AUSTRALIAN OILSEEDS FEDERATION INCORPORATED

FINAL REPORT

"Chlorophyll in canola"

AOF 15-1

1 May 2000 to 30 June 2002

Dr R.J. Mailer, Agricultural Research Institute, Wagga Wagga (02 69381818)

NSW Agriculture

ISBN 0 7347 1421 1

Fig 1. (*Cover*). Samples of canola oil from canola seed samples containing increasing levels of chlorophyll of 6, 23, 35, 53 and 81 mg/kg respectively.

AUSTRALIAN OILSEEDS FEDERATION INCORPORATED

R&D PROJECT 2000/2002

Project Title: Chlorophyll in canola

Reference number given by AOF to Preliminary Proposal: AOF 15-1

Organisation: NSW Agriculture

Administrative Contact:

Title	First name	Surname
Mr	Graham	Denney

Address:
NSW Agriculture, Locked Bag 21, ORANGE NSW 2800

Phone: (02) 6391 3219
Fax: (02) 6391 3327

Email: graham.denney@agric.nsw.gov.au

Principal Investigator:

Title	First name	Surname
Dr	Rodney	Mailer

Address:
Wagga Wagga Agricultural Institute, PMB, Wagga Wagga, NSW 2650

Phone: 02 69381818
Fax: 02 69381809

Email: rodney.mailer@agric.nsw.gov.au

Starting date # : 1 / 5 /2000

Finishing date # : 30 / 06 /2002

Project Outline and Budget

	Process	Criterion	Cost	Total
1	Genotype by environmental relationship	Analyse canola samples from site x cultivar x year. Approx 100 samples.	100 x $31	$3100
2	BHC's study	Obtain samples from bulk handlers and canola trials/crops with the worst levels of chlorophyll/green seed. Approx 100 samples.	100 x $31	$3100
3	Green seed screening test	Determine the relationship between number of green seeds and spectrophotometric reading. Say 50 samples tested for green seed by dip test and compared with spectrophotometric test. Seed colour photographs, relationship graphs.	50 x $10	$1500
4	NIR calibration	Develop a calibration for NIR rapid analysis to be used for screening future canola trials	78 x $31 = 5 days x $0.57/min =	$2418 $1197
5	Analysis of chlorophyll from 2000/01 breeding trials	5,000 canola trial samples will be analysed from 2000 breeding trials and results analysed against sites and cultivars.	Analysis, reports Biometrical analysis	$2,000 $500
6	Travel	Meeting with coordinators Sample collection		$1,200
7	*TOTAL*			***$15,015***

Abstract

The short growing period for canola in Canada is a problem as incomplete maturity often results in green seed. The chlorophyll in the seed is transferred to the oil during processing, resulting in green oil. The colour is then difficult and expensive to remove. Recent reports suggest that Australian canola may also undergo periodic harvests which result in green seed. Frost damage in particular has been reported as the major cause. This study was designed to determine the degree of the problem, the reasons and possible solutions.

Summary

1. Genotype by environmental relationship. Analyse canola samples from site x cultivar x year.

Samples from 1997 and 1998 trials were analysed to determine chlorophyll content. The results indicated a wide range of concentrations particularly with some cultivars such as Range (5-27 mg/kg) and Rainbow (3-32 mg/kg). The study was repeated in 1999 and 2000, encompassing seven cultivars from six sites, in replicated trials. The results in 1999 ranged from 0 to 15.1 mg/kg and in 2000 from 0 to 24.5 mg/kg in the seed. No samples exceeded 30 mg/kg.

In 1999, samples of frost-damaged seed from WA were tested for chlorophyll with all of the samples being less than 30 mg/kg and only a few above 20 mg/kg. Again in 2000, frost damaged samples were obtained from WA and were also low in chlorophyll with levels below 5 mg/kg.

The study indicated that chlorophyll levels in Australian canola were low, with only a few samples exceeding 30 mg/kg over the four years of testing.

2. Bulk Handling Company seed. Obtain samples from bulk handlers and canola trials/crops with the worst levels of chlorophyll/green seed.

BHCs throughout the Australian canola growing regions provided 800 samples for chlorophyll analysis. Although generally the concentration was low, 21 samples from the Temora site were consistently high with chlorophyll levels of between 11 to 67 mg/kg and many exceeding 30 mg/kg in the seed. The variety Pinnacle had the highest concentration of chlorophyll. The level of chlorophyll was attributed to plant stress in the region in that year.

This study was not unbiased as the samples had been selected as green seed by the receival site. Additionally, the samples were limited in number and many were unknown cultivars. However, the range and intensity of chlorophyll indicated that there is a possibility of high levels of green seed to occur at specific sites. No other seed samples obtained from BHCs in 2000 had significant levels of green seed.

3. Green seed screening test. Determine the relationship between number of green seeds and spectrophotometric reading.

Seed samples obtained from Canada and BHCs in 2000 were used to compare the green seed test with the chlorophyll spectrophotometric test described previously. Despite counting either “distinctly dark green seeds” or “partially green seeds” no accurate relationship could be formed between the two. The green seed test was able to identify canola samples with potentially high levels of chlorophyll. Several pale green seeds contributed as much to the chlorophyll content as two distinct green seeds.
The test is considered to be limited in usefulness in obtaining an accurate measure of chlorophyll, or for identifying potential problems with green oil, and indicates the need for a rapid test at receival points.

