PROJECT REPORT No. OS61

IDENTIFYING THE FACTORS DETERMINING THE CHLOROPHYLL CONTENT OF UK RAPESEED

JANUARY 2003

Price: £4.25

PROJECT REPORT No. OS61

IDENTIFYING THE FACTORS DETERMINING THE CHLOROPHYLL CONTENT OF UK RAPESEED

by

I.J. BINGHAM[1], S.P.J. KIGHTLEY[2] AND K. WALKER[1]

[1]SAC, Division of Plant and Crop Science, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA
[2]NIAB, Huntingdon Road, Cambridge, CB3 0LE

In collaboration with:
A. Guttridge (Cargill plc) and B. Edmunds (Unitrition)

This is the final report of a one year project which started in July 2001. The work was funded by a grant of £20,597 from HGCA (project no. 2518).

CONTENTS

	Page no.
Abstract	1
Summary	2
Technical Detail	6
1. Introduction	6
2. Aims and Objectives	7
3. Materials and Methods	9
4. Results	13
5. Discussion	26
6. Conclusions and Recommendations	34
7. Acknowledgements	35
8. References	36
Appendix A	38

ABSTRACT

During ripening of oilseed rape seed, chlorophyll pigments are steadily broken down. Any chlorophyll remaining at harvest is extracted along with the oil during the crushing process. Since the chlorophyll interferes with processing, it must be removed during refining. Chlorophyll concentrations in oil extracted from UK-produced rapeseed are usually higher (50%) than those from seed imported from continental Europe. In recent years, they have been especially high (up to 3 fold higher), leading to problems during refining and increasing refining costs. The higher concentrations in UK-produced seed, compared to seed from continental Europe, threatens the competitiveness of the UK industry, as importing seed or crude oil with a lower chlorophyll concentration becomes an increasingly attractive option for the crushers and refiners. Research was undertaken to investigate the possible causes of the high concentrations in the UK. The objective was to determine the extent of any variation between varieties, variety types (hybrids versus conventional lines), sites and seasons.

Seed samples from 13 varieties harvested in 2001 were collected from the recommended list trials at 5 sites, and analysed for chlorophyll. Varieties differed significantly in their seed chlorophyll concentration. The most popular UK variety, Apex, had the highest concentration. Collectively, restored hybrids had lower concentrations than conventional lines or varietal associations. Differences in maturity class between varieties did not account for much of the variation in seed chlorophyll concentration. There were differences between sites, but no clear effect of latitude on the chlorophyll concentration.

Large seasonal fluctuations in the chlorophyll concentration of commercial crude oil were observed. 2001 was a relatively low chlorophyll year (30 mg per kg oil) whereas 1999 and 2000 were high years (60 and 50 mg per kg respectively). Concentrations of chlorophyll in samples of seed from commercial crops in 2000 and from recommended list trials in 1999, were correspondingly high. The high concentrations appear to be related to periods of particularly dry weather between swathing and harvesting. It is possible that premature harvesting in periods of unsettled weather may also contribute to the problem.

A brief survey of oilseed rape agronomy indicated that the major differences between the UK and continental Europe are the varieties grown and the method of harvest. In Europe most crops are directly combined, very few are swathed or desiccated beforehand. In France, warm dry weather leading up to harvest is the norm indicating that dry weather does not inevitably lead to high seed chlorophyll concentrations.

There may be scope for reducing the chlorophyll concentration to values found in European seed, through use of lower chlorophyll varieties (both hybrids and conventional lines) and through possible improvements in harvest timing or method. Further research is needed to determine the effects of harvest method and timing on the process of chlorophyll breakdown during seed ripening under UK conditions before firm recommendations can be made.

SUMMARY

Chlorophyll and other related pigments are present, in high concentrations, in the seeds of oilseed rape at the end of seed development and before ripening. During ripening, the chlorophyll pigments are steadily degraded, but some may remain at harvest. Most of the residual chlorophyll is then extracted along with the oil during the commercial crushing process. Chlorophyll pigments in oil lead to a number of problems. They can interfere with processing, they can result in rancidity and they produce an undesirable colour. The pigments, therefore, have to be removed during refining which increases costs significantly and results in a loss of oil.

Crushers and refiners have recently voiced concern about higher than usual concentrations of chlorophyll in crude oil from UK-produced seed. Even in a normal ‘low chlorophyll’ year, concentrations in oil from UK seed are typically 50% higher than those in oil extracted from seed imported from neighbouring European countries (France, Germany and Denmark). In recent years, they have been as much as 3 times greater. As the main competition for UK rapeseed comes from continental Europe, the high chlorophyll concentrations seriously weaken the competitiveness of the UK industry. The crushers and refiners are particularly concerned that the high concentrations seen in recent years may reflect a growing trend associated with some change in agronomic practice.

In order to ensure UK growers remain competitive, it is important to provide the industry with information on the best way to achieve consistently low chlorophyll concentrations in the domestic crop. However, before agronomic solutions to the problem can be offered, it is first necessary to establish what factors contribute to the high chlorophyll concentrations in UK produced seed.

Several possible explanations for the high concentrations were proposed, including: changes in the varieties being grown, changes in variety type (i.e. hybrids versus conventional varieties), and the use of lower seed rates with the hybrids leading to more branching and immature pods at harvest. In addition, seasonal variations in weather patterns during the harvest period may contribute to high residual chlorophyll, either by interfering with the process of chlorophyll breakdown or by encouraging premature harvesting.

Aims and objectives

Research was undertaken, in a pilot project, to investigate the factors determining the chlorophyll concentration of UK rapeseed. There were two elements to the project. The first was to compare the agronomy of oilseed rape in the UK with that in neighbouring European countries in order to

identify possible reasons why European seed may have lower chlorophyll concentrations. The second was to determine the extent of variation in UK seed associated with variety, variety type, site and season.

Approach and Key Results

To provide information for the review of agronomic practice, a questionnaire was sent to three leading agronomists in France, Germany and Denmark. These are countries that export significant amounts of rapeseed to the UK. Information was sought on a range of factors including varieties grown, seed rates used, harvest methods, and typical weather conditions during harvest. The most striking difference between the UK and the other European countries is in the harvest method. A much larger proportion of the crop is directly combined compared with the UK, relatively little is swathed and hardly any is desiccated. Another obvious difference is in the actual varieties grown.

Cargill plc and Unitrition provided data for chlorophyll concentrations in crude oil. Concentrations in oil extracted from seed harvested in 2001 were in the range 30-40 mg per kg of oil (when measured and expressed as pheophytin, the main chlorophyll pigment in oil). From the 1999 and 2000 harvests, the concentrations were 60 and 50 mg per kg oil respectively. During this period, concentrations in oil from seed imported from continental Europe were, typically, between 17 and 19 mg per kg. An important target for the UK, therefore, is to reduce the base levels in a 'low chlorophyll' year, such as 2001, to those of European seed and to minimise the seasonal fluctuations.

The Recommended List (RL) trials provided the opportunity to sample and analyse seed from a common group of varieties grown to an identical protocol in contrasting agro-climatic regions of the UK. In the harvest year 2001, seed was collected from 13 varieties at 5 different RL trial sites. The sites were located in Scotland, Northumberland, Shropshire, Norfolk, and Hampshire.

Varieties differed significantly in their seed chlorophyll concentration at harvest. When averaged across sites, Apex had the highest concentration of 5.6 mg per kg seed weight and Pronto (2.2 mg per kg) and Fortress (2.4 mg per kg) the lowest. The varietal differences were independent of differences in maturity rating. There were also significant differences between sites, with Morley in Norfolk producing the highest concentration (averaged across varieties, 5.4 mg per kg) and Edinburgh the lowest (1.8 mg per kg). However, there was no discernible relationship between latitude of the site and the seed chlorophyll concentration.

Restored hybrids had lower concentrations than conventional lines and varietal associations. This is contrary to expectation since the lower seed rate used with hybrids would be expected to lead to greater branching and more immature seeds at harvest. The concentrations found in seed from the RL trials were comparable to those found in seed collected from commercial crops grown in the same geographical regions.

Although the differences between varieties in 2001 appear to be small, choice of variety could have an important effect on the concentration in crude oil. The concentration found in Apex (5.6 mg per kg seed weight) equates to a concentration in crude oil of 33 mg per kg, whilst that in Pronto (2.2 mg per kg seed weight) equates to a concentration in crude oil of only 19 mg per kg. Thus greater production of low chlorophyll varieties such as Pronto, Fortress and Escort, in place of Apex, could help reduce the concentration towards values obtained from imported seed.

The extent of seasonal variation on seed chlorophyll concentrations was investigated by analysing seed of selected varieties taken from RL trials conducted in Aberdeenshire between 1995 and 1999. The seed had been stored dry, in the dark and at cool temperatures. Under these conditions the chlorophyll was considered to be relatively stable. Chlorophyll concentrations were low in 1996 and 1998, but high in 1995 and 1999. The high concentrations appear to be associated with dry weather during seed ripening. No rain fell between swathing and harvesting in 1995 and 1999.

Why dry weather should lead to high concentrations was not investigated in the present study. From the crusher's experience, high concentrations are not usually found in oil from French seed, yet harvesting in warm dry conditions is the norm for France. It is possible that high concentrations result from rapid drying of the seed after swathing, preventing chlorophyll breakdown and effectively trapping it in the seed. The rate of seed drying may be slower in standing crops, allowing a longer period for chlorophyll degradation. More chlorophyll might become trapped if swathing is done prematurely. Another factor that might contribute to high seed chlorophyll concentrations is premature harvesting. This might occur in periods of unsettled weather when growers are anxious to get harvesting completed.

Conclusions and implications

The major factor contributing to the high chlorophyll concentrations experienced by crushers in recent years appears to be the weather pattern during seed ripening and harvesting. We propose that high concentrations could result from a slower breakdown of chlorophyll during seed ripening in swathed crops in dry weather, or a tendency for premature harvesting before the chlorophyll has fully degraded. There may be scope for reducing the seasonal fluctuations in chlorophyll concentration through improved harvest technique or timing. However, further research is needed to establish the effects of swathing on the rate of chlorophyll degradation under UK conditions, before an agronomic solution can be recommended. Encouragement of the wider adoption of low chlorophyll varieties, both conventional lines and hybrids, would help reduce concentrations, even in a low chlorophyll year.

