability in the percentage of seed falling into each size class between different cultivars or locations. In fact, the greatest difference was between the two years of the study. In 1989, a much greater percentage of small seed was produced by all cultivars at all locations. The percentage of medium sized seed did not vary as greatly and the percentage of large seed decreased in 1989. This was likely an environmental effect. Average chlorophyll levels did not change as drastically from one year to the next however. The percentage of seed falling into each size class in each year is presented in Tables A6 and A7 in the Appendix.

In the first year of the study, thousand seed weights were also determined for each size class in each group. These are summarized in Table A8 in the Appendix and indicate, not surprisingly, that the larger seed is also heavier. Therefore seeds could be separated into different classes based either on size or weight.

In summary, the smallest seeds in any given sample do contain the highest chlorophyll levels. A correlation between the seed chlorophyll content and the percentage of small seed present is not supported by this study however. Seed size was found to vary from year to year to a much greater degree than seed chlorophyll content. Either seed size or seed weight could be used to separate seeds into different size classes.

5.0 SUMMARY AND CONCLUSIONS

These studies have investigated a number of factors affecting the level of chlorophyll in canola seed at harvest, in order to determine how best to reduce the chlorophyll problem.

It was found that canola seed can be frozen and stored for up to one month in the freezer, either in the pods or after removal, without a significant change in chlorophyll content. Samples can therefore be stored for later analysis without concern for chlorophyll degradation.

The swathing study investigated the contribution of seed from the side branches to the chlorophyll problem and the effect of rapid drying conditions on the final chlorophyll content of the seed. Seeds from side branches contained 1.5 to 2 times as much chlorophyll as seeds from the main stems. The indeterminate flowering habit of canola results in the formation of immature seed on the branches which can contribute significantly to the chlorophyll problem.

Plants which were dried rapidly in a drying room contained seed chlorophyll levels 1.5 to 6 times higher than plants which were swathed and allowed to mature in the field. Rapid drying apparently halts chlorophyll degradation due to the unavailability of moisture.

Factors such as branching and rapid drying contribute to the formation of large amounts of small seed. These seeds are the most immature on the plant. As a result, when harvest and dry down halt seed chlorophyll degradation, these seeds tend to fix chlorophyll at a higher level.

The rate of chlorophyll degradation was investigated in four cultivars of B. napus - Westar, Regent, Tribute and Global. The rates were not significantly different under the same environmental conditions. This suggests the same metabolic pathway is active in all cultivars tested. Late seeding resulted in slower rates of chlorophyll degradation, caused at least in part by the lower temperatures during the ripening period late in the growing season.

The time at which chlorophyll degradation is initiated is dependent upon the time that the cultivar requires to mature. Cultivars which begin chlorophyll breakdown later are more likely to be exposed to adverse environmental conditions, such as low temperatures, during seed ripening. As a result, these cultivars tend to be harvested prematurely and contain high seed chlorophyll levels.

Seed collected from all cultivars in the "Agroman" trials was analyzed to investigate the contribution of genotype and environment to the level of chlorophyll in canola seed at harvest. Both the genotype of the plant and environmental variables affect the chlorophyll content of canola seed. B. napus cultivars contained significantly higher seed chlorophyll levels than *B*. *campestris* cultivars. Among the *B*. *campestris* cultivars tested, there were no significant differences in the final chlorophyll levels, and all cultivars achieved acceptably low (<24 ppm) chlorophyll levels. There was considerable variation among the *B*. *napus* cultivars with and without triazine tolerance. Stellar tended to contain particularly high seed chlorophyll levels while Delta, Hero and Hyola 40 contained quite low levels.

A positive correlation was established between the number of days to maturity and the final seed chlorophyll content of the cultivars in the study. Premature harvest of late maturing cultivars results in the fixation of seed chlorophyll at high levels. However, exceptions to this also occur, indicating that the length of growing season required is not the only factor contributing to final seed chlorophyll levels.

The seed chlorophyll level at harvest for each cultivar varied considerably by location indicating that the environment has a significant effect on seed chlorophyll degradation. Environmental variables that may be involved include temperature, available moisture and length of growing season. Management practices may also contribute to the cultivar by location interaction.

A significant interaction also exists between the genotype of the plant and the environment. While some cultivars ranked consistently high or low in terms of seed chlorophyll content at all locations, other cultivars changed rank considerably at different sites. Therefore both the genotype of the plant and the environment in which it is grown contribute significantly to seed chlorophyll levels at harvest. Certain cultivars which consistently contain high or low levels of chlorophyll can be identified. Similarly, locations which regularly yield seed with high or low chlorophyll contents can be identified. However, in order to adequately assess the performance of any given cultivar it must be tested at each location of interest over a number of years.

The final chlorophyll level reached in harvested canola seed is therefore dependent on a number of factors. The genotype of the plant is one such factor, both the species and the cultivar within the species. Agronomic practices including late seeding, low seeding rates and swathing during periods of high temperature all contribute to the formation of small immature seed which may contain high levels of chlorophyll. The environment also plays a significant role in the seed chlorophyll level reached at harvest and there is a significant interaction between the environmental conditions and the genotype of the plant. A better understanding of the factors that affect the level of chlorophyll in canola seed is the first step in reducing the chlorophyll problem.

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6.0 APPENDIX

Table A1: Growth Stage Key for *Brassica* Crops

STAGE		DESCRIPTION OF PLANT
0		Preemergence
1		Seedling
2		Rosette
	2.1	First true leaf expanded
	2.2	Second 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 open, lower pods elongating
	4.3	Lower pods starting to fill
	4.4	Flowering complete, seeds enlarging
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: Growth Stage Data for Field Material

1988

EARLY SOWING - MAY 17th

	emerge	3.1	4.1	5.3	5.4	5.5
Westar	May 27	June 21	June 27	Aug 2	Aug 5	Aug 15
Tribute	May 27	June 20	June 27	Aug 2	Aug 8	Aug 15
Regent	May 30	June 22	June 29	Aug 5	Aug 10	Aug 22
Global	May 27	June 29	July 4	Aug 10	Aug 15	Aug 24

LATE SOWING - JUNE 7th

	emerge	3.1	4.1	5.3	5.4	5.5
Westar	June 17	July 11	July 20	Aug 17	Aug 26	Oct 3
Tribute	June 20	July 13	July 20	Aug 19	Aug 26	Oct 3
Regent	June 20	July 13	July 20	Aug 19	Aug 26	Oct 3
Global	June 19	July 20	July 29	Aug 29	Sept 13	NA

1989

FIRST SOWING - MAY 10th

	emerge	3.1	4.1	5.3	5.4	5.5
Westar	May 19	June 19	June 25	July 28	July 31	Aug 14
Tribute	May 19	June 19	June 25	July 28	July 31	Aug 14
Regent	May 19	June 19	June 27	Aug 3	Aug 8	Aug 21
Global	May 19	June 21	July 1	Aug 8	Aug 14	Aug 24