4. NIR calibration. Develop a calibration for NIR rapid analysis to be used for screening future canola trials

A Foss NIR 6500 spectrophotometer was used to develop a calibration for the estimation of chlorophyll in intact canola seed. This has been used by others (Tkachuk *et al*., 1998; Daun et al., 1994) for ground seed. Calibration samples were obtained from the Canadian Grain Commission's Grain Research Laboratory, Winnipeg, Canada, Australian Breeder's Trials and Australian Bulk Handling Companies. Chlorophyll on the calibration set was determined by AOCS method Ak 2-92. A NIR spectrophotometer with a spinning sample module was used and a calibration established with 97 canola samples ranging from 1 to 66 mg/kg chlorophyll. The instrument was operated in reflectance mode using WINISI software (FOSS Pacific). Partial least squares (PLS) regression was used to develop a calibration from the second derivative of the log 1/R of the spectra. A linear regression (RSQ) of 0.959 was obtained for the set with a standard error of prediction (SEP) of 3.076. The NIR method allowed analysis of 200-300 samples per day.

5. Analysis of chlorophyll from 2000 / 01 breeding trials

Breeders' seed was selected from the National Brassica Improvement Project (NBIP) and NSW Agriculture seed trials in 1999/2000. The trials included 8489 samples from 64 individual variety trials and 179 lines from throughout Australia. The trials represented 6 groups including early, mid, and late maturing lines and triazine tolerant types. The study was repeated in 2000/2001 with a further 7220 samples from 75 trials and again incorporating six groups. Samples in both years were analysed by NIR.

1999/2000: Chlorophyll concentration averages exceeded 10 mg/kg at only 5 sites and no site averaged more than 20 mg/kg. There was a wide range of chlorophyll levels within groups. The National early-maturity group, had the greatest range in individual lines with concentration from 0.5 to 51.0 mg/kg. Groups 2 and 4, both NSW Agriculture trial sites, had the least variation from 0.0 to 19.3 and 0.7 to 20.0 mg/kg respectively.

Chlorophyll levels for lines in Group 1 (national early maturity trial) and Group 6 (Triazine Tolerant trial) were generally higher than chlorophyll levels for lines in the other groups. Ten sites in the NBIP had lines with chlorophyll levels greater than 20 mg/kg with 91 samples above 20 mg/kg and of these, 19 were above 30 mg/kg.

There were some significant cultivar effects with some lines being very variable. PACN175 for example, had a chlorophyll concentration ranging from 2.6 mg/kg at Tamworth to 42.7 mg/kg at Newdegate. Newdegate had the highest concentration of chlorophyll from 4 to 51 mg/kg.

2000/2001: Chlorophyll concentration averages in 2000 exceeded 10 mg/kg at only one site and no site averaged more than 15 mg/kg. Group 6 (triazine tolerant) had the greatest range and highest value from 2.1 to 14.7 mg/kg. The maximum level measured was 43.1 mg/kg chlorophyll at Lameroo in South Australia.

6. Conclusion

Generally chlorophyll levels are insignificant in comparison to the levels found in Canada and should not cause refining problems for the industry. Despite this, particular batches of samples obtained from some sites, such as Temora, have shown that regions may produce large quantities of green seed under particular environmental conditions. For this reason, a screening technique for receival sites appears to be justified.

The current green seed test does not appear to give an adequate indication of potential problems in canola seed. A more direct test for chlorophyll content, such as NIR, would be more desirable.

BODY OF THE REPORT

Introduction

Chlorophyll in canola seed is generally broken down to secondary pigments and by-products during the maturation process. In some cases, canola seed has been shown to retain the chlorophyll intact. In these cases the cotyledons can be seen to be green when the seed coat is removed. As the chlorophyll is oil soluble, when oil is extracted, it retains a green colour, the intensity being proportional to the concentration of chlorophyll in the seed.
There are several reasons that chlorophyll is retained in the seed during maturation and generally it is due to stress related causes in which the plant dies prematurely. These causes include frost damage (Daun *et al*.,1985) or severe water stress. Under these conditions the chlorophyll will remain in the seed indefinitely. Samples in this laboratory have been analysed two years after receival and shown to still contain the same amount of chlorophyll as at the time of receival.
Chlorophyll retention in canola is a major problem in Canada where canola is harvested in time to escape the onset of winter. In these cases crops can be damaged and killed by frost before they reach maturity. In some cases, oil extracted from these crops may contain chlorophyll in excess of 100 mg/kg and have a dark green appearance. Australian canola matures into increasingly hotter drier conditions of summer and generally dries off to produce a yellow oil with virtually no chlorophyll. However, there have been occasions in which crops have been water stressed or frost damaged, and in which green oil has resulted.