TECHNICAL DETAIL

1. Introduction

Embryos of fully developed oilseed rape seed contain chloroplasts and large concentrations of chlorophyll and other related pigments (Johnson-Flanagan and Thiagarajah, 1990; Ward et al. 1994)). During seed ripening the chlorophyll pigments are steadily degraded and changes in pigment composition occur. In green seed, chlorophyll *a*, chlorophyll *b* and pheophytin *a* are the major pigments (Ward et al. 1994), while pheophytin *b*, pheophorbides, methylpheophorbides and pyropheophorbides are present in minor quantities. In ripe seed, the pigments are mostly chlorophyll *a* and chlorophyll *b* (Endo et al. 1992; Ward et al. 1994). The ratio of chlorophyll *a* to *b* can vary, but usually falls within the range 2 to 4:1 (Ward et al. 1992). In the commercial crushing process, the chlorophyll pigments are extracted with the oil and chlorophylls *a* and *b* are broken down into their derivatives (predominantly pheophytin and pyropheophytin *a* and *b* respectively; Endo et al. 1992; Suzuki and Nishioka, 1993). Thus the ‘chlorophyll’ in crude oil consists of a mixture of chlorophyll derivatives. During routine analysis of oil these derivatives are not usually separated, but are measured together and referred to collectively as ‘chlorophyll’ or ‘chlorophyll pigments’.

Chlorophyll pigments in oil can cause a number of problems. They can absorb light promoting oxidation, which leads to rancidity (Usuki et al. 1984; Kiritsakis and Dugan, 1985). The pigments bind to the active site of the nickel catalyst thus interfering with hydrogenation (Abraham and deMan, 1986) and their presence in oil can result in a colour that is unacceptable to consumers. Pigment concentrations in crude oil must be reduced during refining. This can be done by blending the oil with oil of lower chlorophyll concentration, or by adsorption of the pigments onto bleaching clay (Carr, 1995), both of which add significantly to the cost of refining. However, greater chlorophyll concentrations require more bleaching clay, which can lead to oil losses as the clay retains some oil. Moreover, chlorophyll *a* derivatives are more easily removed from oil with bleaching clay than *b* derivatives (Suzuki and Nishioka, 1993).

In recent years crushers and refiners have experienced problems with higher than usual concentrations of chlorophyll in crude oil from UK produced seed. The high concentrations have interfered with the refining process and increased costs. During the same period, oil from seed imported from Continental Europe was found to have much lower concentrations. Refiners have also reported that batches of oil with comparable concentrations of chlorophyll may differ in the ease with which the pigments can be removed. Some batches may be particularly resistant to bleaching (Brian Edmunds, personal communication). This seems to be a more occasional, but nevertheless serious, problem associated with certain sources of seed, and could be related to differences in the ratio of chlorophyll *a* and *b* derivatives in the oil.

The industry is concerned that the high concentration of chlorophyll seen in recent years represents a growing trend, possibly resulting from a change in oilseed rape agronomy in the UK. Several hypotheses can be proposed to account for an increase in seed, and consequently crude oil chlorophyll concentration from the domestic crop.

1. A wider adoption of hybrid varieties, and in particular varietal associations, with their extended flowering period, resulting in a wider variation of within-plant seed maturity and more green seeds at harvest.
2. A move to lower seed rates, possibly in association with the use of hybrids, leading to a wider variation of within-plant seed maturity at harvest.
3. The introduction of new varieties (conventional lines or hybrids) with inherently slower rates of chlorophyll degradation and hence greater residual concentrations at harvest.
4. A steady move away from the dominant UK variety Apex, with its noted good harvesting characteristics, to varieties that are more difficult to harvest. This could lead to problems in achieving the optimum timing of harvest.
5. A series of years where the weather pattern during seed development and ripening was conducive to chlorophyll retention in the seed.
6. A series of years where unsettled weather forced growers to harvest early before chlorophyll had time to fully degrade.

If the UK industry is to remain competitive, it is important that chlorophyll concentrations in crude oil from UK seed are reduced, as far as possible, to those from European seed.

2. Aims and Objectives

The aim of the project was to investigate the factors determining the residual chlorophyll content of UK rapeseed, and in particular to test the hypotheses outlined above.

The specific objectives were:

- To conduct a brief review to compare oilseed rape agronomy in the UK with that in the other major producing and exporting countries of Europe.
- To determine the extent of variation in seed chlorophyll content at harvest associated with site, variety, variety type (conventional versus hybrid) and season.

The purpose of the review was to identify possible reasons for the lower chlorophyll concentrations in oil extracted from seed produced in Continental Europe compared with UK seed. To investigate the extent of variation in seed chlorophyll associated with site, variety and variety type, seed from selected varieties in the Recommended List (RL) trials (harvested in 2001) was analysed for chlorophyll. The seed was collected from trial sites at 5 different locations in the UK. These concentrations were compared with those in seed sampled from commercial crops grown in the same regions. Seasonal variation was investigated by comparing the chlorophyll concentration of seed sampled from Recommended List trials conducted in Aberdeenshire between 1995 and 1999.

This project provides information on the possible causes of recent high chlorophyll concentrations in UK grown rapeseed. Further research will be needed to determine whether there are agronomic solutions to the problem.

3. Material and Methods

3.1 Survey

A project steering group was established consisting of representatives from Cargill plc, Unitrition (BOCM-Pauls), NIAB and SAC. The group identified France, Germany and Denmark as being major sources of imported European rapeseed. A questionnaire (Appendix A) was sent to agronomists with expert knowledge of oilseed rape production in their respective countries.

3.2 2001 Recommended list trial sites and seed samples

Five sites from the RL trials of harvest year 2001 were selected from contrasting regions of the UK (Table 1). The locations represent major areas of oilseed rape production and were selected to cover the range of UK agri-climatic zones. From each of the sites, thirteen varieties, representing different variety types and maturity dates were selected for study (Table 2). All the varieties are grown commercially.

Table 1. Location of RL trial sites sampled in 2001

Trial site	County	Region
ADAS Bridgets	Hampshire	South
ADAS Morley	Norfolk	South East
Harper Adams	Shropshire	Midlands
NIAB Cockle Park	Northumberland	North
SAC Edinburgh	East Lothian	North

In July and August of 2001, samples of seed (approx. 200 g) were collected from each of the three replicate plots per variety. The samples were taken directly off the combine and immediately dispatched to SAC for analysis. Upon receipt, the seed moisture content was determined and found to vary from 9 to 12 % depending on the trial site. Those above 10% were dried over an air stream at 21^{o}C to a moisture content of approximately 9%. For each site/variety combination, a 100 g sub-sample was taken from each replicate plot, mixed thoroughly and dispatched to Cargill plc for determination of seed oil content. The remaining seed lots were then stored in the dark at 4^{o}C to await chlorophyll analysis.

*Table 2. Varieties and variety types sampled from RL trials in 2001. Maturity refers to the general maturity rating averaged for all RL sites over a 4 year period; ml, moderately late; me, moderately early; e, early; ve, very early. * The top cross variety Spirit, is grown under the same husbandry as a restored hybrid and is included with the other restored hybrids for statistical purposes.*

Variety	Variety-type	Maturity
Apex	Conventional	ml
Canberra	Conventional	me
Escort	Conventional	me
Fortress	Conventional	me
Gemini	Varietal association	ml
Herald	Conventional	ve
Lipton	Conventional	ml
Madrigal	Conventional	ml
Pronto	Restored hybrid	e
Recital	Conventional	me
Royal	Restored hybrid	e
Spirit	Top cross hybrid*	ml
Synergy	Varietal association	ml

3.3 Samples from commercial crops

Samples of seed were collected from commercial crops harvested in 2001. These were taken from farm-saved seed sent to NIAB for glucosinilate analysis. Samples from farms located in the same regions as the trial sites were identified. The farmers involved were then contacted to request permission to use their seeds in the study and to obtain details of harvest technique. The seed samples were sent to SAC for chlorophyll analysis. In order to maintain confidentiality, the name and address of the consenting farmers was withheld by NIAB. Only regional location and harvest details were supplied. Samples of seed from commercial crops harvested in 2000 were also supplied anonymously by NIAB. Upon receipt at SAC, all samples were stored in the dark at 4°C prior to analysis.

3.4 RL trials 1995-99

SAC has stocks of seed harvested from RL trials conducted in Aberdeenshire, dating back to 1993. These have been stored air-dry, in a dark shed at cool ambient temperature. Under these conditions chlorophyll is thought to be relatively stable an unlikely to degrade substantially (J. Daun, personal communication). Samples from a small number of representative varieties harvested in 1995, 1996 1998, and 1999 were analysed for chlorophyll.

3.5 Seed chlorophyll analysis

A method for the rapid extraction and determination of chlorophyll from small seed samples was developed following Tkachuk et al. (1988). Approximately 2 g of seed were weighed to the nearest 0.1 mg and transferred to a 20 ml glass McCartney bottle. Solvent (n-heptane:ethanol, 3:1 v/v, 10 ml) was added to each bottle and the seed ground with an Ultraturrax homogenizer (IKA, Germany) for 60 sec. After homogenizing, the bottles were capped and centrifuged at 3000 rpm for 10 min in an MSE Mistral 3000i refrigerated centrifuge (MSE, UK). The sample and solvent were then allowed to stand for 2 h before analysis. A 4 ml sample of the optically clear supernatant was transferred by pipette to an optical cuvette with 1cm path length and its absorbance measured at wavelengths 665, 630 and 700 nm in a UV/visible spectrophotometer with a bandwidth <2 nm (Cecil CE2040, Cecil Instruments, UK). The chlorophyll concentration of the seed was calculated as:

$$\text{Chlorophyll, mg kg}^{-1} = \frac{k \times A_{corr} \times V}{m \times l}$$

where:

A_{corr} (the corrected absorbance) = $A_{665} - [(A_{700} - A_{630})/2]$
A_{665}, A_{700} and A_{630} are the absorbance at 665, 700 and 630 nm respectively.
k = 13 mg l^{1} cm
l = path length in cm
m = weight of seed as received (i.e. at storage moisture content) in g
V = volume of solute in ml

The method expresses the content of chlorophyll-related pigments in the seed as chlorophyll *a*. A_{665} is the absorbance of the sample at the wavelength of the absorbance maximum for chlorophyll *a*. Absorbance at 630 and 710 nm are used to correct for background. The absorbance coefficient *k* was taken from the American Oil Chemists Society (AOCS) Official Method Ak 2-92 (Firestone, 1998).

3.6 Determination of chlorophyll in crude oil

The absorbance of optically clear samples of commercial crude oil was measured at 630, 670 and 710 nm in a cuvette of 10 mm path length using a UV/visible spectrophotometer (Cecil CE2040). The content of chlorophyll-related pigments was calculated (after AOCS Official Method Cc 13i-96, Firestone, 1998) as:

$$C = \frac{345.3 \times (A_{670} - 0.5\ A_{630} - 0.5\ A_{710})}{l}$$

where:

C = the concentration of chlorophyll pigments expressed as mg pheophytin *a* kg^{-1} oil
A = the absorbance of the sample at the specified wavelengths, nm
l = the pathlength in mm

The method uses a calibration based on the absorption coefficient of pheophytin *a*, and the wavelength (670 nm) of its absorbance maximum.

