CHLOROPHYLL IN AUSTRALIAN CANOLA

M. M. Vonarx and R.J. Mailer

NSW Agriculture, Wagga Wagga Agricultural Institute,
Private Mail Bag, Wagga Wagga, NSW 2650, Australia

ABSTRACT

Chlorophyll in canola is an important quality factor as it produces an undesirable colour in the oil and may also promote oxidation of the oil in the presence of light. Until recently chlorophyll was not perceived to be a problem in Australia. However in recent years it has become obvious that some canola deliveries contain above the acceptable standard of 30 mg/kg in oil. Green seeds and consequently green oil has been a problem in the Canadian canola industry for many years, mainly due to their climatic conditions.

The aim of this study was to determine if there is a problem with chlorophyll in Australian canola, the extent of this problem and ultimately if it is cultivar and /or environmentally related. An NIR calibration was developed to be able to screen the numerous trial samples that pass through the laboratory each year. The initial study included 6 cultivars grown over two years at six sites. No chlorophyll problem was apparent with an average of 1.5 mg/kg in 1997 and 2.3 mg/kg in 1998. In subsequent years, samples from receival sites were found to contain chlorophyll levels as high as 70 mg/kg, although these were rare.

INTRODUCTION

Green seeds and consequently green oil has been a problem in the Canadian canola industry for many years, mainly due to their climatic conditions (Daun, 1982). In Australia it has only been in the last few years that any significant number of green seeds have been obvious. Chlorophyll is undesirable in canola because it is extracted with the oil, giving the oil a green colour. Removing the colour is expensive and time consuming (Mag, 1983). Chlorophyll pigments remaining in the processed oil have also been associated with increased oxidation causing rancidity (Tautorus, 1993) and difficulties in hydrogenation (Abraham, 1986). Several Australian canola seed deliveries during 1999-2000 harvest were found to contain in excess of the Australian standard (Australian Oilseed Federation Trading Standards). Often the delivery containing green seed can be blended with other deliveries to meet the standard but crushers may reject seed that contains green seed above that limit. In 1999 several batches of green canola oil were reported by processors.

The most common cause of green seed in *Brassica* napus is stress, whether biotic (eg blackleg) or environmental (frost or adverse weather conditions) (Canola Guide Magazine- 1999, p66). It is suggested that more chlorophyll is present after a plant has suffered stress because the plant regresses, switching back to biosynthesis and chlorophyll production, instead of degradation, which normally occurs as the plant matures.

At present in Canada and Australian, chlorophyll concentration is estimated by counting the number of green seeds in a canola sample. This is done by transferring 100 seeds onto a strip of masking tape. The seeds are then crushed using a paint roller and the number of distinctly green seeds are counted. The interpretation of what is a green seed is very subjective and is likely to vary between individuals . The actual relationship between distinctly green seeds and chlorophyll in the seed has been shown to be relatively poor (Daun 1982).

A quick efficient method is required in industry to screen canola samples for chlorophyll. Near-infrared reflectance (NIR) spectroscopy is widely used to determine quality components in canola

grains, initially using ground seed and later using whole seeds (McGregor, 1990). It has been shown that to determine chlorophyll an instrument must be capable of detecting an absorption maxima near 670 nM (Tkachuk, 1988). Preliminary studies on chlorophyll using whole seed were carried out by Daun (1994).

This paper examines chlorophyll results obtained from canola samples grown in Australia over three years, a GRDC funded project. These samples were used to develop a calibration for the NIR and subsequently monitor samples from the 'National Brassica Breeding Project', analysed by this laboratory annually. The results were used to determine whether chlorophyll is cultivar and /or environmentally related and the degree of the problem in Australia.

MATERIALS AND METHODS

Canola seed with higher levels of chlorophyll used for this study, was obtained from the Grains Research Laboratory, Winnipeg Canada. Other samples were trial samples from the 'National Brassica Improvement Project', seed from the Bulk Handling Companies, as well as grower samples. Chlorophyll was determined using the official Method of the American Oil Chemists' Society (AOCS) Ak2-92, extracting chlorophyll from ground seed and measuring it on a spectrophotometer.

The results obtained from all the analysis were then used to set up a calibration on a Foss NIRSystems 6500 Near Infra Red Spectrophotometer. The instrument was operated in reflectance mode. Unground seeds were scanned and the analysis carried out using WINISI software (FOSS). Partial least squares (PLS) regression was used to develop a calibration from the second derivative of the log 1/R of the spectra. This calibration was then used to screen samples from trials sown in 2000 and the results statistically analysed for cultivar and/or site effect on chlorophyll production.