Australian Standards: The Australian Oilseed Federation has adopted the Canadian methods of analysis and standards for acceptance for green seed. The measurement of chlorophyll is described in the AOCS Standards Ak 2-92 (1998) and incorporates "Swedish Tubes", stainless steel cylinders unavailable commercially in Australia. The method is specific and is not generally available at canola receival sites. Currently, only the Wagga Wagga Agricultural Institute (WWAI) has access to the tubes and the test method in Australia. Samples have been analysed at both the Canadian Grain Commission and at WWAI to ensure that results from both laboratories were consistent. A comparison between the two laboratories is shown in Table 1.

Table 1. Comparison of analytical results for chlorophyll in canola seed from Canada and WWAI

Site	Sample	WWAI	Canadian Grain Commission
Canada	CN9HF28	30	33
Canada	CN9CC15	83	81
AOCS proficiency test	STD #4	12	13
Canada	CN9CL13	43	41
Canada	CN9CL12	36	35
Canada	CN9HF21	6	6

To allow receival sites to estimate rapidly the seed chlorophyll content, a "green seed" test has been instigated. This involves a random sample of 100 seeds which are crushed and observed for green cotyledons (Fig. 1 & 2). If there are green seeds, four more random samples of 100 seeds are selected and an average number of green seeds per 100 is determined. More than two green seeds per 100 are considered above the limit and subject to negotiation between the buyer and seller.

1. *Genotype by environmental relationship - Analyse canola samples from site x cultivar x year.*

In 1998 a number of samples were analysed from 1997 and 1998 trials to determine the degree of chlorophyll retention. Several samples of each cultivar were tested from each site. Although the cultivars were consistent at each site tested, there was considerable difference between sites. Range (5-27 mg/kg) and Rainbow (3-32 mg/kg) showed the greatest variability.

The investigation was repeated with a more complete set of samples from trials in 1999 and 2000, encompassing seven cultivars from six sites throughout the canola-growing region (Table 2a & b).

Table 2a. Average chlorophyll content (mg/kg) of a selection of samples from S4 mid maturity replicated research trials including seven cultivars x five sites during 1999/2000 (Wallendbeen was not harvested in 1999).

Cultivar	Wagga Wagga		Junee Reefs		Boree Creek		Ariah Park		Wongan Hills	
Replicate	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
Oscar	6.5	7.6	6.2	4.7	2.2	3.9	3.5	3.9	5.3	7.8
Dunkeld	8.9	8.6	14.2	14.3	1.0	1.6	6.8	2.5	15.1	8.4
Rainbow	10.7	6.9	7.1	5.6	0.3	0.0	2.6	1.2	7.0	15.7
Surpass 600	6.6	12.2	10.3	10.4	0.0	3.5	1.6	3.2	7.7	14.2
Pinnacle	2.0	4.8	8.7	7.5	0.0	1.3	1.4	6.7	nr	Nr
Grouse	11.6	4.7	7.7	6.5	0.0	0.5	1.7	1.4	7.3	3.7
Scoop	6.8	10.1	8.5	11.1	2.6	3.8	4.6	nr	11.7	3.7

Table 2b. Average chlorophyll content (mg/kg) of a selection of samples from S3 mid maturity replicated research trials with seven cultivars x six sites during 2000/01.

Cultivar	Wagga Wagga		Junee Reefs		Boree Creek		Ariah Park		Wongan Hills		Wallendbeen	
Replicate	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
Oscar	3.6	4.9	6.8	9.9	2.6	4.9	5.0	5.5	1.6	0.0	2.4	3.3
Dunkeld	0.9	2.1	6.5	5.1	0.0	0.6	0.9	2.6	3.7	2.5	5.3	1.8
Rainbow	7.3	4.6	3.5	2.5	3.9	0.1	2.2	4.1	nr	nr	4.3	5.9
Surpass 600	0.7	1.4	6.6	3.8	1.5	1.6	2.8	1.1	1.3	2.3	3.3	4.3
Pinnacle	2.9	4.4	1.7	3.8	2.7	4.7	24.5	11.0	nr	nr	3.2	3.1
Grouse	6.8	3.3	5.6	4.1	0.9	2.5	9.3	4.9	0.0	0.0	7.4	4.0
Scoop	4.8	6.1	9.2	4.6	5.3	5.6	10.5	5.5	1.3	0.0	nr	nr

nr - no result

Frost damaged samples: In 1999 a further set of samples, which were severally frost-damaged, were received from WA (Paul Carmody) for chlorophyll test. All of the samples tested were less than 30 mg/kg with only a few above 20 mg/kg. Again in 2000, frost damaged samples were obtained from WA and were tested for chlorophyll. These samples were also low in chlorophyll with levels below 5 mg/kg.

Overall, the study indicated that chlorophyll levels in Australian canola were generally low, with only a few samples exceeding 30 mg/kg over the three years of testing. In most cases, concentrations were less than 10 mg/kg at which level no green colour can be detected in the oil.

2. Bulk Handling Company (BHC) study: Obtain samples from bulk handlers and canola trials/crops with the worst levels of chlorophyll/green seed.