3.7 Laboratory extraction and analysis of oil

The oil content of rapeseed was determined after extraction with petroleum ether in a Soxhlet apparatus following a method based on AOCS Official Method Am 2-93 (Firestone, 1998). Briefly, seed lots were ground, 10 g sub-samples were then weighed to the nearest 0.1 mg, and extracted in 40/60 petroleum ether for 4 h. The samples were then reground in a Retch mill and returned to the Soxhlet apparatus for a further extraction of 4 h. After removing residual solvent in a vacuum oven, the oil content was determined gravimetrically. Chlorophyll pigments in the Soxhlet extracted oil were determined as above for crude oil.

3.8 Statistical analysis

Data were analysed using standard parametric and non-parametric techniques where appropriate. All statistical routines were carried out using Minitab release 12.1 (Minitab Inc, USA).

4. Results

4.1 Survey

A questionnaire was completed by each of the experts contacted, but they differed in the thoroughness of their response. Details of the responses are given in Appendix A. The main features are summarised below.

- High chlorophyll concentrations in seed and crude oil are generally not considered to be a problem in France, Germany and Denmark. Seed chlorophyll is not routinely measured in any of the countries surveyed.
- The vast majority of the national crop is directly combined in France, Germany and Denmark. In contrast to the UK, there is relatively little swathing or desiccation practised.
- Weather patterns during harvest in Germany and Denmark are similar to those of the UK. In France, dry and sunny weather is the norm, though there are exceptions (e.g. year 2000 in W. France).
- Choice of varieties differs between countries. Hybrids account for a larger proportion of the national crop in Germany and Denmark, than the UK and France.
- Very little spring rape is grown in France and Germany. In the UK and Denmark a similar percentage of the total crop is made up of spring varieties (10-18%).
- Seed rates for both conventional lines and hybrids tend to be lower in France and Germany (up to 50%) than the UK.
- There is little regional variation in agronomy except in France.

4.2 Chlorophyll analysis - technique development and calibration

Preliminary experiments in which replicate samples of seed were extracted in heptane:ethanol demonstrated that the technique had acceptable levels of repeatability, giving coefficients of variation of 10-20% (data not shown). The short homogenization time ensured that heating was minimised and evaporation of solvent negligible. The absorption spectrum (Fig. 1) for the extract had a peak at 665 nm, which is consistent with the major pigment being chlorophyll *a*.

Samples of seed of known chlorophyll concentration, gifted by the Canadian Grain Commission, were used to determine the extraction efficiency of the technique. Regression analysis indicated that there was a linear relationship between the known chlorophyll

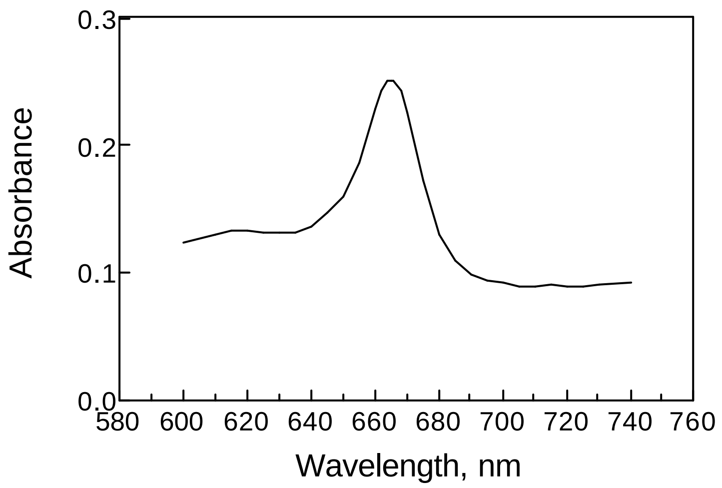

Fig. 1. Absorption spectrum for a heptane:ethanol extraction of chlorophyll pigments from seed

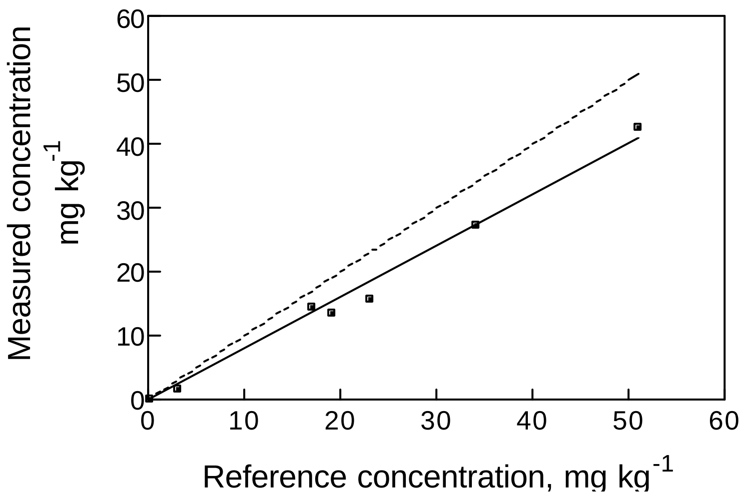

Fig. 2. Relationship between the seed chlorophyll concentration of reference samples supplied by the Canadian Grain Commission and that measured on heptane:ethanol extracts using the extraction technique developed in the present study. Each point is the mean of 3 separate extracts from the same seed lot. Line fitted by regression forced through the origin

y = 0.80x r^2 = 0.996. Broken line shows 1:1 relationship indicating complete extraction.

concentration and that measured with the current technique, over a wide range of concentrations (Fig. 2). The slope of the relationship (0.80; significantly less than 1.00, $P < 0.05$), implies that the technique extracted 80% of the seeds' chlorophyll, as measured by the Canadian Grain Commission (CGC) using the AOCS Official Method Ak 2-92 (Firestone, 1998). The latter involves solvent extraction in a ball mill for up to 1 h. All values of seed chlorophyll concentration reported in the present study are those obtained using the rapid extraction technique described above (section 3.5), and are *not* corrected for the lower recovery of chlorophyll with this method.

Seed samples from the RL trials harvested in 2001 were subject to heptane:ethanol extraction and Soxhlet extraction. The chlorophyll (expressed as pheophytin *a*) concentration of laboratory extracted oil (Soxhlet) was linearly related ($P < 0.001$, Fig. 3) to the seed chlorophyll concentration (estimated as chlorophyll *a* in heptane:ethanol), but there was some scatter in the data ($r^2 = 0.85$). Expressing the seed chlorophyll concentration on an oil weight, rather than seed weight, basis (Fig. 4) removes variation associated with differences in oil content between seed lots. However, this failed to increase the amount of variation accounted for by the regression relationship ($r^2 = 0.85$). The concentration of chlorophyll-related pigments in Soxhlet extracted oil, was only 20% of that measured by heptane:ethanol extraction (Fig. 4).

From here on in, the term seed chlorophyll refers specifically to chlorophyll extracted in heptane:ethanol, and expressed as chlorophyll *a* on a seed weight basis. Unless otherwise stated, oil chlorophyll (crude and Soxhlet-extracted) refers to concentrations measured and expressed as pheophytin *a*, per unit weight of oil.

4.2 Chlorophyll concentration of commercial crude oil

Fig. 5 shows the range of chlorophyll concentrations found in commercial crude oil during 1999/2000 and 2001/2002. These values are typical of those obtained from plants crushing UK-grown seed. From August 1999 through to June 2000, concentrations ranged from 50-60 mg kg^{-1} oil. These were derived from seed harvested in the summer of 1999. From May to July of 2001, concentrations were also high, averaging about 50 mg kg^{-1}; this was from seed from the 2000 harvest. From September 2001 to the spring of 2002, concentrations fell to values between 30 and 40 mg kg^{-1} (seed from 2001 harvest).

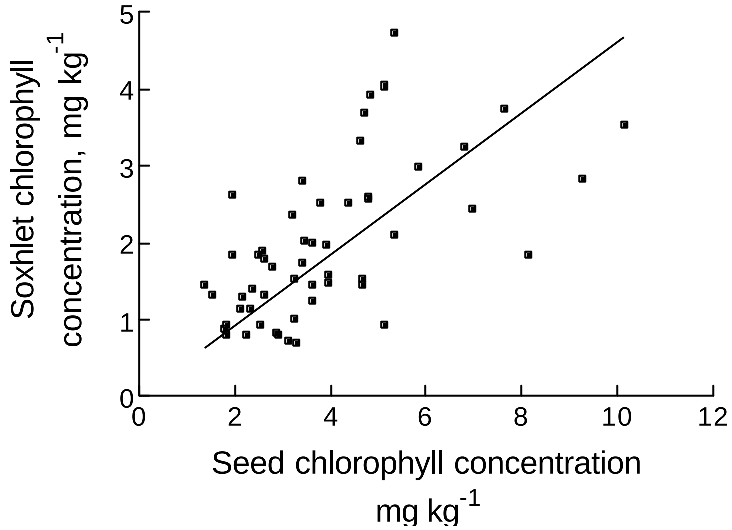

Fig. 3. Relationship between seed chlorophyll concentration measured on heptane:ethanol extracts and expressed on a seed weight basis and that measured in oil extracted using a Soxhlet apparatus. Line fitted by regression y = 0.46x, r^2 = 0.85. Samples were from the RL trials of 2001 and each point represents a single variety/site combination. Seed chlorophyll concentrations are means of separate extractions of seed from three replicate plots per variety/site combination and expressed on a seed weight basis; Soxhlet concentrations are from a single analysis of a blended sample of seed from the same three plots.

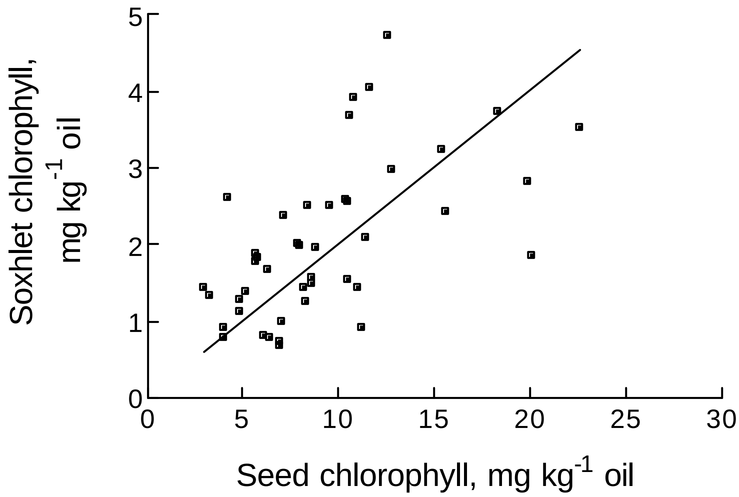

Fig. 4. Relationship between seed chlorophyll concentration expressed on a seed oil basis and that measured in oil extracted using a Soxhlet apparatus. Line fitted by regression y = 0.20x r^2 = 0.85. Details of samples as for Fig. 3.