SECOND SOWING - MAY 24th

	emerge	3.1	4.1	5.3	5.4	5.5
Westar	June 5	June 29	July 4	Aug 8	Aug 14	Aug 24
Tribute	June 5	June 29	July 4	Aug 8	Aug 14	Aug 28
Regent	June 5	June 29	July 5	Aug 11	Aug 14	Sept 5
Global	June 5	July 3	July 10	Aug 14	Aug 21	Sept 5

THIRD SOWING - JUNE 9th

	emerge	3.1	4.1	5.3	5.4*	5.5
Westar	June 23	July 13	July 19	Aug 17	---	---
Tribute	June 23	July 10	July 17	Aug 17	---	---
Regent	June 23	July 13	July 19	Aug 24	---	---
Global	June 23	July 17	July 24	Aug 28	---	---

* plants were swathed after 5.3

Table A3: Daily Weather Conditions Recorded During the 1988 Field Study

DATE	TEMPERATURE HIGH (C)	LOW (C)	MEAN (C)	RAIN (mm)	SOLAR (cal/cm)	GDD
05/17	25.4	6.0	16.9	0.0	641.0	
05/18	30.0	13.7	19.1	8.9	440.0	
05/19	18.0	11.1	14.0	5.3	239.0	
05/20	14.0	11.2	12.9	0.0	205.0	
05/21	19.7	9.9	13.9	0.0	429.0	
05/22	25.9	7.3	17.6	0.0	690.0	
05/23	21.3	11.0	16.9	0.0	686.0	
05/24	24.5	5.4	15.7	0.0	676.0	
05/25	30.9	11.4	20.5	0.0	550.0	
05/26	25.9	7.7	18.3	0.0	548.0	
05/27	31.1	12.4	22.2	0.0	619.0	
05/28	30.0	14.0	21.9	5.1	465.0	
05/29	32.9	15.7	24.6	0.0	619.0	
05/30	31.7	18.3	25.7	0.0	547.0	
05/31	34.5	21.1	27.9	0.0	633.0	
06/01	33.9	21.4	26.6	0.0	569.0	
06/02	31.0	18.9	25.2	0.0	664.0	
06/03	32.2	17.2	25.5	0.0	666.0	
06/04	36.5	14.2	26.8	0.0	683.0	
06/05	36.1	21.9	29.3	0.0	694.0	
06/06	35.6	20.8	28.9	0.0	706.0	
06/07	37.3	19.3	28.8	0.0	679.0	
06/08	26.0	14.3	20.5	0.0	670.0	
06/09	27.3	8.7	19.1	0.0	717.0	
06/10	32.9	13.7	23.8	0.0	659.0	
06/11	36.6	17.5	26.8	7.9	642.0	
06/12	26.0	18.1	22.6	0.0	364.0	
06/13	25.5	15.9	20.8	0.0	704.0	
06/14	17.0	13.8	14.9	8.4	107.0	
06/15	17.9	11.4	14.7	1.3	429.0	
06/16	23.9	7.3	16.7	0.0	709.0	
06/17	32.0	12.8	22.4	3.3	588.0	
06/18	30.6	17.5	24.4	12.2	457.0	
06/19	30.0	17.8	24.9	0.0	712.0	
06/20	33.4	14.3	24.4	0.3	634.0	
06/21	32.7	22.5	26.9	0.0	609.0	
06/22	26.0	15.9	20.9	0.0	668.0	
06/23	28.3	13.7	20.5	6.1	446.0	
06/24	34.5	17.3	24.2	0.0	527.0	
06/25	25.6	15.5	20.3	0.0	567.0	
06/26	28.2	13.1	21.3	0.0	638.0	
06/27	33.8	16.2	25.3	2.3	623.0	
06/28	25.1	16.4	20.3	2.5	485.0	
06/29	26.0	11.0	19.0	0.0	741.0	

Table A3 (continued)

DATE	HIGH	LOW	MEAN	RAIN	SOLAR	GDD
06/30	28.7	9.2	20.3	0.0	736.0	
07/01	28.4	14.6	20.8	0.0	480.0	
07/02	29.8	14.3	22.6	0.0	701.0	
07/03	32.3	17.9	25.4	0.0	533.0	
07/04	31.0	17.1	25.4	0.0	606.0	
07/05	31.5	19.4	23.7	22.6	325.0	
07/06	29.3	21.1	24.4	1.3	316.0	
07/07	27.0	19.1	22.9	1.5	616.0	
07/08	31.0	15.6	23.8	0.0	619.0	
07/09	23.0	16.5	20.3	0.0	642.0	
07/10	20.1	11.8	16.0	0.0	434.0	
07/11	24.4	10.2	18.0	0.0	591.0	
07/12	29.5	10.7	19.5	12.2	564.0	
07/13	27.5	15.8	21.0	0.0	678.0	
07/14	22.2	13.1	18.4	1.0	315.0	
07/15	26.5	16.3	21.0	0.0	682.0	
07/16	26.8	12.9	19.9	0.0	374.0	
07/17	27.3	12.8	20.6	0.0	638.0	
07/18	27.4	15.5	21.3	0.0	597.0	
07/19	27.5	12.8	19.8	1.3	484.0	
07/20	28.0	13.4	20.7	0.0	627.0	
07/21	29.6	15.0	22.8	0.0	579.0	
07/22	34.7	14.8	25.4	0.0	659.0	
07/23	28.1	16.2	23.0	1.0	413.0	
07/24	27.1	13.5	20.9	0.0	593.0	
07/25	31.7	15.8	23.5	0.0	440.0	
07/26	32.3	18.7	25.0	0.0	602.0	
07/27	35.3	19.3	27.7	0.0	642.0	
07/28	36.4	21.8	27.5	0.3	439.0	
07/29	29.4	17.4	23.1	12.7	550.0	
07/30	29.6	14.6	22.5	0.0	629.0	
07/31	26.7	16.5	21.7	15.5	582.0	
08/01	26.9	14.0	20.3	0.0	356.0	*
08/02	25.0	15.8	20.8	0.0	236.0	15.8
08/03	25.4	17.9	21.4	0.0	553.0	32.2
08/04	25.8	14.0	20.5	0.0	446.0	47.7
08/05	34.2	12.5	23.8	0.0	605.0	66.5
08/06	38.9	16.4	28.2	0.0	540.0	89.7
08/07	28.5	14.8	22.9	0.3	427.0	107.6
08/08	25.4	9.5	18.4	0.0	569.0	121.0
08/09	31.8	16.2	23.7	0.0	544.0	139.7
08/10	37.3	17.6	27.1	0.0	500.0	161.8
08/11	29.3	16.7	23.7	0.0	512.0	180.5
08/12	28.8	16.8	21.1	4.3	363.0	196.6
08/13	26.2	17.8	21.8	0.0	242.0	213.4
08/14	31.2	17.2	23.9	0.0	546.0	232.3
08/15	35.6	16.9	26.5	0.0	557.0	253.8
08/16	29.1	20.1	24.3	0.0	358.0	273.1

Table A3 (continued)