In 2000, 800 samples were received from BHCs in Victoria, NSW, SA and WA for analysis. The Temora site had reported high levels of green seed. Generally, chlorophyll levels were low, (<20 mg/kg) except at the Temora site. Several samples grown in areas around Temora had relatively high chlorophyll levels of between 11 and 67 mg/kg (Table 3).

Although the samples shown in Table 3 are a limited subset of the total 800 samples tested from canola sites across Australia, they show clearly that with suitable conditions, a single region may produce canola seed with consistently high chlorophyll. This may result in reduced opportunities to blend green seed with mature seed to obtain acceptable chlorophyll levels.

Table 3. Canola samples received at Temora BHC in 1999/2000

Site	**Cultivar**	**Chlorophyll concentration (mg/kg)**
Cootamundra	Pinnacle	21
Greenthorpe	Oscar	11
Harden	Oscar	29
Cootamundra	Oscar	19
Old Junee	Unknown	12
Cootamundra	Oscar	44
Temora	Unknown	28
Harden	Oscar	35
Temora	Unknown	13
Boorowa	Unknown	27
Temora	Unknown	26
Mirrool	Unknown	37
Cootamundra	Pinnacle	67
Grenfell	Clancy	25
Frogmore	Unknown	17
Temora	Unknown	13
Cowra	Oscar	11
Henty	Charlton	18
Junee	Pinnacle	39
Temora	Unknown	33
Temora	Unknown	14

3. Green seed screening test. Determine the relationship between number of green seeds and spectrophotometric reading.

Seed samples obtained from Canada and BHCs in 2000 were used to compare the green seed test with the chlorophyll spectrophotometric test described previously. Despite counting either "distinctly dark green seeds" or "partially green seeds" no accurate relationship could be formed between the two. The green seed test was only useful in identifying canola samples with green seed and a potential to cause oil discolouration after extraction. It was shown that several pale green seeds contributed as much to the overall chlorophyll content as two distinct green seeds. This was also the finding of Daun (1982) and Daun and Symons (2000) who showed poor correlation between green seeds and chlorophyll content due to several factors including operator perceptions of "greenness".

Fig 1. Seed sampling ruler and paint roller used to flatten seed.

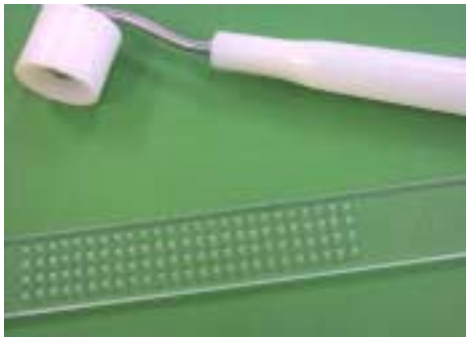
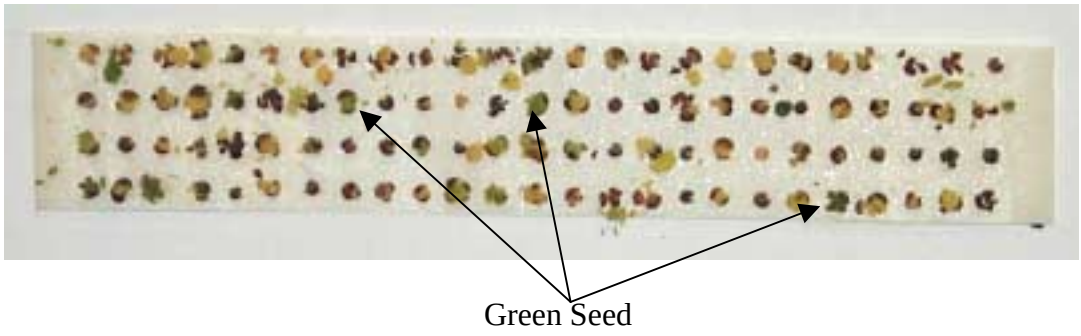


Fig 2. Flattened seed on masking tape



4. NIR calibration: Develop a calibration for NIR rapid analysis to be used for screening future canola trials.

A Foss NIR 6500 spectrophotometer, used to screen breeders seed samples through the WWAI canola research laboratory, was used to develop a calibration for the estimation of chlorophyll in intact canola seed. This has been achieved by others (Tkachuk et al., 1998; Daun et al., 1994) using ground seed.

Seed samples: Calibration of the NIR spectrophotometer required canola seed with a range from low to high levels of chlorophyll. Due to the limited availability of canola seed with high chlorophyll in Australia, calibration samples were supplemented with seed from the Canadian Grain Commission's Grain Research Laboratory, Winnipeg, Canada. Commercial canola seed was obtained from Australian Bulk Handling Companies.

Analysis: Chlorophyll was determined using the official spectrophotometric method of the American Oil Chemists' Society (AOCS) Ak 2-92. Results have previously been compared to other laboratories and through the American Oilseed Chemists' Proficiency Program to ensure accuracy and reliability of the analysis.