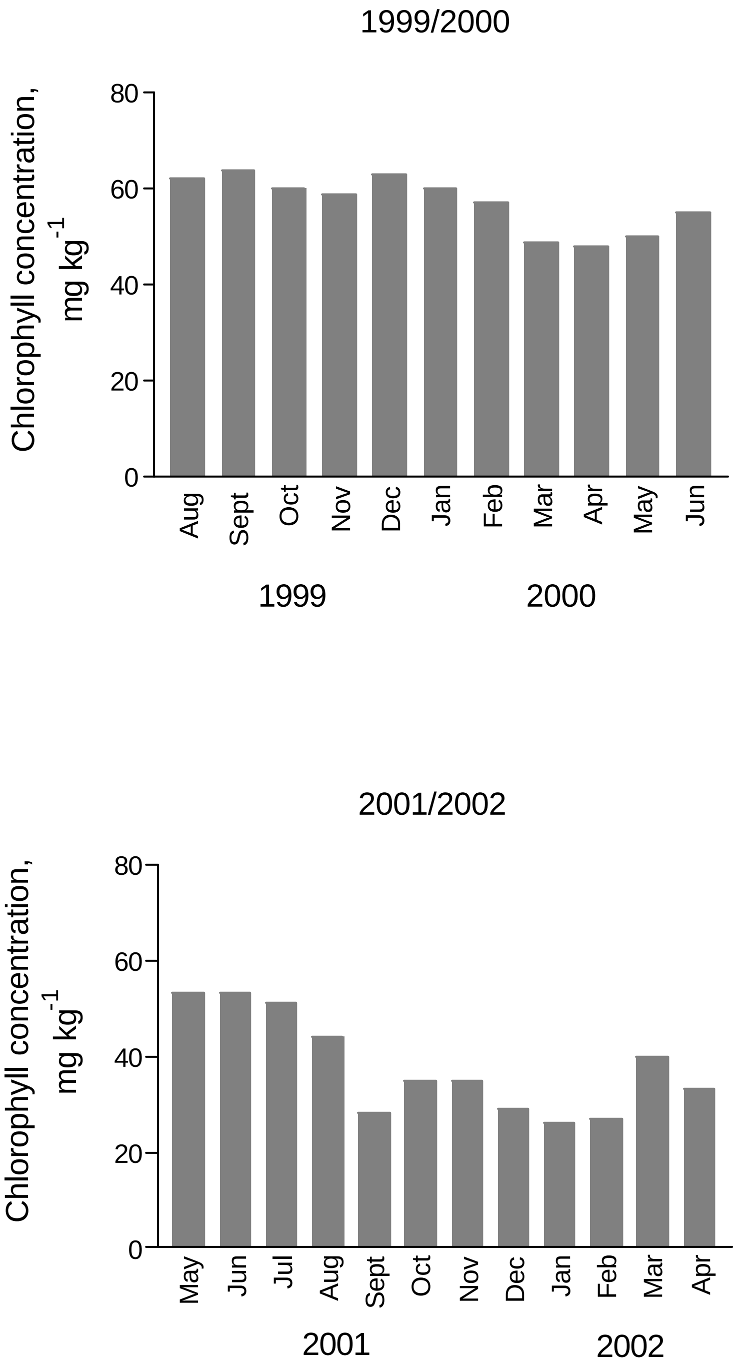

Fig. 5. Concentrations of chlorophyll in samples of crude oil from a commercial plant crushing UK-produced seed during 1999/2000 and 2000/2001 (source Unitrition).

The concentration of chlorophyll in crude oil extracted from UK grown-seed harvested in 2001 compared favourably with that from Australian seed (Fig. 6a), but was about 38% higher than that from French seed. Samples of seed from batches recently crushed (spring 2002) were provided by Cargill plc and Unitrition and analysed for chlorophyll. The relationship between the seed chlorophyll concentration and that measured in the crude oil is shown in Fig. 6b.

The measured values only cover a narrow range and were obtained from seed harvested in 2001. Seed samples from batches crushed in 1999 and 2000 are no longer available for analysis. However, some data have been obtained from Unitrition for the concentration of chlorophyll in a Soxhlet extraction of seed that can be broadly related to the concentration found in crude oil at the time. The likely seed chlorophyll concentrations have been estimated from the Soxhlet extraction data, using the relationship in Fig. 3, and in Fig. 6b they are plotted against values for crude oil chlorophyll. The results must be interpreted cautiously because the Soxhlet analyses were conducted at a different laboratory and on a different batch of seeds from that used to relate Soxhlet extracted chlorophyll to seed chlorophyll. For this reason the regression analysis in Fig 6b has been restricted to the measured values. Estimated values are shown relative to an extrapolation of the regression line beyond the measured values. The chlorophyll concentration in crude oil is lower than would be predicted from the extrapolated regression relationship. This suggests that either the relationship between seed chlorophyll and crude oil chlorophyll is not linear (which would be in contrast with results of Daun, 1982), or that there is some error in the estimates of seed chlorophyll from the Soxhlet data. Nevertheless, the results show that seed chlorophyll concentrations of between 10 and 15 mg kg^{-1} equate with concentrations in crude oil of 50-60 mg kg^{-1}.

4.3 RL trials harvested in 2001

Values of seed chlorophyll concentration did not follow a statistically normal distribution. However, analysis of variance conducted on the original data and on data after $\log_e$ transformation gave similar results. Thus, for ease of interpretation, only the non-transformed results are presented here. Varieties differ in their rate of development. This could influence the seed chlorophyll concentration since all varieties at a given RL trial site are harvested on the same date. Maturity data were available for replicate plots at each site, except Harper Adams. The maturity index was therefore included as a co-variate in the analysis. For the Harper Adams data set, a mean value for each variety, taken from across the other sites, was used. Table 3 gives the adjusted means after correcting for the effects of maturity. In most cases adjusted means differed from the non-adjusted means (i.e. before correcting for maturity) by <1-2% and in no case by more than 6%.

Table 3. Effects of variety and site on seed chlorophyll concentrations (mg kg^{-1} seed weight) in the RL trials of 2001. Each value is the mean of three replicate plots adjusted for the effects of maturity in an analysis of co-variance. Sites are HA, Harper Adams; CP, Cockle Park; Mor, Morley; Ed, Edinburgh; Bri, Bridgets. Sites were harvested on different dates; varieties at a given site were harvested on the same date. In each case plots were swathed and combined.

	Site					
Variety	HA	CP	Mor	Ed	Bri	Var mean
Apex	4.60	6.61	10.08	2.67	3.84	5.56
Canberra	3.48	5.14	5.24	1.93	2.24	3.61
Escort	2.21	6.85	3.28	0.94	1.45	2.95
Fortress	2.00	3.33	3.23	1.23	1.96	2.35
Gemini	3.41	2.48	7.58	1.60	4.65	3.94
Herald	2.29	4.13	3.90	1.57	2.07	2.79
Lipton	2.52	4.71	4.31	2.21	3.34	3.42
Madrigal	2.64	4.74	4.91	1.28	2.97	3.31
Pronto	1.91	2.06	4.01	1.41	1.65	2.21
Recital	3.32	3.74	5.86	2.54	2.99	3.69
Royal	2.51	2.53	4.76	1.99	2.51	2.86
Spirit	2.70	3.69	5.26	2.12	3.19	3.39
Synergy	3.56	2.29	8.04	1.83	4.56	4.06
Site mean	2.86	4.02	5.42	1.79	2.88	
SED						
var	0.492	site	0.305	var x site	1.099	
prob	0.000	prob	0.000	prob	0.006	
df	12	df	4	df	48	

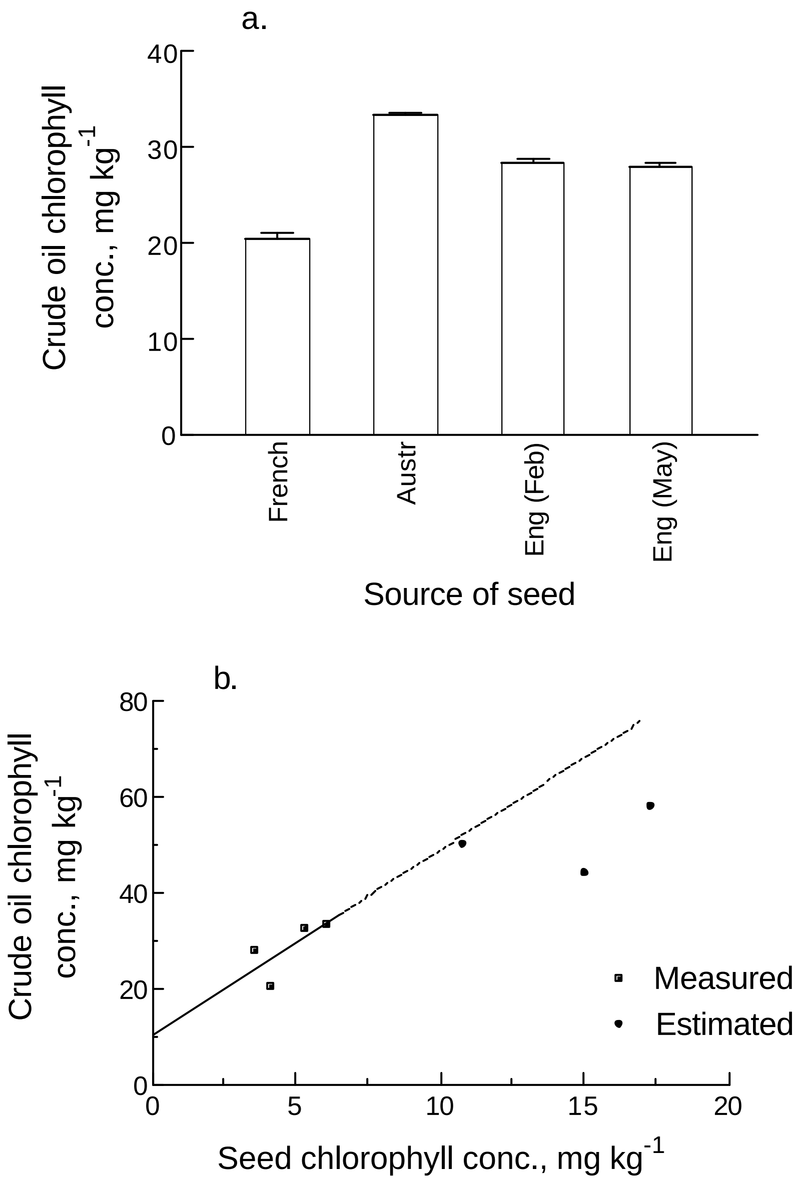

Fig 6. a) Concentrations of chlorophyll in oil from a commercial plant crushing seed from different sources (France, Australia, and England) in 2002. Separate lots of English seed were crushed in February and May. In the case of the English and French seed, the seed was from the year 2001 harvest. Values are mean $\pm$ SE of measurements made every 2-3 days during the crushing period (approx 1 month). b) The relationship between seed chlorophyll concentration and that found in crude oil from commercial crushers. Measured values are for seed samples from the 2001 harvest (supplied by Cargill plc and Unitrition). Estimated values are for seed chlorophyll concentrations measured on Soxhlet extractions of seed from the 1999 harvest and converted to seed chlorophyll concentrations using the relationship in Fig 3. Solid line fitted by regression to measured values only $y = 3.85x + 10.30$ $r^2 = 0.51$. Broken line is an extrapolation of the fitted line beyond the measured values.