DATE	HIGH	LOW	MEAN	RAIN	SOLAR	GDD
08/17	26.6	18.5	22.1	0.0	528.0	290.2
08/18	27.4	16.7	21.1	0.0	370.0	306.3
08/19	28.3	12.3	20.3	0.0	410.0	321.6
08/20	28.5	14.8	21.2	1.3	409.0	337.8
08/21	28.4	18.5	22.1	7.9	210.0	354.9
08/22	27.4	15.1	20.5	0.0	494.0	370.4
08/23	20.2	15.3	17.8	0.0	200.0	383.2
08/24	27.2	10.9	19.2	1.5	503.0	397.4
08/25	21.5	13.8	17.5	0.0	354.0	409.9
08/26	23.7	10.2	15.6	1.0	242.0	420.5
08/27	14.6	10.0	12.1	0.3	166.0	427.6
08/28	17.6	10.9	13.8	0.0	232.0	436.4
08/29	21.5	12.3	16.0	0.0	464.0	447.4
08/30	26.2	8.5	17.4	0.0	507.0	459.8
08/31	26.0	13.2	19.0	0.0	376.0	473.8
09/01	27.1	10.5	18.8	0.0	439.0	487.6
09/02	22.8	13.4	17.9	0.0	324.0	500.5
09/03	26.4	13.9	19.7	0.0	478.0	515.2
09/04	21.9	11.3	16.5	0.0	476.0	526.7
09/05	18.9	6.9	13.3	0.0	497.0	535.0
09/06	26.7	2.9	15.2	0.0	485.0	545.2
09/07	27.1	13.2	19.4	0.0	265.0	559.6
09/08	18.9	11.6	15.6	0.0	374.0	570.2
09/09	18.7	8.6	14.2	1.0	448.0	579.4
09/10	17.0	2.6	10.3	9.4	169.0	584.7
09/11	11.7	9.1	10.4	28.0	NA	590.1
09/12	15.7	7.2	11.5	0.0	NA	596.6
09/13	20.2	8.9	14.6	0.0	NA	606.2
09/14	22.6	3.9	13.3	0.0	NA	614.5
09/15	23.1	10.2	16.7	0.0	NA	626.2
09/16	15.1	12.9	14.0	0.9	NA	635.2
09/17	22.6	9.2	15.9	0.0	NA	641.1
09/18	12.4	7.4	9.9	13.0	NA	651.0
09/19	10.3	4.0	7.2	1.8	NA	653.2
09/20	10.2	3.2	6.7	0.6	NA	654.9
09/21	10.8	6.4	8.6	0.2	NA	658.5
09/22	17.6	7.0	12.3	0.4	NA	665.8
09/23	17.4	4.2	10.8	0.0	NA	671.6
09/24	19.3	4.6	12.0	0.0	NA	678.6
09/25	12.5	2.7	7.6	0.0	NA	681.2
09/26	17.4	7.6	12.5	0.0	NA	688.7
09/27	10.3	4.7	7.5	0.0	NA	691.2
09/28	12.9	6.4	9.7	0.0	NA	695.9
09/29	17.4	4.3	10.9	0.0	NA	701.8
09/30	24.0	8.6	16.3	0.0	NA	713.1
10/01	14.4	2.2	8.3	0.0	NA	716.4
10/02	14.1	-0.1	7.0	1.2	NA	718.4
10/03	7.7	-3.8	2.0	0.0	NA	718.4

Table A3 (continued)

DATE	HIGH	LOW	MEAN	RAIN	SOLAR	GDD
10/04	8.5	-6.5	1.0	0.0	NA	718.4
10/05	11.8	-2.6	4.6	0.0	NA	718.4
10/06	19.8	-1.0	9.4	0.0	NA	722.8
10/07	20.5	4.5	12.5	0.0	NA	730.3

* GDD calculated from August 2nd

Table A4: Daily Weather Conditions Recorded During the 1989 Field Study

DATE	TEMPERATURE HIGH (C)	LOW (C)	MEAN (C)	RAIN (mm)	SOLAR (cal/cm)	GDD
05/10	27.3	16.6	23.8	0.0	329.4	
05/11	27.0	13.1	20.4	0.0	575.9	
05/12	27.8	12.9	20.6	0.0	651.5	
05/13	29.5	8.1	20.3	0.0	663.2	
05/14	30.2	10.1	21.0	0.0	519.0	
05/15	30.4	12.8	22.0	0.0	362.2	
05/16	30.9	17.4	24.4	0.0	475.6	
05/17	24.5	14.5	19.3	15.5	256.5	
05/18	27.2	13.3	19.6	0.0	650.8	
05/19	18.0	8.8	14.7	12.7	104.9	
05/20	20.1	7.5	14.0	0.0	491.4	
05/21	26.3	7.6	17.6	0.0	499.1	
05/22	22.7	8.1	16.0	0.0	341.9	
05/23	22.3	9.6	15.8	0.0	615.4	
05/24	12.8	9.6	11.5	1.0	92.4	
05/25	9.7	4.2	7.6	5.3	204.7	
05/26	15.2	3.4	8.8	0.0	686.0	
05/27	23.0	3.2	14.8	2.5	426.9	
05/28	13.6	7.0	10.5	0.0	621.4	
05/29	16.8	6.0	11.1	0.0	581.8	
05/30	21.1	9.4	15.0	0.0	498.1	
05/31	23.8	10.3	17.4	0.0	658.9	
06/01	26.3	10.2	18.1	1.5	510.3	
06/02	19.3	9.2	14.3	0.0	652.5	
06/03	23.3	5.0	13.8	0.0	648.3	
06/04	16.9	1.6	11.1	0.0	418.9	
06/05	21.6	10.9	16.2	3.6	647.7	
06/06	16.5	8.3	13.2	15.0	171.5	
06/07	15.0	8.6	11.6	23.9	290.1	
06/08	19.1	5.2	12.9	0.0	728.0	
06/09	23.1	5.4	15.6	0.0	724.0	
06/10	24.5	8.0	17.9	0.0	513.7	
06/11	22.7	12.2	17.2	8.6	228.2	
06/12	16.5	10.5	13.7	35.3	59.3	
06/13	19.2	8.6	13.4	7.9	446.5	
06/14	22.5	7.3	15.9	0.0	724.0	
06/15	25.4	9.1	18.1	0.0	672.4	
06/16	26.7	13.8	20.5	0.0	725.0	
16/17	23.7	14.6	19.1	1.0	410.3	
06/18	28.0	13.3	20.7	0.0	686.0	
06/19	30.8	13.4	23.0	0.0	722.0	
06/20	29.7	20.9	24.2	0.0	429.6	
06/21	24.7	16.9	20.3	0.0	410.5	
06/22	24.7	15.8	20.3	0.0	668.1	
06/23	22.4	14.1	17.3	3.8	361.2	

Table A4 (continued)