Calibration of the NIR spectrophotometer: A Foss NIR Systems 6500 Near InfraRed Spectrophotometer with a spinning sample module was utilised for this study. A calibration was established using a set of 97 canola samples selected from Canada, Australian Bulk Storage Companies and Breeders' Trials ranging from 1 to 66 mg/kg chlorophyll. The instrument was operated in reflectance mode using unground seeds. WINISI software (FOSS Pacific) was utilised. Partial least squares (PLS) regression was used to develop a calibration from the second derivative of the log 1/R of the spectra.

A linear regression (RSQ) of 0.959 was obtained for the calibration set of 97 samples with a standard error of prediction (SEP) of 3.076. The NIR method allowed analysis of 200-300 samples per day.

5. Analysis of chlorophyll from 1999/2000 and 2000/01 breeding trials.

For comparison of chlorophyll levels between breeding lines and across a range of environments, Breeders' seed was selected from the National Brassica Improvement Project (NBIP) and NSW Agriculture seed trials in 1999/2000. The trials included 8489 samples from 64 individual variety trials (Table 4) and 179 lines (Table 5) from canola growing areas throughout Australia. All trials were replicated and samples for chlorophyll were taken from each plot. These trials were separated into 6 groups including early, mid, late maturing and triazine tolerant types.

The comparison was repeated in 2000/2001 with a further 7220 samples from 75 trials from four States in Australia and again incorporating six groups.

Samples were analysed by NIR. The NIR6500 was calibrated using samples analysed by the official AOCS method Ak2-92. This method allowed rapid analysis of samples from the breeding trials in 2000 and in 2001.

Chlorophyll concentration 1999/2000: From the 64 sites analysed, mean chlorophyll concentration exceeded 10 mg/kg at only 5 sites and no site averaged more than 20 mg/kg (Table 4a). Although the means for individual sites were generally low, there was a wide range of chlorophyll levels within groups. For example, Group 1, the National early-maturity group, had the greatest range with

concentrations from 0.5 to 51.0 mg/kg. Groups 2 and 4, both NSW Agriculture trial sites, had the least variation from 0.0 to 19.3 and 0.7 to 20.0 mg/kg respectively.
Chlorophyll levels for lines in Group 1 (national early maturity trial) and Group 6 (Triazine Tolerant trial) were generally higher than chlorophyll levels for lines in the other groups. Ten sites in the NBIP had lines with chlorophyll levels greater than 20 mg/kg with 91 samples above 20 mg/kg and of these, 19 were above 30 mg/kg.
For the 3 lines in each group with the lowest (across sites) concentration of chlorophyll, Table 4c presents the fitted value from the analysis, the raw mean and the incidence of that line. Table 4d is the equivalent for the three lines with highest (across sites) concentration of chlorophyll. The (across sites) means for all these lines were all less than 20 mg/kg but some lines were quite variable. PACN175 had a chlorophyll concentration ranging from 2.6 mg/kg at Tamworth to 42.7 mg/kg at Newdegate.

Chlorophyll concentration 2000/2001: In 2000, average chlorophyll concentration exceeded 10 mg/kg at only one site and no site averaged more than 15 mg/kg (Table 5). Group 6 (triazine tolerant) had the greatest range and highest value from 2.1 to 14.7 mg/kg. The maximum level measured was 43.1 mg/kg chlorophyll at Lameroo in South Australia.

6. Biometrical analysis:

Analytical results from breeders trial samples grown in 1999 and in 2000 were provided to Ms B. Orchard (WWAI) for statistical analysis. The results of this analysis have been published in the Australian Journal of Experimental Agriculture (in press). The analysis showed that from six sample groups, environment was the major determinant of chlorophyll (37.5-57.6%) with ‘variety' (7.4 – 22.3%) and ‘site by variety' (7.6-14.1%) being of similar importance.

7. Travel :

Progress reports have been presented to the AOF Standards meeting throughout the project in Sydney and Melbourne with a final summary, which will be provided to the AOF on 26/09/2002.

8. Summary

The analysis of over 8489 samples from trials in 2000 and a further 7220 samples in 2001, from throughout the Australian canola-growing region has provided considerable data regarding chlorophyll levels in Australian canola. The study has determined that chlorophyll concentration can often exceed the acceptable industry standards. Significantly, 10 of the 64 sites in 2000 contained lines that exceeded 20 mg/kg of chlorophyll. Ninety-one of those samples were above 20 mg/kg.

The results shown in Tables 4 and 5 would indicate that the problem is not associated purely with the maturity groups as all show some varieties with high chlorophyll. The sites with high levels in 1999 were also well distributed including Katanning and Newdegate (WA), Rutherglen (Vic.) and Turretfield (SA). Genotype had a significant effect, with some lines showing consistently high chlorophyll. Despite this, it does not seem to be related to genetic material from a particular breeding program as the highest chlorophyll lines included lines from several individual-breeding programs.
In a preliminary project carried out in 1997 and 1998, analysis of samples from selected sites showed variability in the samples analysed. However, the average chlorophyll level was only 1.5 mg/kg and

2.3 mg/kg respectively. This study did not identify the problem which has been experienced at Australian receival points. From 800 samples received and analysed from Bulk Handling Companies in Victoria, NSW, SA and WA in 1999 and 2000, results ranged from 0 to 67 mg/kg with an average of 24 mg/kg. However, this was not a random sample as Bulk Handlers had selected samples to send for analysis.