Although the chlorophyll concentrations were generally low, varieties differed significantly even after correcting for differences in maturity index. Apex had the highest concentration, and Pronto the lowest, when averaged across sites. The mean chlorophyll concentration also differed significantly between sites. Morley returned the highest and Edinburgh the lowest concentration when averaged across varieties. Edinburgh is the most northerly site and Bridgets the most southerly. Thus there was no obvious effect of latitude on the seed chlorophyll concentration in 2001. There was, however, a significant variety x site interaction. Although the rankings of certain varieties such as Apex and Pronto were relatively consistent between sites, others were less consistent. For example Synergy was amongst the highest ranking varieties at Morley, Harper Adams and Bridgets, but amongst the lowest at Cockle Park.

After varieties were grouped according to variety-type, conventional and varietal associations were found to have comparable chlorophyll concentrations when averaged across sites (Table 4). Restored hybrids, as a group, had significantly lower concentrations, even after differences in maturity were taken into account (through inclusion of maturity index as a co-variate). However, the ranking of variety-types was not consistent between sites, resulting in a significant site x variety-type interaction. Varietal associations had the lowest seed chlorophyll amongst the groups at Cockle Park, but the greatest at Harper Adams, Morley and Bridgets. However, given the overall low concentrations involved, these variety-type x site interactions seen in 2001 have little practical relevance.

Table 4. Effects of variety type and site on seed chlorophyll concentrations (mg kg^{-1} seed weight) in the RL trials of 2001. Each value is the mean of three replicate plots adjusted for the effects of maturity in an analysis of co-variance. Site names are given in Table 3.

	Site					Var type	
Variety type	HA	CP	Mor	Ed	Bri	mean	SE
Conventional	2.91	4.69	5.3	2.1	2.85	3.57	0.14
Varietal association	3.34	2.04	7.59	1.87	4.35	3.84	0.28
Restored hybrid	2.34	2.52	4.65	1.84	2.78	2.83	0.23
Site mean	2.87	3.09	5.85	1.94	3.33		
SE			0.24				

Site; P = 0.000, df 4
Var type; P = 0.008, df 2
Site x var type; P = 0.000, df 8

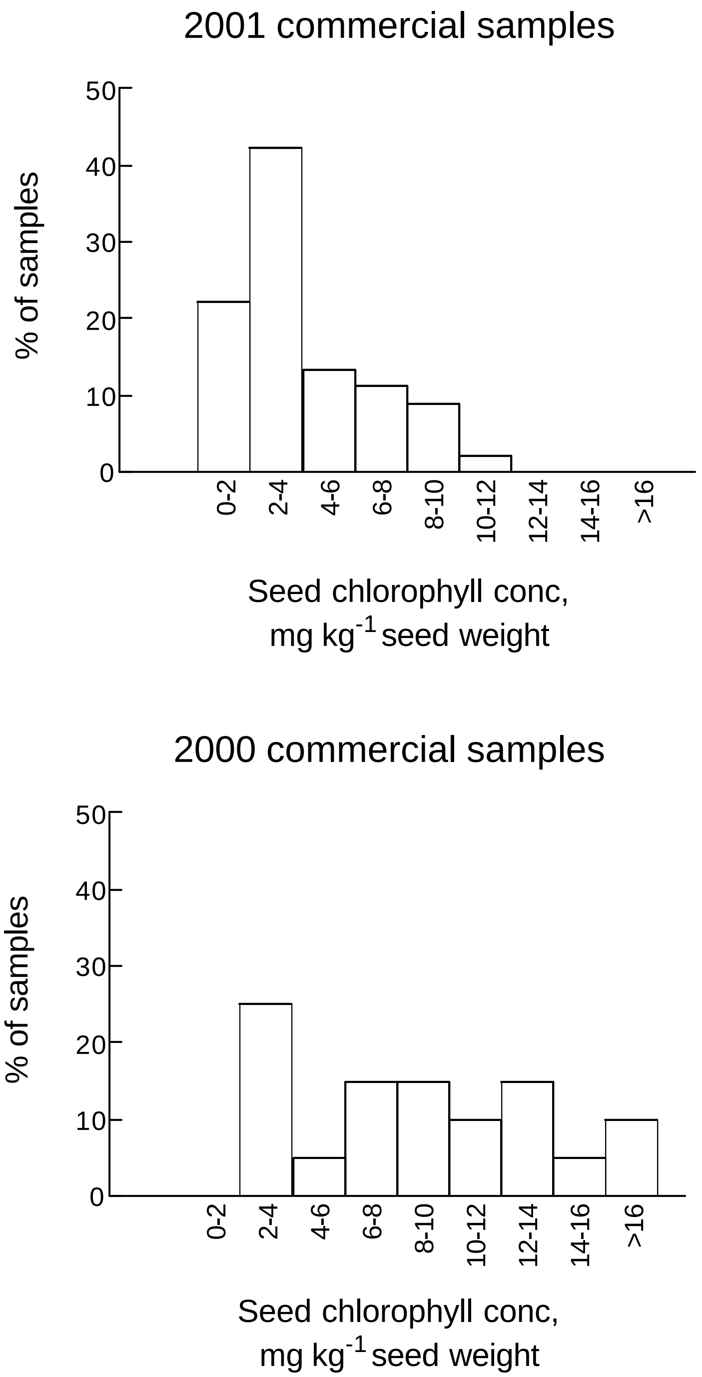

Fig. 7. Chlorophyll concentrations in seed taken from commercial crops in the harvest years 2001 and 2000. Mean for 2001, 3.94, median 3.24; mean for 2000, 9.77, median 9.31. Median values for the two years were significantly different at $P < 0.001$ in a Mann Whitney U test. Number of seed samples in 2001 was 45; number in 2000 was 20.

4.4 Commercial crops harvested in 2001

Samples of seed were obtained from commercial crops grown in the same regions as the RL trials. No attempt was made to control for variety, harvesting technique or other husbandry factor in the selection regime. In general, chlorophyll concentrations were comparable with those found in the RL trials (Fig. 7). The majority of samples had concentrations of 2 to 4 mg kg^{-1}, and only one sample (2% of the total) was above 10 mg kg^{-1}.

When grouped according to region or harvest method, chlorophyll concentrations did not differ significantly between groups (Table 5). Although a number of varieties were represented in the samples, just four dominated; Fortress, Escort, Apex and Madrigal. The mean chlorophyll concentration of these varieties differed significantly (Table 5) and was greatest for Apex.

Table 5. Seed chlorophyll concentrations of 45 samples of seed collected from commercial crops in 2001 and grouped according to harvest method and region of production. Values are means $\pm$ SE for the group. Minimum number of samples per group was 8. For varieties, mean concentrations for the 4 most common varieties are shown. Data analysed by one analysis of variance (variety) or the Kruskal Wallis test (harvest method and region). Varieties followed by a different letter are significantly different as shown by LSD (P = 0.05). Harvest methods are; DC, direct combined; Desicc, desiccated; Swath, swathed.

Factor	Seed chlorophyll, mg kg^{-1}				P
Harvest method	DC	Desicc	Swath		
	3.71 $\pm$ 0.62	4.41 $\pm$ 0.64	4.03 $\pm$ 0.65		ns
Region	South	South East	Midlands	North	
	2.87 $\pm$ 0.58	3.35 $\pm$ 0.49	5.12 $\pm$ 1.20	5.19 $\pm$ 0.78	ns
Variety	Apex	Escort	Fortress	Madrigal	
	6.33 $\pm$ 1.36 a	2.57 $\pm$ 0.57 b	3.69 $\pm$ 0.60 ab	3.66 $\pm$ 0.38 ab	0.022

4.5 Commercial crops harvested in 2000

Samples from commercial crops harvested in 2000 displayed a greater range of seed chlorophyll concentrations than those of 2001 (Fig. 7). 40% of samples had seed concentrations greater than 10 mg kg^{-1}, with some as high as 27 mg kg^{-1}. The median concentration (9.31 mg kg^{-1}) was significantly greater than that of 2001 harvested crops (3.24 mg kg^{-1}) (Fig. 7).

4.6 RL trials 1995-99

Stocks of seed from the RL trials conducted in Aberdeenshire were not complete, which meant that in some years only one or two replicates were available for analysis. This is because the stocks have been used for a number of purposes, and consequently samples have been depleted. Seed from the harvest year 1997 had deteriorated and was not analysed. Further, because of the steady turnover of varieties in the trials system, not all were available each year. As a result, the data have not been analysed statistically. Nevertheless, clear differences between years and varieties are apparent (Table 6). The lowest chlorophyll concentrations were found in 1996. In 1998 concentrations were again generally low, with the exception of Apex (although we cannot be certain how representative this one sample of Apex is). Concentrations were greater in years 1995 and 1999. The latter was characterised by having particularly high concentrations, with all varieties exceeding 10 mg kg^{-1}.

In years conducive to the retention of high chlorophyll concentrations, Apex appeared to be the most susceptible variety. To a lesser extent, Lipton also appeared to be susceptible (see years 1999 and 1998). There was no consistent difference between the chlorophyll concentrations of Synergy sown at 70 and 120 seeds m^{-2} (Table 6).