DATE	HIGH	LOW	MEAN	RAIN	SOLAR	GDD
06/24	26.6	10.9	17.0	53.9	427.2	
06/25	24.5	14.0	18.8	1.3	609.1	
06/26	19.3	13.3	16.3	2.0	379.3	
06/27	22.8	12.0	17.1	0.8	565.5	
06/28	25.3	12.5	18.8	0.0	570.9	
06/29	22.9	16.4	19.2	14.5	185.8	
06/30	30.6	16.7	23.9	0.0	699.1	
07/01	31.6	18.0	25.3	0.0	701.0	
07/02	27.5	19.6	24.4	0.0	521.9	
07/03	26.8	14.5	21.3	0.0	715.0	
07/04	32.6	14.6	24.5	0.0	679.9	
07/05	27.9	21.1	24.2	13.0	361.8	
07/06	25.0	16.4	20.7	0.0	641.8	
07/07	27.9	13.7	21.4	0.0	627.2	
07/08	24.8	17.9	21.0	2.3	247.8	
07/09	27.8	16.9	21.6	0.0	545.9	
07/10	24.0	16.3	20.1	0.0	552.1	
07/11	28.9	16.5	22.1	0.0	577.8	
07/12	24.5	17.4	20.9	13.5	439.3	
07/13	27.6	15.9	21.8	0.0	617.1	
07/14	28.5	14.9	21.5	10.9	526.2	
07/15	28.9	14.4	22.4	0.0	672.3	
07/16	30.6	15.1	23.6	0.0	660.3	
07/17	30.4	18.0	24.1	1.3	582.3	
07/18	29.1	16.5	23.2	0.0	597.6	
07/19	31.7	19.1	25.4	0.0	588.2	
07/20	33.0	17.1	25.7	0.0	628.0	
07/21	34.1	17.2	26.2	0.0	634.9	
07/22	32.6	16.6	25.6	0.0	593.6	
07/23	31.5	18.0	25.1	0.0	588.1	
07/24	30.5	20.5	25.4	0.0	420.6	
07/25	35.6	20.8	27.2	0.0	608.2	
07/26	27.7	17.5	23.3	1.5	636.0	
07/27	22.6	14.5	18.3	0.0	512.6	
07/28	22.6	15.0	19.2	0.0	366.9	
07/29	25.2	17.5	20.8	0.0	253.2	
07/30	28.7	18.5	23.0	0.0	498.0	**
07/31	26.4	15.8	21.9	0.0	293.7	16.9
08/01	37.5	20.9	28.8	0.0	572.4	40.7
08/02	35.1	23.0	28.2	2.0	509.2	63.9
08/03	32.7	19.4	24.0	17.8	405.8	82.9
08/04	28.0	16.3	22.0	0.0	574.5	99.9
08/05	18.2	13.0	15.0	1.0	119.9	109.9
08/06	23.5	9.9	17.4	0.0	527.6	122.3
08/07	26.2	11.0	19.8	0.0	582.9	137.1
08/08	28.9	14.3	21.4	0.0	550.9	153.5
08/09	32.0	12.0	22.4	0.0	585.5	170.9
08/10	33.7	13.9	24.0	0.0	573.1	189.9

Table A4 (continued)

DATE	HIGH	LOW	MEAN	RAIN	SOLAR	GDD
08/11	33.4	15.8	24.5	0.0	541.1	209.4
08/12	31.9	18.3	24.4	15.5	523.4	228.8
08/13	21.9	14.7	17.9	0.3	119.1	241.7
08/14	21.9	12.3	16.6	0.0	339.4	253.3
08/15	25.4	8.1	17.4	0.0	585.7	265.7
08/16	28.3	9.8	19.6	0.0	575.7	280.3
08/17	29.5	13.0	21.5	0.0	422.0	296.8
08/18	24.3	18.1	20.4	26.2	207.6	312.2
08/19	19.9	12.2	17.6	4.8	173.1	324.8
08/20	15.9	9.9	13.0	0.0	260.2	332.8
08/21	22.9	8.2	16.4	0.0	365.3	344.2
08/22	28.3	13.5	20.7	0.0	476.0	359.9
08/23	30.5	15.2	23.0	0.0	518.0	377.9
08/24	30.9	17.7	24.1	0.0	517.1	397.0
08/25	25.1	18.2	21.8	0.0	267.4	413.8
08/26	24.7	14.9	20.7	0.0	446.8	429.5
08/27	23.8	10.1	17.7	0.0	394.8	442.2
08/28	22.3	15.8	18.3	0.8	261.2	455.5
08/29	15.9	10.0	14.0	5.6	154.3	464.5
08/30	19.5	6.0	12.9	0.0	323.5	472.4
08/31	20.6	11.7	16.0	0.0	399.3	483.4
09/01	22.8	10.1	16.5	0.0	518.4	494.9
09/02	26.9	6.8	17.3	0.0	483.7	507.2
09/03	23.1	12.8	17.7	0.3	224.5	519.9
09/04	23.5	14.0	18.2	0.0	467.2	533.1
09/05	29.2	11.4	19.6	0.0	488.4	547.7

** GDD calculated from July 31st

Table A5: Days to Maturity for Cultivars in the "Agroman" Trials

CULTIVAR	# YEARS TESTED	DAYS TO MATURITY
B. napus		
Alto	2	95
Celebra	3	93
Delta	2	93
Global	3	100
Hero	3	89
Hyola 40	3	91
Legend	2	93
Profit	3	91
Regent	4	94
Stellar	3	94
Topas	2	102
Vanguard	3	91
Westar	5	92
Triazine tolerant		
ASC-N4-TT	2	84
OAC Triton	5	94
OAC Triumph	2	96
SV8525953	3	90
Tribute	4	93
B. campestris		
Colt	2	82
Horizon	2	82
Parkland	3	78
Tobin	5	82

Table A6: Percentage of Seed Falling Into Each Size Class In the 1988 "Agroman" Trials

	SMALL		MEDIUM		LARGE	
	%	STE	%	STE	%	STE
Loc:Bagot						
Global	3.40	0.44	44.75	3.34	51.85	3.53
Regent	5.98	1.20	76.75	1.88	17.30	3.05
Tribute	5.13	0.52	77.75	1.54	17.13	2.02
Triton	5.75	1.71	71.38	4.45	22.90	6.06
Westar	2.83	0.38	76.88	1.98	20.28	2.35
Loc:Mariapolis						
Global	3.05	0.66	56.13	4.90	40.80	5.58
Regent	2.53	1.06	56.70	3.72	40.73	4.76
Tribute	0.80	0.19	36.15	6.00	63.05	6.13
Triton	1.73	0.50	38.05	8.40	60.20	8.88
Westar	0.73	0.08	22.73	2.53	76.55	2.60
Loc:Melita						
Global	3.83	0.38	61.90	1.81	34.25	1.89
Regent	2.50	0.66	58.40	4.05	39.08	4.38
Tribute	1.53	0.10	44.88	3.48	53.65	3.40
Triton	2.00	0.38	51.45	4.41	46.53	4.61
Westar	1.18	0.27	37.38	4.51	61.45	4.53
Loc:Roblin						
Global	0.85	0.09	44.38	4.08	54.80	4.17
Regent	1.73	0.38	70.13	1.99	28.18	2.38
Tribute	2.43	0.41	76.05	1.50	21.50	1.78
Triton	1.47	0.12	65.20	0.75	33.33	0.78
Westar	1.10	0.25	60.60	2.04	38.28	2.16
Loc:Shoal Lake						
Global	10.70	1.91	72.20	0.51	17.05	1.57
Regent	8.95	1.18	80.78	2.06	10.30	2.08
Tribute	10.25	1.42	80.63	1.23	9.13	1.22
Triton	13.50	1.84	78.80	1.05	7.70	2.56
Westar	4.88	0.83	78.05	2.61	17.08	3.14
Loc:Waskada						
Global	10.08	0.54	71.50	1.40	18.43	1.56
Regent	4.98	0.38	81.05	0.70	13.95	0.74
Tribute	1.93	0.23	57.25	1.99	40.83	2.14
Triton	11.70	5.82	68.73	6.22	19.55	1.01
Westar	1.40	0.24	50.55	2.47	48.08	2.68