The variable Australian environment is the major factor in annual variations in quality and agronomic performance of canola lines. Selection for more tolerant canola varieties to improve quality consistency has been the aim of Australian breeders for several years. Screening for new breeding lines involves selection for a range of traits, both agronomic and quality types. Increased oil and protein content and reduced glucosinolate and erucic acid concentration have been major selection criteria in the past. However, the use of NIR spectrometry allows for multiple trait analysis on large numbers of samples in a relatively short time and at low operating cost. The possibility of selecting for cultivars low in chlorophyll is enhanced through knowledge that there is a strong cultivar effect. Breeding programs need to be aware of the genetic influence in the retention of high chlorophyll in mature canola. A positive selection pressure may now be applied to remove the lines that show a tendency toward high chlorophyll content over the range of growing environments in the canola growing areas.

Acknowledgments

The Australian Oilseed Federation and NSW Agriculture funded the project. The National Brassica Improvement Project is funded by the Grains Research and Development Corporation (GRDC).

References

Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th edition., AOCS Press, Champaign, 1998.

Daun JK (1982) The Relationship between Rapeseed Chlorophyll, Rapeseed Oil Chlorophyll and Percentage Green Seeds. *Journal of the American Oil Chemists' Society* 59, 15-18.

Daun JK, and Symons S (2000) How Green Is Green? Sampling and Perception in Assessing Green Seeds and Chlorophyll in Canada. *Journal of American Oil Chemists' Society* 77, 1209-1213.

Daun JK, Clear KM, Williams PC (1994) Comparison of Three Whole-Seed Near Infrared analysers for Measuring Quality Components of Canola Seed. *Journal of the American Oil Chemists' Society* 71, 1063-1068.

Daun JK, Clear KM, Mills JT (1985) Effect of frost damage on the quality of canola (*B. napus*). *Journal of the American Oil Chemists' Society* 62, 715-719.

Tkachuk R, Mellish VJ, Daun JK, Macri LJ (1998) Determination of Chlorophyll in Ground Rapeseed Using a Modified Near Infrared Reflectance Spectrophotometer. *Journal of the American Oil Chemists' Society* 65, 381-385.

Table 4a. List of trial sites and chlorophyll minimum, maximum and mean for each site in 2000. The trials consisted of 6 groups: Group 1 (NBIP early-maturity), Group 2 (NSW early-maturity), Group 3 (NBIP mid-maturity), Group 4 (NSW mid-maturity), Group 5 (NBIP late-maturity), Group 6 (NBIP triazine-tolerant cultivar). Western Australia (W), New South Wales (N), South Australia (S), Victoria (V).

Group 1	min	max	mean
Merredin W	1.8	13.4	4.7
Minnipa S	0.5	21.4	6.7
Turretfield S	3.1	27.8	9.1
Condobolin N	1.8	14.1	6.1
Horsham V	5.0	19.6	9.3
Lameroo S	2.2	15.6	5.9
Newdegate W	4.0	51.0	18.9
Tamworth N	2.0	11.4	3.6
Walpeup V	1.0	18.9	4.9
Wagga Wagga N	2.5	20.9	8.1
Wongan Hills W	1.0	15.0	5.4

Group 2	min	max	mean
Bogan Gate N	0.0	9.7	3.8
Condobolin N	0.9	9.4	3.1
Coonamble N	2.0	9.7	4.1
Tamworth N	1.5	8.7	3.4
Wagga Wagga N	2.8	19.3	6.6
Thuddungra N	1.3	7.4	3.6

Group 3	min	max	mean
Avondale W	1.5	7.9	4.0
Lake Bolac V	0.7	7.5	3.5
Turretfield S	2.0	18.1	7.2
Bordertown S	3.2	16.3	9.5
Horsham V	3.4	16.2	6.6
Katanning W	2.0	31.7	15.5
Suntop N	1.7	5.8	3.9
Wongan Hills W	1.8	6.0	3.1
Wagga Wagga N	2.5	13.1	6.2
Tamworth N	1.0	21.0	5.7

Group 4	min	max	mean
Ariah Park N	2.7	17.0	6.7
Bingara N	1.2	13.0	6.2
Boree Creek N	0.7	14.1	5.6
Borenore N	1.9	17.8	4.8
Condobolin N	1.4	7.3	3.3
Finley N	1.0	12.1	3.3
Grenfell N	0.8	9.7	4.0
Junee Reefs N	3.9	20.0	10.2
Pine Ridge N	2.3	12.4	5.1
Suntop N	2.4	7.1	4.0
Tamworth N	0.4	9.8	3.9
Wagga Wagga N	2.2	14.4	6.1
Beargamil N	1.8	12.8	5.3
Coolamon N	2.5	10.4	4.7
Combaning N	1.7	11.2	4.8
Dunedoo N	1.9	7.2	3.8
Merriwa N	1.9	6.4	3.2
Thuddungra N	1.5	8.1	3.9
Yass N	0.5	7.4	3.4