Table 6. Seed chlorophyll concentrations (mg kg^{-1} seed weight) of selected varieties from RL trials in Aberdeenshire between 1995 and 1999. For 1996, 1998 and 1999 each value is the mean of 3 replicate plots except where no SE is given in which case it was unreplicated. For 1995, values are means of 3 separate extractions, on samples from the same plot. MI (mean maturity index for the variety) measured at the site from 1-9 (late-early).

	1995			1996			1998			1999		
Variety	Chl	*se*	MI	Chl	se	MI	Chl	Se	MI	Chl	se	MI
Apex	35.61	*2.49*	4.7	2.15	0.44	5.0	21.91		8.3	47.45	3.03	6.0
Falcon	7.63	*0.52*	4.7	1.55	0.62	6.0						
Gazelle	6.28	*1.04*	5.3	1.03	0.08	7.0						
Synergy 120	6.53	*1.18*	4.0	3.39	1.95	6.7	1.92	0.07	7.3			
Synergy 70				1.73	0.76	5.0	2.34	1.04	7.0	10.12	1.74	5.0
Herald							5.52		7.3	16.17	1.13	6.7
Lipton							6.08	0.36	7.7	24.63	2.94	7.0
Pronto							2.92	0.81	7.0	11.59	0.69	7.3
Mean	14.01			1.97			6.78			21.99		
Swath/Harvest interval, d	7			16/17			10			9		

5. Discussion

5.1 Comparison of chlorophyll concentrations and standards

Before embarking on a comparison of chlorophyll concentrations in seed and crude oil, it is important to be clear about what is being measured. In mature seed, the major chlorophyll pigments are chlorophyll *a* and *b* in a ratio of approximately 2 to 4:1, plus minor quantities of pheophytin *a*. (Daun and Thorsteinson 1989; Endo et al., 1992; Cenkowski et al., 1993; Ward et al. 1994). Crude oil contains very little chlorophyll *a* or *b*, as the pigments are degraded during the extraction process to their respective pheophytins (through the removal of Mg) and pyropheophytins (Daun and Thorsteinson 1989; Endo et al., 1992; Suzuki and Nishioka, 1993). These pigments absorb light at similar wavelengths, but with different absorption coefficients to chlorophyll *a* and *b* (Endo et al. 1992).

In the UK, Cargill plc and Unitrition measure and express chlorophyll-related pigments in crude oil using a spectrophotometer calibrated against pheophytin *a*, as outlined in the AOCS Official Methods (Cc 13i-96, Firestone, 1998). This calibration was developed using only two samples, differing in pigment content, which were blended in different ratios (J. Daun, personal communication). The Canadian and Swedish oilseed industries measure chlorophyll in oil in the same way, but calculate the concentration from a calibration against pure chlorophyll *a* (AOCS Official Methods 13d-55, Firestone, 1998). This gives a value 3.33 times lower than that based on pheophytin.

Trading specifications for Canola oils have limits of 25 to 30 mg kg^{-1} (as chlorophyll *a*). No such limits have been set for the UK. However, refiners began to voice concerns when the chlorophyll concentration (as pheophytin *a*) in crude oil rose to around 60 mg kg^{-1} (equivalent to approximately 18 mg kg^{-1} as chlorophyll *a*) as the chlorophyll pigments interfered, to an unacceptable extent, with the refining process. Desirable concentrations would be those no greater than the norm for a good year (i.e. 30-40 mg kg^{-1} pheophytin; 9-12 mg kg^{-1} as chlorophyll *a*) and preferably less. Although these concentrations are somewhat lower than the trading standards operating in Canada, they are greater than those consistently found in crude oil from the UK's main competitors in Continental Europe.

During 1999 and 2000, concentrations in crude oil from UK-grown seed, harvested in 1999, were around 60 mg kg^{-1} (Fig 5). From the crop harvested in 2000, concentrations were 50-55 mg kg^{-1} (Fig 5, May-Jul 2001). By contrast, those from seed imported from Europe were in the order of 17-19 mg kg^{-1} (A. Guttridge, personal communication). This year, concentrations in oil from UK seed harvested in 2001 have been in the acceptable range (30-40 mg kg^{-1}, Fig 5b, September 2001 onwards). They are comparable with those from Australian seed, though higher than those from French seed (22 mg kg^{-1} Fig. 6a). Thus, UK growers can produce seed that yields acceptable concentrations of chlorophyll in crude oil, but in order to be able to compete with European imports it is necessary minimize the risk of high concentrations arising. Indeed it is desirable to try and reduce the concentration to values close to or lower than those from European seed

5.2 Seed versus crude oil chlorophyll

Chlorophyll concentrations in seed and crude oil are linearly related (Daun, 1982). For the purpose of grading Canola seed, a concentration of 22 mg kg^{-1} seed weight equates to approximately 25 mg kg^{-1} in crude oil (expressed as chlorophyll *a*) (Canadian Grain Commission, 2002). Based on the relationship reported by Daun (1982), the same seed chlorophyll concentration would equate to a concentration in oil of 35 mg kg^{-1}. From Fig 6b we would predict that a seed chlorophyll concentration of 22 mg kg^{-1} would result in a concentration in crude oil of 95 mg kg^{-1} pheophytin (28 mg kg^{-1} as chlorophyll *a*). If a correction is made for the incomplete extraction of chlorophyll in the present study (Fig. 2), the predicted concentration in oil is 116 mg kg^{-1} (35 mg kg^{-1} as chlorophyll *a*). Thus our estimates lie between those currently used by the Canadian oilseeds industry and those reported in the early North American literature. The agreement is remarkable given the limited number of samples available in the present study. It provides us with confidence in our ability to predict potential chlorophyll problems for crushers from rapid solvent extraction of seed.

Chlorophyll concentrations measured in Soxhlet extractions of seed correlated with those found in heptane:ethanol extractions (Fig. 3). However, the quantities extracted by Soxhlet were considerably lower. This is because Soxhlet extraction, unlike cold solvent extraction and the commercial crushing process, is effective at removing much of the chlorophyll from the oil. Consequently, seed analysis using cold solvent extraction is preferable to Soxhlet extraction as it provides a more sensitive, and rapid, means of predicting potential problems for crushers.

Based on Fig 6b, seed chlorophyll concentrations of 10 mg kg^{-1} and above would result in undesirable concentrations in oil (i.e. >50 mg kg^{-1} as pheophytin).

5.3 Potential causes of high chlorophyll concentrations in UK rapeseed

A number of hypotheses were put forward to explain the perceived trend towards higher chlorophyll concentrations in crude oil in recent years (section 1). When developing these hypotheses it was assumed that the high concentrations in crude oil resulted from higher than usual residual chlorophyll concentrations in UK rapeseed, rather than any change in the extent to which the chlorophyll was extracted in the crushing process. This seems a reasonable assumption. Firstly, whilst a small amount of chlorophyll may remain in the meal, most is normally extracted along with the oil (Endo et al., 1992). To have a significant impact on the concentration in crude oil a change in the proportion of the chlorophyll extracted from the seed would need to be large. Secondly, the results from the present study suggest a strong association between seed chlorophyll in the national crop and crude oil chlorophyll. High concentrations in oil were experienced in years where high seed chlorophyll concentrations were found (Figs 5 and 7, Table 6). The hypotheses proposed to account for an increase in seed chlorophyll concentrations are dealt with below.

5.3.1 Seed rates and varieties

Varieties clearly differ in their seed chlorophyll concentration at harvest. In RL trials all varieties are harvested on the same date. However, varietal differences cannot be ascribed simply to differences in crop maturity at harvest, for three reasons. Firstly, varietal differences were still evident after the chlorophyll concentrations were adjusted (within the statistical analysis) for the effects of maturity (Table 3). Secondly, in the RL trials at Aberdeen, Synergy, was one of the latest maturing varieties, but had one of the lowest concentrations (Table 6). This is contrary to expectation; later maturing varieties would be expected to have more immature pods at harvest, and hence higher average seed chlorophyll concentrations, than earlier maturing ones. Finally, varietal differences were also found amongst samples collected from commercial crops (Table 5). In principle, the timing of harvest of these crops would be determined by the developmental stage of the particular variety, rather than an average for all varieties as is the case in the RL trials. There may, however, be operational reasons why it is not possible to harvest some commercial crops at the optimum stage.

There is no evidence to support the hypothesis that the apparent rise in chlorophyll concentration in recent years is the result of the wider adoption of hybrids and the use of lower seed rates. In 2001, varietal associations had similar concentrations to conventional lines, when averaged across the RL sites, whilst restored hybrids had significantly *lower* concentrations. Moreover, there was no obvious difference in seed chlorophyll concentration from crops of Synergy sown at 70 and 120 seeds m^{-2} (Table 6).

Collectively these results suggest that there is genetic variation amongst UK varieties in seed chlorophyll concentration at harvest that is unrelated to maturity or crop density. Genotypic differences in chlorophyll content at harvest have been reported for some North American *Brassica napus* L. varieties (Ward et al. 1995; Brown et al. 1999). Here too, the genetic differences could not be explained by differences in crop maturity at harvest. They could result from varietal differences in the rate of chlorophyll clearing during seed maturation (Ward et al. 1995). Our findings that restored hybrids have lower seed chlorophyll concentrations than conventional lines, and that varying the seed rate has no significant effect (at least over the range 70 to 120 seeds m^{-2}), are consistent with results from experiments conducted under Canadian conditions (Van Deynze et al. 1992).

The adoption of new, high chlorophyll-retaining varieties, cannot explain the high concentrations found in crude oil over the last few years (hypothesis 3, section 1).
Apex has been the dominant variety grown in the UK since 1994 (Sylvester-Bradley et al. 2002) and of the varieties tested has the highest chlorophyll concentration (Tables 3, 5 and 6). Some of the newer varieties, especially the restored hybrids such as Pronto, have relatively low seed chlorophyll concentrations. However, because their market share at present is small, their impact on the chlorophyll concentration of oil extracted from the national crop will be low.

5.3.2 Year to year variation

Over the years during which Apex has dominated the market, there have been marked fluctuations in seed chlorophyll concentration. High concentrations were found in 1995, 1999 and 2000, but not in 1996, 1998 and 2001 (Table 6, Fig. 7). In the Aberdeen RL trials, a general increase in concentration was observed across varieties in 1999 and to a lesser extent in 1995 compared to 1996 and 1998, but Apex was the variety that responded most with concentrations substantially greater than the others. This suggests there may be a strong genotype x environment interaction in seed chlorophyll concentration. However, it is not clear whether these data for Apex are representative of the UK as a whole. Apex is not particularly well suited to conditions in the N of Scotland and yields are usually below average. It will be necessary to compare Apex with other varieties in replicated experiments conducted at several locations in the UK, and over several years, to establish the true extent of any genotype x environment interaction.