Table A7: Percentage of Seed Falling Into Each Size Class In the 1989 "Agroman" Trials

	SMALL		MEDIUM		LARGE	
	%	STE	%	STE	%	STE
Loc:Beausejour						
Global	18.30	2.68	45.28	3.14	36.40	5.53
Regent	24.13	5.06	48.73	2.01	27.15	6.07
Tribute	40.98	5.54	46.85	3.16	12.13	4.25
Triton	24.33	0.50	52.90	1.09	22.80	1.38
Westar	15.80	1.50	55.23	1.28	29.03	2.33
Loc:Mariapolis						
Global	70.80	2.86	27.00	2.70	2.23	0.35
Regent	55.28	3.48	39.98	2.83	4.75	0.66
Tribute	77.88	2.17	20.93	1.99	1.20	0.18
Triton	55.68	5.79	39.93	4.72	4.40	1.37
Westar	35.28	6.73	57.78	5.77	6.98	1.63
Loc:Melita						
Global	32.13	8.42	39.80	1.56	28.07	9.68
Regent	40.47	5.03	43.37	1.99	16.17	3.24
Tribute	36.25	2.54	48.25	1.33	15.53	1.29
Triton	50.40	7.65	40.17	3.89	9.43	3.79
Westar	21.23	4.90	43.80	2.77	34.97	7.47
Loc:The Pas						
Global	38.73	3.89	46.40	2.52	14.88	1.62
Regent	44.57	2.34	43.93	1.89	11.53	0.61
Tribute	38.00	5.36	49.57	3.34	12.43	2.24
Triton	40.73	1.84	45.80	0.40	13.40	1.42
Westar	21.28	0.64	48.75	0.88	29.98	1.46
Loc:Shoal Lake						
Global	66.58	4.05	28.83	3.22	4.65	0.88
Regent	64.08	1.24	31.43	0.90	4.45	0.41
Tribute	72.05	6.28	25.95	5.70	1.95	0.56
Triton	32.15	0.94	53.45	0.31	14.38	0.85
Westar	32.25	8.39	53.38	5.06	14.40	3.45
Loc:Teulon						
Global	26.10	2.02	47.63	2.72	26.28	4.56
Regent	41.90	7.91	45.03	4.62	13.08	3.29
Tribute	40.05	3.60	47.40	1.27	12.60	2.54
Triton	32.17	4.48	49.03	0.28	18.80	4.76
Westar	23.30	2.54	50.35	3.12	36.33	5.44

Table A8: Thousand Seed Weights for Seeds of Varying Size From the 1988 "Agroman" Trials

	SMALL		MEDIUM		LARGE	
	WT(g)	STE	WT(g)	STE	WT(g)	STE
Loc:Bagot						
Global	1.32	0.036	3.02	0.085	4.68	0.054
Regent	1.49	0.040	2.71	0.071	3.98	0.119
Tribute	1.51	0.046	2.84	0.033	4.11	0.071
Triton	1.49	0.121	2.51	0.219	3.90	0.206
Westar	1.53	0.069	2.89	0.086	4.01	0.046
Loc:Mariapolis						
Global	1.63	0.027	2.99	0.045	4.48	0.074
Regent	1.54	0.034	3.06	0.062	4.35	0.047
Tribute	1.56	0.087	3.23	0.052	4.77	0.133
Triton	1.46	0.054	2.91	0.061	4.57	0.063
Westar	1.37	0.022	3.04	0.064	4.74	0.056
Loc:Melita						
Global	1.54	0.043	2.93	0.061	4.60	0.034
Regent	1.51	0.040	2.96	0.064	4.14	0.036
Tribute	1.57	0.029	3.09	0.032	4.49	0.078
Triton	1.42	0.060	2.99	0.036	4.21	0.073
Westar	1.59	0.147	3.10	0.061	4.57	0.055
Loc:Roblin						
Global	1.62	0.032	3.37	0.021	4.55	0.039
Regent	1.75	0.039	3.10	0.043	4.20	0.041
Tribute	1.80	0.025	3.00	0.040	4.02	0.035
Triton	1.74	0.015	3.06	0.012	4.25	0.034
Westar	1.72	0.085	3.23	0.023	4.32	0.057
Loc:Shoal Lake						
Global	1.71	0.050	2.97	0.024	4.03	0.090
Regent	1.72	0.051	2.69	0.046	3.89	0.164
Tribute	1.72	0.043	2.85	0.043	4.08	0.117
Triton	1.67	0.065	2.69	0.079	3.78	0.160
Westar	1.81	0.036	2.88	0.073	4.03	0.023
Loc:Waskada						
Global	1.65	0.038	3.06	0.110	4.46	0.094
Regent	1.66	0.066	3.02	0.051	4.16	0.025
Tribute	1.63	0.029	3.31	0.093	4.66	0.072
Triton	1.87	0.186	3.01	0.135	4.16	0.055
Westar	1.67	0.023	3.28	0.029	4.53	0.072

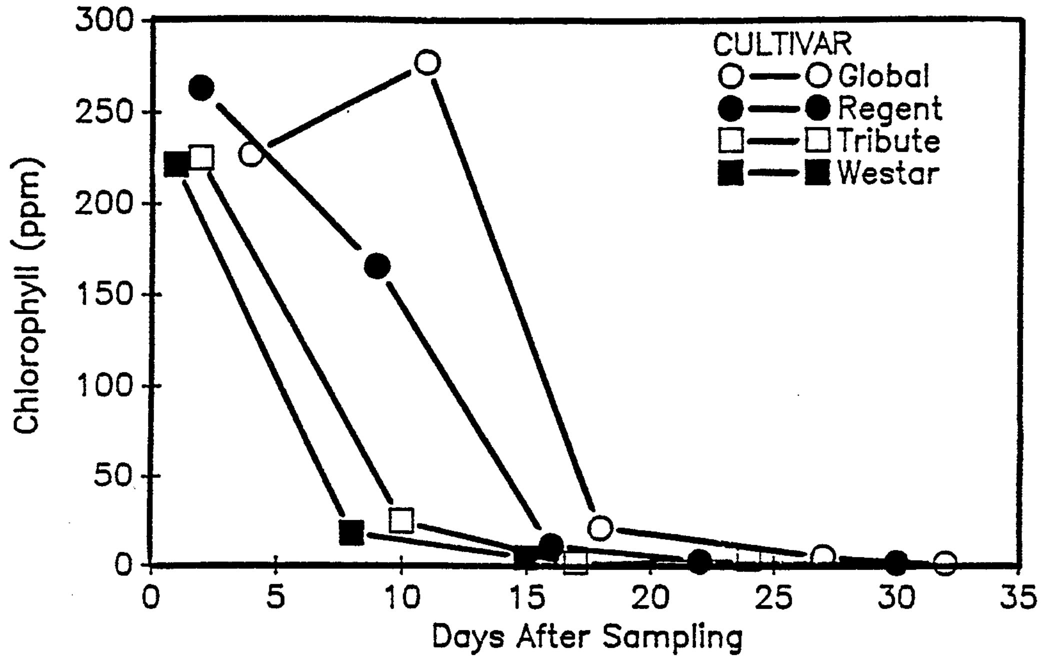


Fig. A1 : Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1988. Chl vs Time.