Group 5	min	max	mean
Wagga Wagga N	2.0	13.8	4.8
Bordertown S	4.3	20.9	10.5
Turretfield S	3.0	22.2	8.6
Esperance W	0.8	12.6	3.6
Horsham V	2.5	14.1	6.5
Lake Bolac V	2.0	13.2	5.4

Group 6	min	max	mean
Lameroo S	0.7	22.5	5.2
Merredin W	2.7	13.3	4.9
Rutherglen V	3.3	36.3	15.5
Struan S	2.1	14.5	5.9
Grenfell N	0.2	12.3	3.2
Hopetoun	2.5	19.0	6.5
Horsham V	1.7	17.1	4.8
Mount Barker W	1.5	13.2	4.0
Tamworth N	1.3	9.5	3.3
Turretfield S	2.0	17.5	5.3
Wagga Wagga N	2.0	24.0	7.9
Wongan Hills W	4.0	19.3	8.9

Table 4b. List of canola cultivars. Cultivar acronyms on breeding lines represent cultivars from individual breeding programs. The trials consisted of 6 groups as described in Table 4a.

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AGC8	PACN164	AGC14	SURPASS600	RO018	PINNACLE
NS03767	BLN1999	RO012	RN14	BLN2359	TO001
AGC7	SURPASS400	NS03788	BLN2005	RO022	TN6
AGC6	PACN162	AGC9	BLN2153	RO019	TN4
RO005	BLN2025	AGC16	SCOOP	AGC17	KAROO
AGC5	BLN2003	AGC11	AGA99-13	OSCAR	PACT2001
BLN2300	BLN2026	AGC15	PURLER	AGC22	TO007
BLN2288	RN2	CHARLTON	BLN2114	BLN2358	AGT1
PACN173	BLN2033	RO010	RN15	AGC23	DRUM
AGC4	AGA99-27	GROUSE	SURPASS600TT	BLN2215	TO002
RO007	44C71	BLN2312	TN4	BLN2216	PACT2003
RO001	MYSTIC	SCOOP	BLN2008	AGC24	PACT2002
BLN2017	BLN2030	RO009	46C03	RAINBOW	AGT2
RO008	BLN2029	BLN2323	NS3094	BLN2355	PACN164
AGC3	BLN2060	AGC13	46C01	BLN2065	AGT5
BLN2299	RAINBOW	BLN2319	46C72	PACY9043	AGT4
PACN170	KAROO	RO014	BLN1981	RO017	TN1
AGC1	BLN2062	AGC12	TM4	RO021	TN7
BLN2311	OSCAR	SURPASS600	INSIGNIA	BLN2200	AGT6
NS03752	BLN1960	DUNKELD	CLANCY	CHARLTON	AGA99-26
PACN175	GEORGIE	NS03741	RIPPER	AGC18	SURPASS600TT
RO006	BLN2054	BLN2354	BLN2137	AGC20	TO004
MYSTIC	MONTY	BLN2256	BLN2006	DUNKELD	AA8-6
RO002	BLN2004	RO015	BLN2173	P98132	AGT3
AGC2	AGA99-04	AGC10	BLN1994	AGC21	TO008
RAINBOW	BLN2017	NS03787	BLN1560	BLN2087	AGA99-27
MONTY	PACN176	BLN2293	BLN1990	AGC19	CLANCY
BLN2298	TN1	BLN2191	PACN178	RO020	TO006
BLN2316	DRUM	RO013	TN6	BLN1888	TM8
RO004	EMBLEM	OSCAR	PACN168	RM22	TO005
RO003		RO011	TROOPER	RN18	TM4
BLN2062		BLN2320	TN7	BLN1766	TO003
PACN176		RO016	PINNACLE	RN19	HYLITE200TT
		NS03793	47C02	BLN2010	
		NS03729	BLN1592		
		RAINBOW	GROUSE		
			TM8		
			DUNKELD		
			AGA99-14		
			CHARLTON		
			BLN2162		
			OSCAR		
			RAINBOW		
			NS3082		
			NS3092		

Table 4c. Three canola lines with the lowest chlorophyll levels of each maturity group and triazine tolerant group.

Group 1	Raw mean	Site present
AGC2	4.88	All
RO008	5.01	All
PACN170	5.07	All
GROUP 2		
AGA99-04	3.28	All
BLN2062	3.20	5 of 6
44C71	3.20	All
GROUP 3		
RO010	3.95	All
RO011	4.37	All
RO014	5.02	All
GROUP 4		
TN4	3.37	All
TN7	3.67	12 of 19
46C01	3.85	All
GROUP 5		
RN18	3.55	2 of 6
BLN2215	4.19	All
OSCAR	4.76	All
GROUP 6		
AGA99-26	4.31	8 of 12
TN7	4.49	8 of 12
TN4	5.21	8 of 12

Table 4d. Three canola lines with the highest chlorophyll levels of each maturity group and triazine tolerant group.