In samples from commercial crops in England (year 2000), high concentrations were associated with several varieties and not exclusively with Apex. If the concentrations found in these commercial crop samples (mean 9.8 mg kg^{-1}), and in the Aberdeenshire RL trials of 1999 (mean after excluding Apex, 15.6 mg kg^{-1}), are representative of the national crop, they would be enough to account for the high chlorophyll concentrations in crude oil observed by the crushers in 1999 and 2000 (Table 6, Fig 6b and 7). Thus the major factor contributing to the problems experienced by crushers appears to be seasonal variation in seed chlorophyll concentrations rather than a change in the varieties or seed rates used by growers.

5.3.3 Weather patterns

The large year to year variation in seed chlorophyll concentrations might be explained by differences in weather patterns during harvest. The data set from Aberdeenshire RL trials is the most useful for exploring the effects of weather conditions, because crops were grown in the same region and under the same husbandry regime in different seasons, and local weather data are available. The high chlorophyll years of 1995, and 1999, were years with low summer rainfall. In particular, there was no rainfall between swathing and harvest in these years (Fig. 8). In contrast, the low chlorophyll years of 1996 and 1998 had regular bouts of rain during crop ripening. Average temperatures between swathing and combining were broadly comparable in all years, although the daily maximum, and the range between maximum and minimum temperature, tended to be larger in 1995 and 1999 (data not shown).

The dry weather of 1999 was not confined to Aberdeenshire. Across the UK a cold June was followed by the driest July since 1911 (Met Office, 2002). In most regions of England, the rainfall was only 30-50% of the long-term (1961-1990) average, and mean monthly temperatures were above average everywhere. Sunshine hours were also well above average. Although there are no seed chlorophyll data for regions other than Aberdeenshire, the 1999 domestic harvest gave high concentrations (60 mg kg^{-1}) in crude oil.

In contrast, 2001 resulted in low seed and crude oil concentrations. July was the wettest since 1993. In England, hot thundery weather occurred in the first week, followed by wet unsettled conditions for weeks two and three and a relatively warm dry final week. In Scotland the weather in July was generally dull and unsettled. August had short periods of warm weather interspersed by cooler wet weather.

The harvest year 2000 was also a relatively high chlorophyll year. Chlorophyll concentrations in crude oil fell between those of 1999 and 2001. Samples of seed from commercial crops showed highly variable chlorophyll concentrations, but, on average, were higher than those in 2001 (Fig. 7). However, for the 2000 crop it is more difficult to establish a link between the higher concentrations and dry weather. The middle of June was dry, but the end of the month was cooler and more unsettled. July was wet in many regions and localised heavy rain caused flooding in some areas in the south, but the third week was particularly sunny and hot. Although July 2000 will be remembered for interruptions to harvest, there was considerable variation between regions. Scotland and Northern Ireland were, on the whole, drier than the long-term average for the month. August was mostly warm but changeable. The high and variable chlorophyll concentrations in 2000 may have resulted from a proportion of crops experiencing dry hot weather at a critical period in the ripening process, such as immediately after swathing. More information is required on when specific crops were harvested and the local weather conditions at the time in order to substantiate this hypothesis.

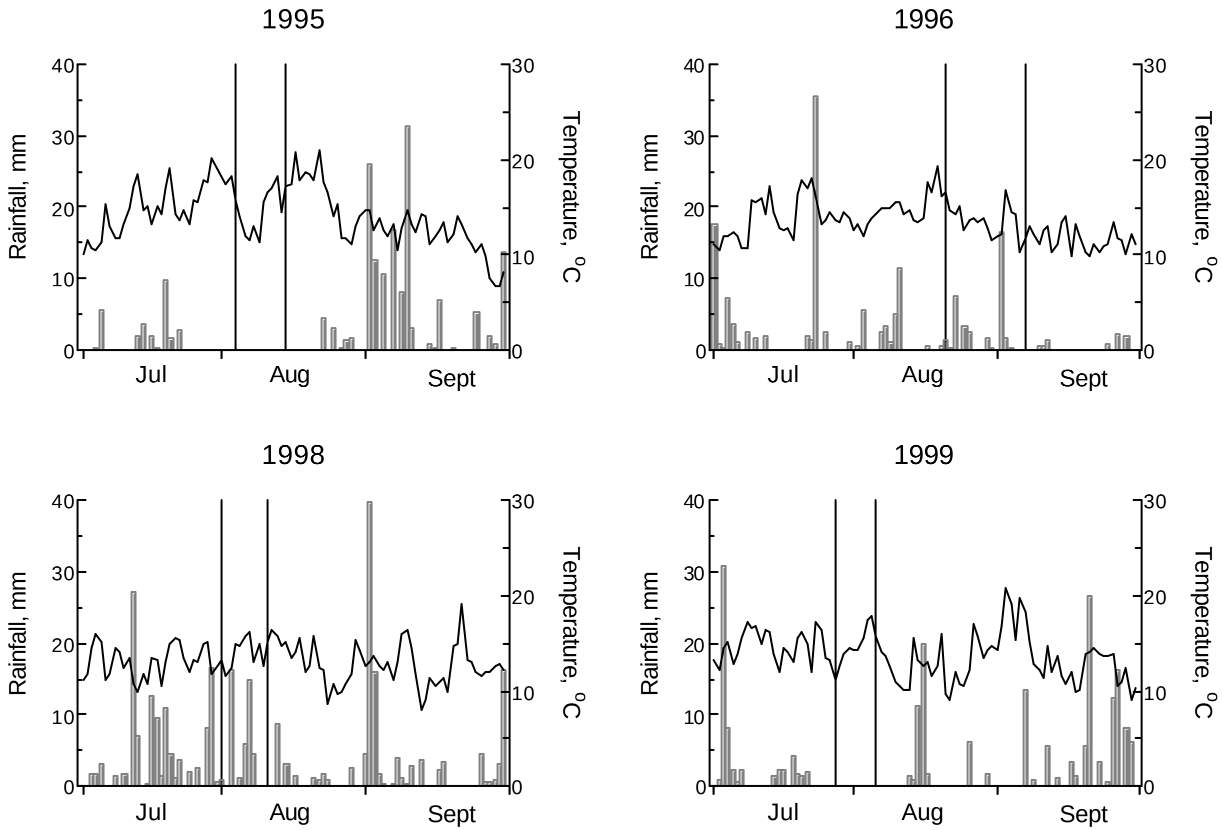

Fig. 8. Weather patterns at the Aberdeenshire RL sites for the harvest years 1995, 1996, 1998 and 1999. Vertical bars are daily rainfall totals; continuous line, mean temperature. Vertical lines indicate the date of swathing (left hand line) and harvesting (right hand line).

5.3.4 A possible mechanism

At present we can only speculate as to why there may be higher residual chlorophyll concentrations in UK seed during drier seasons. Loss of chlorophyll from the seed is thought to result largely from enzymatic degradation (Johnson-Flanagan and Spencer, 1996). It occurs in an exponential fashion and is most rapid when the seed moisture content is high (Ward et al. 1992; Cenkowski et al 1993; Ward et al. 1995). Rapid drying of the seed can inhibit metabolism and limit the breakdown of chlorophyll, resulting in high concentrations at harvest (Ward et al. 1992). Frequent re-wetting of the seed during ripening, as would occur in an average year with frequent light rainfall, could prolong the period of chlorophyll clearing. Indeed, artificial conditioning of prematurely harvested seed at warm temperatures and high humidity can promote chlorophyll degradation (Cenkowski et al. 1993). However, since there are no data available for rates of chlorophyll degradation under UK conditions, it is unclear whether drying in the field is sufficiently rapid for significant chlorophyll trapping to occur.

Another explanation might be that, in dry summers, farmers are tempted to harvest crops early before the chlorophyll has had time to degrade, either to get the job completed, or because of concerns over the risk of pod shatter. Interestingly, in the RL trials at Aberdeen, the year with the lowest seed chlorophyll concentrations, was the one with the longest interval between swathing and harvesting (Table 6). Premature harvesting during spells of unsettled weather may also contribute to high chlorophyll concentrations in some crops.

Excessively high seed chlorophyll concentrations do not appear to be inevitable in dry weather. Harvesting in warm dry weather is the norm in France and, in the crusher's experience, the seed produces low chlorophyll concentrations in crude oil. This suggests that there may be agronomic solutions for producing seed with low chlorophyll concentrations. One of the main differences between the agronomy of oilseed rape in the UK and France is the harvest technique (Appendix A). In France, unlike the UK, the vast majority of the crop is directly combined. We found no effect of harvest method on the chlorophyll concentration of commercial samples harvested in 2001, but this was a 'low chlorophyll' year (Table 5). In drier summers, the rate of seed moisture loss may be greater in swathed compared with standing crops, leading to a greater chlorophyll concentration at harvest. This could be exacerbated by the reliance on a diminishing number of contractors for swathing. Swathing prematurely, particularly in dry weather, may lead to greater chlorophyll trapping. Seed chlorophyll concentrations were greater in swathed compared with directly harvested crops in Northern Idaho (USA) and the difference increased the earlier the swathing (Brown et al.

1999). In contrast, other studies (in Canada) have found either no difference between swathed and directly cut crops, or that swathing accelerated the rate of chlorophyll degradation in maturing seed (Cenkowski et al. 1989; 1993). The effect of harvest technique and timing on chlorophyll degradation and retention probably depends on the prevailing climatic conditions during the time of crop maturation. High seed moisture contents facilitate chlorophyll degradation, but low temperatures slow the rate (Ward et al. 1992). Further research is needed to determine the rate of chlorophyll clearing under UK climatic conditions and the effects of swathing on the process. This would reveal whether there is scope through harvest timing and practice, to reduce the seasonal variation in chlorophyll concentration and ensure low concentrations in UK crops at harvest.

Whilst the main problem with high chlorophyll concentrations comes from seasonal variation in weather conditions, even in a low chlorophyll year concentrations in oil extracted from UK seed are generally higher than those in oil from European seed. There is potential for reducing the concentration significantly through a change in variety. In 2001 the mean concentration for Apex in RL trials was 5.56 mg kg^{-1} (Table 3) and in commercial crops was 6.33 (Table 5). This equates to a concentration in crude oil of about 33 mg kg^{-1} (Fig. 6b), the norm for a low chlorophyll year in the UK. If the lower chlorophyll varieties such as Pronto or Escort (2.2-3 mg kg^{-1}, Table 3 and Table 5) dominated the market instead of Apex, then in 2001 the chlorophyll concentration in crude oil would have been about 19-22 mg kg^{-1}. This is comparable with the concentrations in oil extracted from European produced seed.