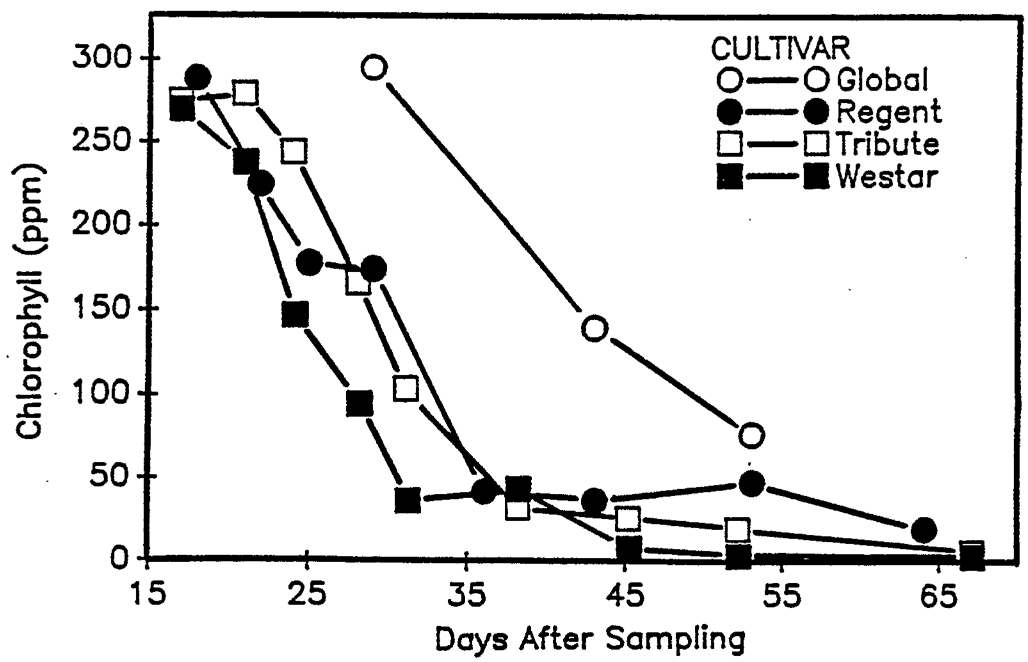


Fig. A2 : Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1988. Chl vs Time.

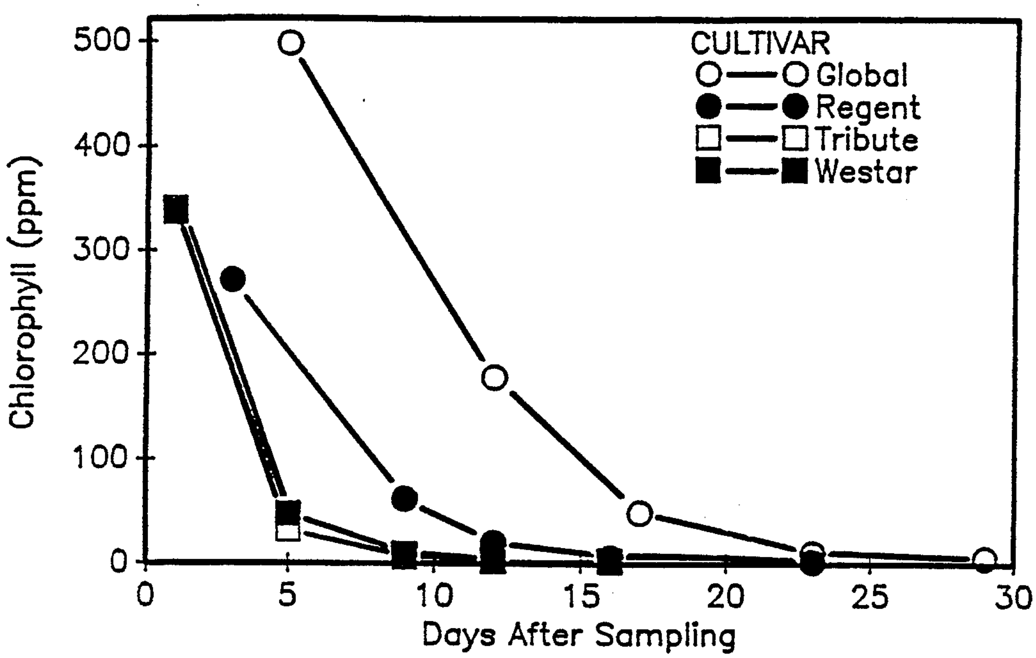


Fig. A3 : Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1989. Chl vs Time.

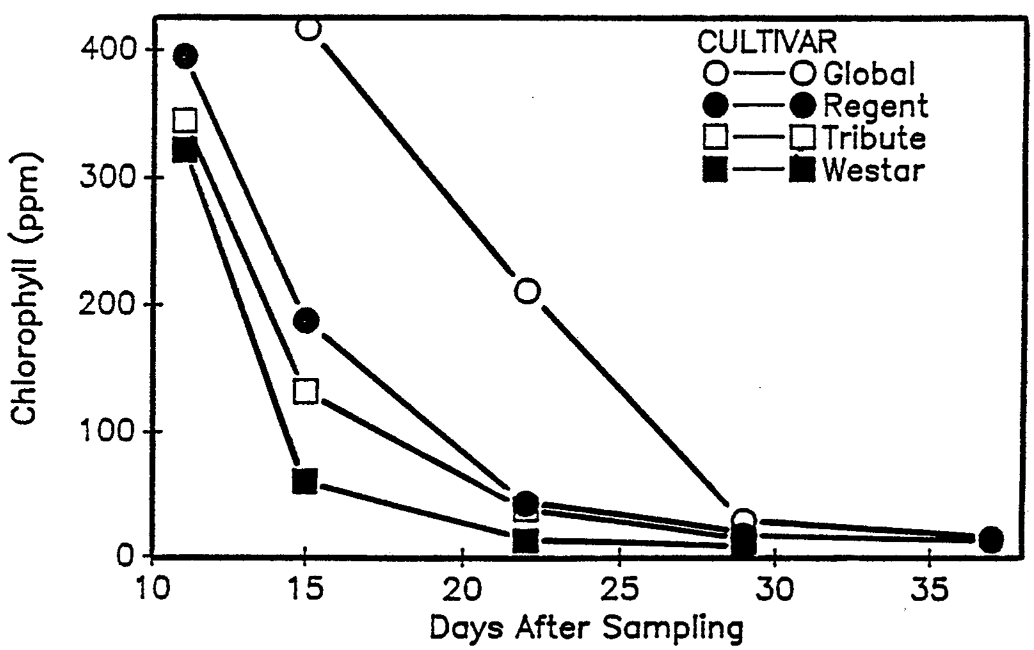


Fig. A4 : Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1989. Chl vs Time.