GROUP 1	Raw mean	Site present
PACN175	13.83	All
PACN173	14.50	All
AGC6	15.20	All
GROUP 2		
PACN162	5.25	5 of 6
BLN2029	5.54	5 of 6
PACN176	6.45	All
GROUP 3		
BLN2256	8.38	All
BLN2293	8.18	All
AGC10	8.73	All
GROUP 4		
NS3082	6.83	All
BLN2162	7.59	12 of 19
PACN178	7.49	All
GROUP 5		
BLN2087	8.73	All
AGC17	9.56	All
AGC20	11.82	
GROUP 6		
HYLITE 200TT	8.59	3 0f 12
PACT2002	11.68	All
PACT2003	13.63	All

Table 5. List of trial sites and chlorophyll minimum, maxmum and mean for each site in 2001. The trials consisted of 6 groups: Group 1 (NBIP early-maturity), Group 2 (NSW early-maturity), Group 3 (NBIP mid-maturity), Group 4 (NSW mid-maturity), Group 5 (NBIP late-maturity), Group 6 (NBIP triazine-tolerant cultivar). Western Australia (W), New South Wales (N), South Australia (S), Victoria (V).

Group 1	**Min**	**Max**	**Mean**
Newdegate W	0.0	9.8	4.2
Tamworth N	0.0	9.8	3.2
Condoblin N	0.0	10.2	4.0
Minnipa S	0.0	11.2	4.4
Horsham V	1.0	13.3	6.2
Moree N	0.0	13.6	5.3
Wagga Wagga N	0.6	18.1	6.8
Geraldton W	5.1	21.1	11.0
Lameroo S	1.5	27.0	9.2
Beulah V	1.0	27.2	8.5

Group 2 (S3)	**Min**	**Max**	**Mean**
Coonamble N	0.0	4.8	1.8
Condobolin N	0.0	6.7	3.6
Bogan Gate N	0.0	7.4	2.7
Tamworth N	0.0	10.0	2.8
Oaklands N	2.8	12.0	7.1
Wagga CCA N	0.2	13.7	6.6
Beckom N	0.0	15.2	7.3
Nyngan N	0.5	15.3	5.7
Moree N	0.0	15.8	5.6
Wagga CBA N	2.0	17.7	6.8
Group 2 (S4)			
Naradhan N	0.0	8.0	3.9
Piliga N	0.0	8.0	0.5
North Star N	0.0	8.2	4.1
Gunnedah N	1.0	10.6	4.6
Thuddungra N	2.5	13.2	6.3
Tottenham N	0.0	15.5	2.9
Trangie N	2.5	20.0	8.6

Group 3 (S2)	**Min**	**Max**	**Mean**
Lake Bolac V	2.5	26.0	9.9
Suntop N	0.0	7.8	3.0
Moree N	0.0	8.7	3.3
Beverly W	0.0	13.6	3.0
Wagga Wagga N	2.0	14.8	7.1
Katanning W	0.0	15.7	5.6
Bordertown S	1.2	17.0	7.4
Horsham V	2.0	20.2	8.0
Struan S	0.6	20.2	7.9

Table 5 (cont.)

Group 4 (S3)	**Min**	**Max**	**Mean**
Aria Park N	1.3	16.4	6.3
Condobolin N	0.0	13.8	5.0
Finley N	0.0	10.6	3.9
Grenfell N	0.0	11.5	3.7
Moree N	0.0	6.1	3.1
Suntop N	0.0	7.3	2.4
Wallendbeen N	0.2	14.2	5.7
Wagga Wagga N	4.0	21.5	8.6
Group 4 (S4)			
Merriwa NSW	0.0	5.5	2.7
Leadville NSW	0.0	5.7	1.6
North Star NSW	0.9	7.5	4.0
Gunnedah NSW	0.0	8.3	3.8
Gilgandra NSW	0.0	9.4	4.8
Ulamambri NSW	0.0	10.3	2.9
Yass NSw	0.0	11.6	4.9
Wakool NSw	0.0	12.1	3.7
Thuddungra NSW	1.7	12.6	5.4
Oaklands NSW	3.3	14.8	7.9
Coolamon NSW	1.8	15.7	5.5
Beckom NSW	3.9	16.7	9.2
Combaning NSW	0.0	17.3	4.6

Group 5 (S2)	**Min**	**Max**	**Mean**
Wagga Wagga N	0.0	9.5	4.7
Bordertown S	0.5	10.7	5.4
Horsham V	0.0	13.3	4.9
Lake Bolac V	3.2	20.7	9.1
Gibson W	2.0	23.6	9.1
Struan S	2.7	25.3	8.6

Group 6 (S2)	**Min**	**Max**	**Mean**
Merredin W	0.0	6.9	2.1
Moree N	0.0	8.1	2.7
Grenfell N	0.0	12.3	3.3
Hopetoun V	0.0	13.2	5.1
Wagga Wagga N	0.7	13.4	5.7
Katanning W	0.3	13.7	5.1
Rutherglen V	1.5	15.5	6.7
Horsham V	1.1	18.3	6.3
Lake Bolac V	3.7	18.8	8.2
Struan S	0.0	20.7	7.1
Lameroo S	1.2	43.1	14.7