6. Conclusions and recommendations

- Seed and crude oil chlorophyll concentrations were low in 2001, but higher in 2000 and 1999. Even in 2001, concentrations in crude oil were greater than those found in oil extracted from seed imported from Continental Europe.
- A major target for the UK industry is to reduce the seasonal fluctuation in concentration and to reduce the base concentration (i.e. that found in a low chlorophyll year) to that of its European competitors.
- In 2001, concentrations differed significantly between RL sites, but there was no clear effect of latitude.
- Varieties grown under the same husbandry regime (RL trials) differed significantly in their seed chlorophyll concentration. The most popular UK variety, Apex, had the highest concentration. These varietal differences were independent of differences in maturity date.

- Of the relatively small number of varieties tested, restored hybrids had significantly lower concentrations than conventional lines and varietal associations.
- The lower seed rate used with hybrids such as Synergy had no significant effect on the chlorophyll concentration.
- Wider production of the low chlorophyll varieties such as Pronto (hybrid), Escort, Fortress and Herald (conventional lines) rather than Apex, would, in a low chlorophyll year, reduce the chlorophyll concentration in crude oil to values found in oil from European seed (i.e. 17-19 mg pheophytin kg^{-1}).
- A major factor contributing to the high concentrations in crude oil in recent years is seasonal variation in weather conditions leading up to, and during harvest. High concentrations appear to be associated with dry weather between swathing and harvest. It is possible that high concentrations might also arise during unsettled weather if farmers are tempted to harvest prematurely.
- The data supporting a link between seed chlorophyll concentration and specific weather conditions during harvest comes from a limited number of sites. Further research is needed over a wider range of site/season combinations with detailed measurement of local weather conditions, in order to substantiate the link.
- Research is needed to investigate the rate of chlorophyll degradation under UK conditions and its relationship to seed drying and seed maturation. The effects of harvest technique and timing on the process needs to be investigated in order to identify whether improved recommendations for harvest practice can help reduce the chlorophyll concentration, particularly in the 'high chlorophyll' years.

7. Acknowledgments

We thank Jim Daun (Grain Research Laboratories, Canadian Grain Commission) for useful discussions and the gift of reference samples, Bill Ashby (BOCM-Pauls) and Elaine Booth (SAC) for advice and supporting data, Neal Garrow (Cargill plc) for carrying out the Soxhlet extractions and analyses, the Biochemistry and Molecular Genotyping Group (NIAB) for providing samples of commercial seed and associated husbandry data, and the HGCA for financial assistance. We are also grateful to Wolfgang. Sauermann (Landwirtschaftskammer Schleswig-Holstein, Germany), André Merrien (CETIOM, France) and Hilmer Sorensen (KVL, Royal Veterinary and Agricultural University, Denmark) for their assistance with the survey. SAC receives financial support from the Scottish Executive Environmental and Rural Affairs Department.

8. References

Abraham V. and deMan J.M. (1986). Hydrogenation of canola oil as affected by chlorphyll. *Journal of the American Oil Chemists Society*. 63: 1185-1188.

Brown J., Davis J.B., Erickson D.A. and Brown A.P. (1999). Effects of swathing on yield and quality of spring canola in Northern Idaho. *Journal of Production Agriculture.* 12:33-37.

Canadian Grain Commission (2002). Quality monitoring of Canadian oilseed crops, pp 1-6. http://www.cgc.ca/Grl/Oilseeds/oilseeds-e.htm

Carr R.A. (1995). Processing the seed and oil. In: *Brassica Oilseeds. Production and Utilization.* pp 267-290. D.S. Kimber and D.I. McGregor (eds) CAB International, Wallingford.

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

Cenkowski S., Sokhansanj S. and Sosulski F.W. (1989). Effect of harvest date and swathing on moisture content and chlorophyll content of canola seed. *Canadian Journal of Plant Science.* 69: 925-928.

Daun J.K. (1982). The relationship between rapeseed chlorophyll, rapeseed oil and percentage green seeds. *Journal of the American Oil Chemists Society.* 59: 15-18.

Daun J.K. and Thorsteinson C.T. (1989). Determination of chlorophyll pigments in crude and degummed canola oils by HPLC and spectrophotometry. *Journal of the American Oil Chemists Society*. 66: 1124-1128.

Endo Y., **Daun J.K. and Thorsteinson C.T. (1992).** Characterisation of chlorophyll pigments present in canola seed, meal and oils. *Journal of the American Oil Chemists Society*. 69: 564-568.

Firestone D. (1998). Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th Edition, AOCS Press, Champaign. Methods Ak 2-92, Cc 13d-55, Cc 13i-96, Am 2-93.

Johnson-Flanagan A.M. and Spencer M.S. (1996). Chlorophyllase and peroxidase activity during degreening of maturing canola (*Brassica napus*) and mustard (*Brassica juncea*). *Physiologia Plantarum.* 97: 353-359.

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

Kiritsakis A. and Dugan L.R. (1985). Studies in photooxidation of olive oil. *Journal of the American Oil Chemists Society*. 62: 892-896.

Met Office (2002). Weather and climate: past weather UK. http://www.met.gov.uk/climate/uk

Suzuki K. and Nishioka A. (1993). Behaviour of chlorophyll derivatives in canola oil processing. *Journal of the American Oil Chemists Society*. 70: 837-841.

Sylvester-Bradley R., Lunn G., Foulkes J., Shearman V., Spink J. and Ingram J. (2002). Management strategies for high yields of cereals and oilseed rape. In: *Agronomic Intelligence: The Basis for Profitable Production.* pp. 8.1-8.17. Proceedings of the HGCA R & D Conference, Coventry, January 2002.

Tkachuk R., Mellish V.J., Daun J.K. and Macri L.J. (1988). Determination of chlorophyll in ground rapeseed using a modified near infrared reflectance spectrophotometer. *Journal of the American Oil Chemists Society.* 65: 381-385.

Usuki R., Endo Y. and Kaneda T. (1984). Prooxidant activities of chlorophyll and pheophytins on the photooxidation of edible oils. *Agricultural and Biological Chemistry.* 48: 991-994.

Van Deynze A.E., McVetty P.B.E., Scarth R. and Rimmer S.R. (1992). Effect of varying seed rates on hybrid and conventional summer rape performance in Manitoba. *Canadian Journal of Plant Science.* 72: 635-641.

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

Ward K., **Scarth R., Daun J.K. and Thorsteinson C.T. (1994).** Characterization of chlorophyll pigments in ripening canola seed (*Brassica napus*). *Journal of the American Oil Chemists Society*. 71: 1327-1331.

Ward K., **Scarth R., Daun J.K. and Vessey J.K. (1995).** Chlorophyll degradation in summer oilseed rape and summer turnip rape during seed ripening. *Canadian Journal of Plant Science.* 75: 413-420.

Appendix A

Survey of oilseed rape agronomy in the UK and countries exporting rapeseed to the UK

Outlined below are the questions included in the survey of European agronomists and a summary of their responses. NR indicates no response to the question.

Q.1 Have you received reports of any rise in seed chlorophyll concentrations in commercial crops in your region?

UK	France	Germany	Denmark
Yes. Reports of high concentrations in crude oil from UK rapeseed. Seed chlorophyll not routinely measured. Possible cause - premature swathing and low sunshine levels in the run up to harvest	No reports. Several possible causes listed which could account for it if it was reported. Variation between pods in canopy; use of hybrids and lower seed rates; later maturing varieties; use of desiccants before full maturity	No	Not considered a problem. It was a few years ago and was thought to be related to immature seed. Now mostly winter OSR is grown which is mature before swathing

Q.2. What harvest method is most commonly used?

Method	% of crop			
	UK	France	Germany	Denmark
Swathing	40	9	1	20
Desiccation,	40	1	1	0
Direct combining	20	90	98	80

Q 3. If swathed or desiccated, what is the normal time span between that and combining?

Harvest method	UK	Germany
Swathing	10-20 days	
Desiccation	10-15 days	
Not specified		8-10 days

Q 4. If you are involved in field trials what harvest method do you use, and have you observed seed chlorophyll content changing in recent years?

UK	France	Germany	Denmark
Swathing	Direct combing	Direct combining 50% Swathing 50%	NR
No change observed – seed chlorophyll not measured	NR	Chlorophyll content not determined	NR

Q 5. How would you describe the typical weather pattern in the run up to harvest? Were any of the of the harvest years 1999, 2000, 2001 unusual in their weather pattern?

UK	France	Germany	Denmark
Mixed	Dry and sunny	Mixed	Mixed
2000 had low sunshine hours	1999 was normal. 2000 was wet on the west side of France. Seed germination in the pod was observed	2001 was wet in June until the middle of July. Lower oil contents than 1999 and 2000 harvests	NR

Q 6. What are the predominant varieties grown and the approximate proportion of the total crop? Estimates are for crops in harvest years 2000 and 2001. Only the dominant varieties are listed. Where the sum is less than 100%, there are several other varieties each with a small share of the market.

UK		France		Germany		Denmark	
Variety	%	Variety	%	Variety	%	Variety	%
Conventional		*Conventional*		*Conventional*		*Conventional*	75
Apex	35	Pollen	26	Express	30		
Fortress	15	Canary	19	Mohican	10		
Escort	15	Capitol	11	Contact	5		
Madrigal	10	Madrigal	6	Prince + Fortress	5		
Contact	10	Columbus	6				
		Nava Jo	4				
Hybrids		*Hybrids*		*Hybrids*		*Hybrids*	25
Pronto	10	Pronto	3	Talent	30		
		Constant	3	Panther	5		
				Maja	5		
				Artus	5		
				Mendel	5		

Q 7. What proportion of spring rape is grown?

UK	10-15 %
France	0.5 %
Germany	0.5 %
Denmark	18 %

Q 8. What is the normal seed rate used for conventional inbred lines and hybrids?
Seed rates are in kg ha^{-1}. They are broad estimates because thousand seed weight differs widely between varieties.

	UK	France	Germany	Denmark
Conventional	4.5 – 6.0	2.0 – 3.0	2.5 – 3.5	NR
Hybrids	3.0 - 3.5	> 1.0	1.8 – 2.3	NR

Q. 9. Does the harvest method or any other factor (e.g. variety, seed rate) differ markedly across the country?

UK	No
France	More early varieties in the South to escape from drought in June/July. More hybrids in the south and south west, less at the east border
Germany	No
Denmark	NR