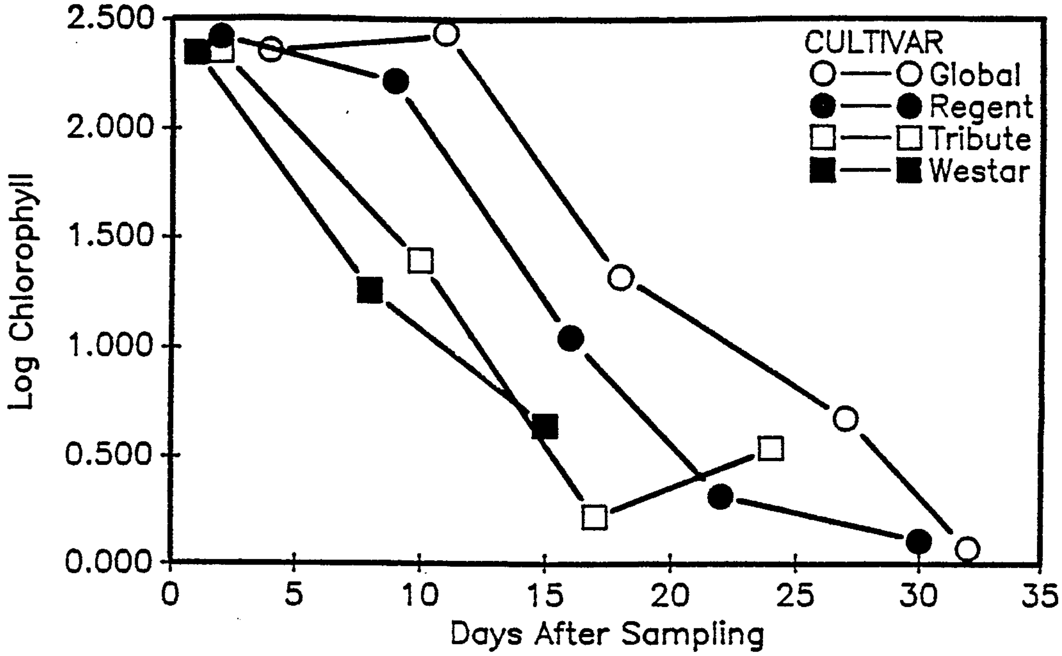


Fig. A5 : Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1988. Logchl vs Time.

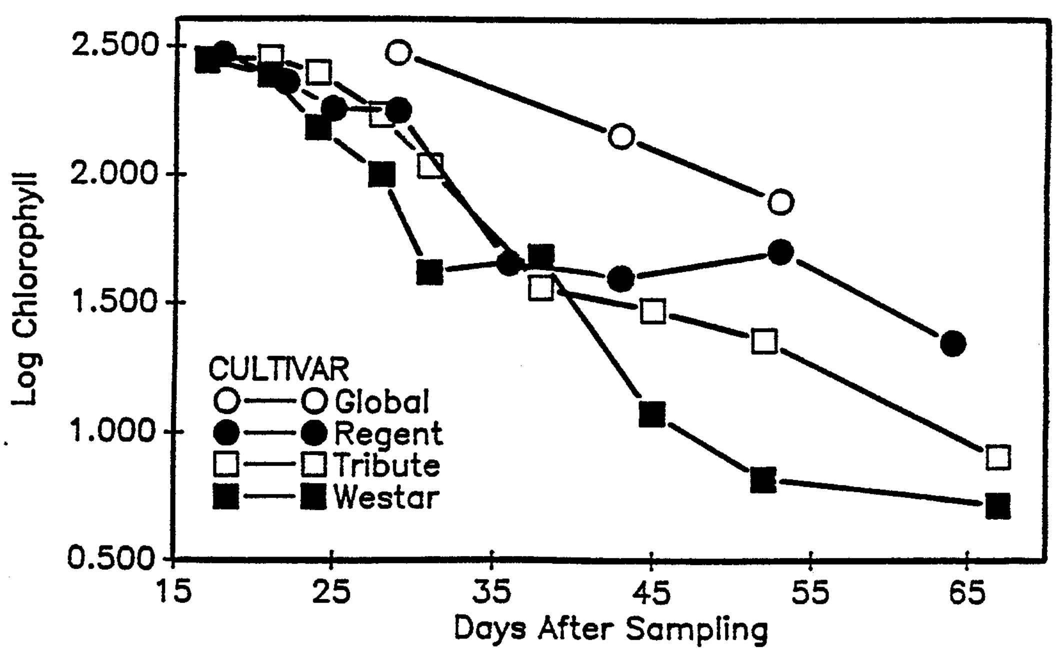


Fig. A6 : Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1988. Logchl vs Time.

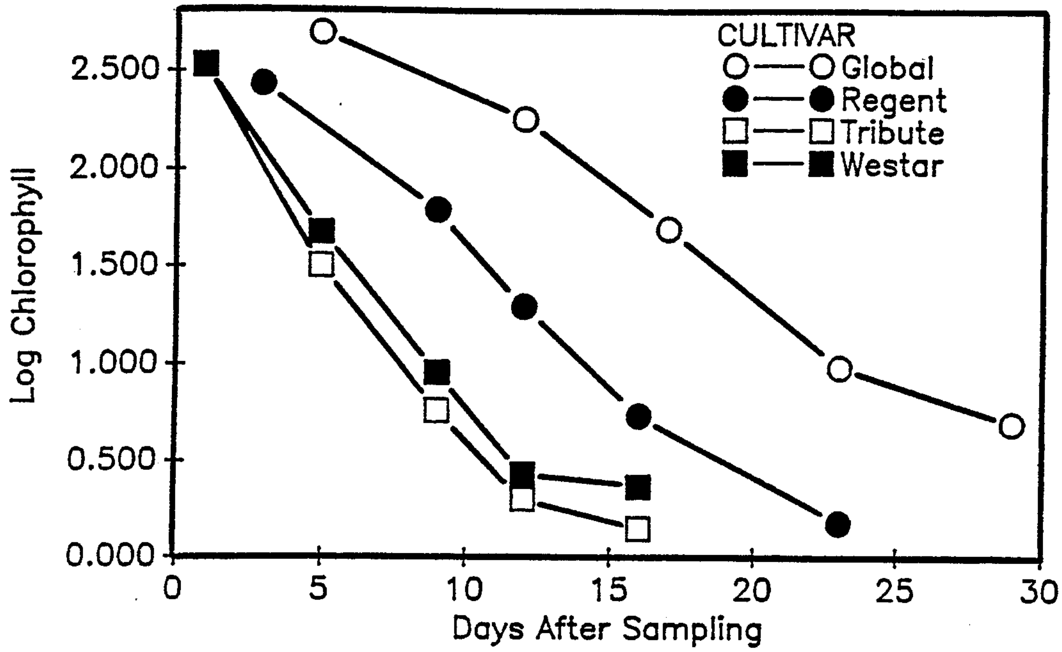


Fig. A7 : Chlorophyll degradation rates in four cultivars of B. napus. Early sowing 1989. Logchl vs Time.

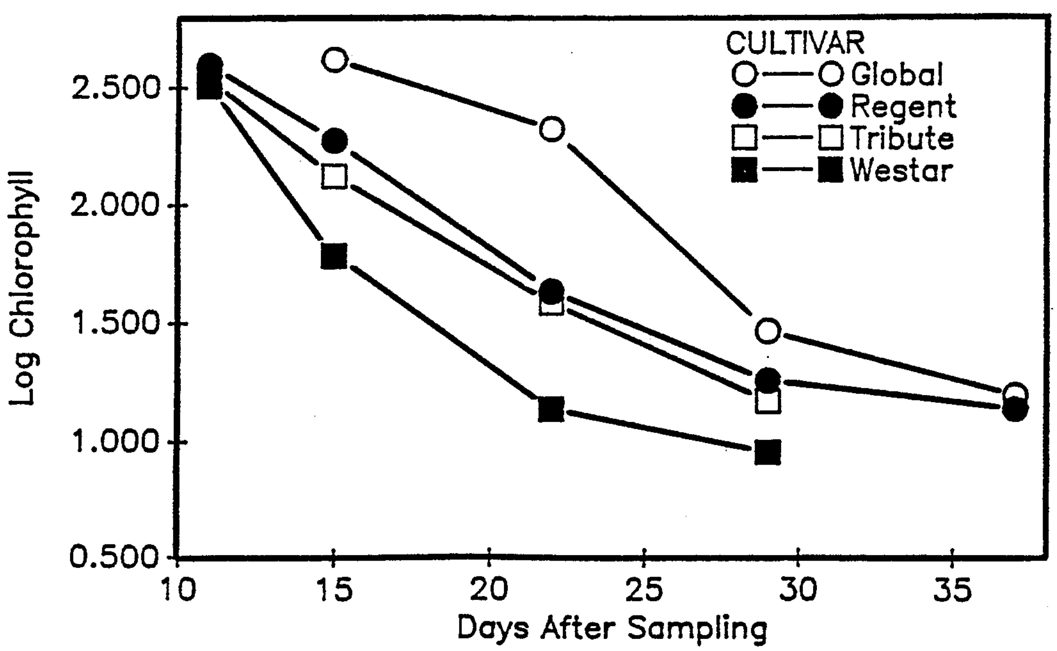
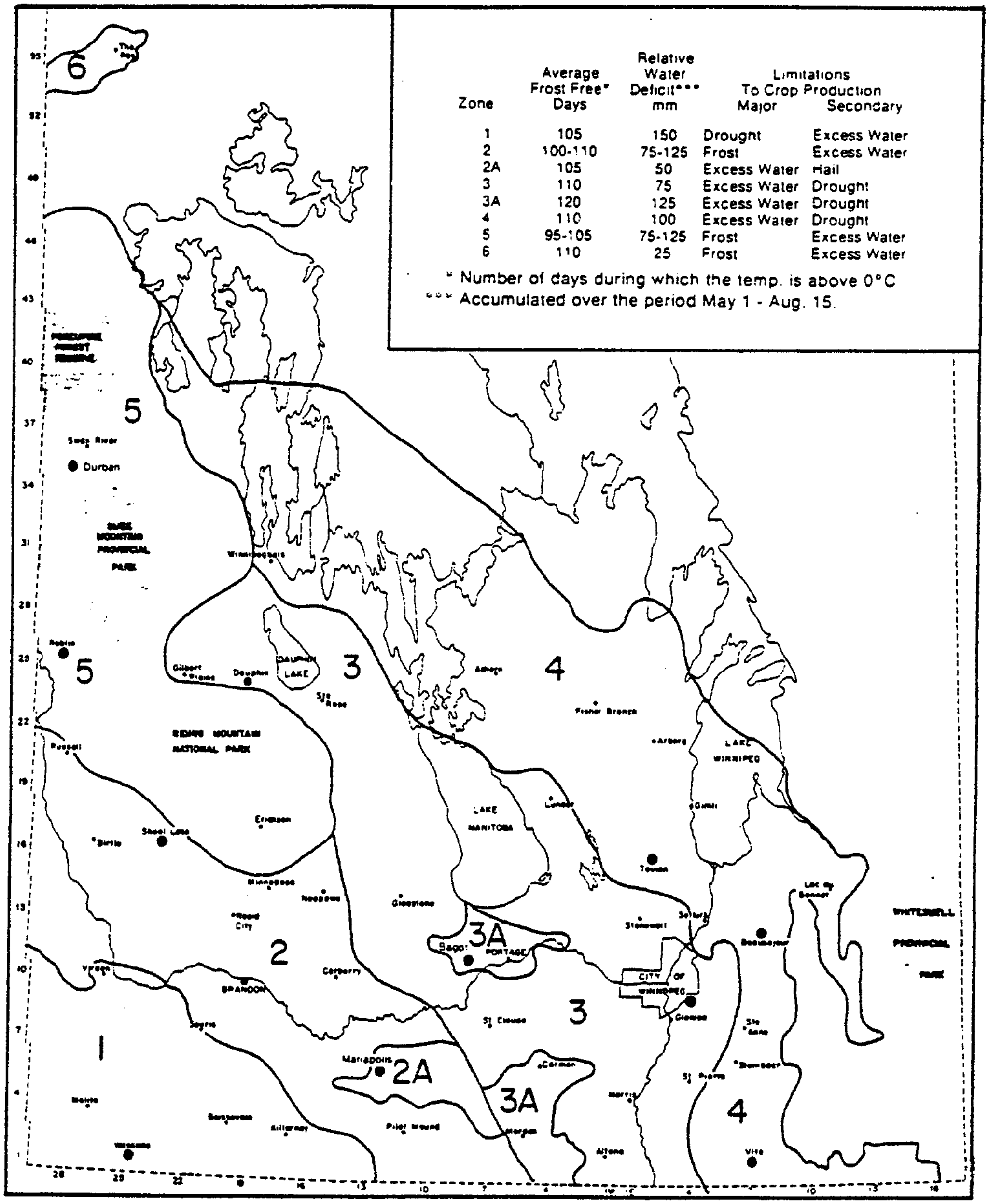


Fig. A8 : Chlorophyll degradation rates in four cultivars of B. napus. Late sowing 1989. Logchl vs Time.



● CROP VARIETY ADAPTATION TRIALS.

Fig. A9 : Crop zones for the "Agroman" field trials.