RESULTS AND DISCUSSION

The first studies on the presence of chlorophyll were carried out in 1997 and 1998 when 6 cultivars grown over six sites were analysed. The results obtained showed that the chlorophyll levels were minimal, (Table 1). The average chlorophyll content for 1997 was 1.5 mg/kg and 1998 2.3 mg/kg. Samples received from receival sites containing excess numbers of green seeds analysed over the next few years showed levels up to 70mg/kg but these were rare.

Table 1 indicates that S2 early, S2 mid and triazine trials have maximum levels of chlorophyll above the standard of 30mg/kg, however the mean levels are low. The maximum levels represent one or two sites only.

Trials	No of Samples	Mean	Min	Max
S2 Early	1084	8	1	51
S3 Early	539	4	0	19
S2 Mid	1051	6	1	32
S3/S4 Mid	2177	5	0	20
S2/S4 Late	591	6	1	22
Triazine	997	6	0	36

Table 1. Chlorophyll levels across trial samples from 2000/2001 season.

The early maturing S2 trials had the highest average chlorophyll levels. Newdegate in WA contributed most to this average as the levels at this site were far above anywhere else. The weather conditions were probably the main contributing factor to these high levels. Weather data obtained, showed that plants were obviously stressed from a early stage of development . At seedling stage,

(late June), canola plants were subject to sand blasting which thinned plots, with well below normal rainfall in June/July and virtually drought-like conditions after July (G. Walton-personal communication). The majority of sites showed very low chlorophyll levels with a few showing higher levels in particular cultivars. There were several cultivars that were consistently higher even when the overall levels were low.

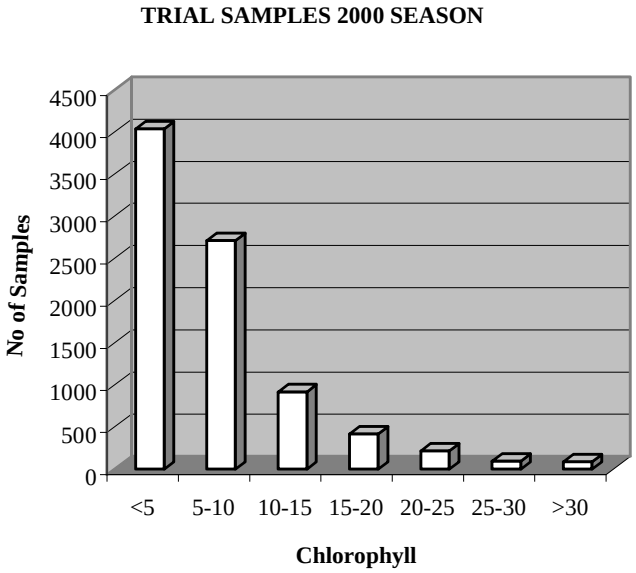

Figure 1. Distribution of chlorophyll levels from all trials across Australia from 2000 season.

Most receival sites did not appear to have a problem with above standard green seed during the 2000/2001 season. However some samples contained levels above 30mg/kg chlorophyll. They were in the minority and obviously any problem had been overcome by blending.

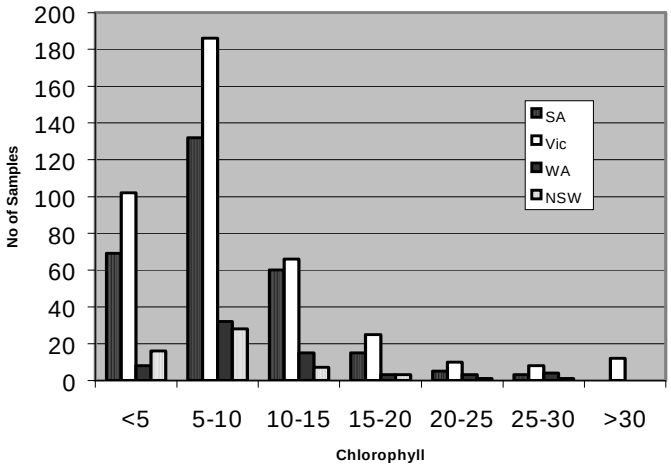

Figure 2. Chlorophyll values from BHA's across Australia for 2000 season

This study has shown a site effect on chlorophyll levels and stress appears to be a major cause. Several cultivars appear to show consistently higher levels of chlorophyll relative to the mean level

of a particular site. At present chlorophyll does not appear to be a major problem in Australia except for individual sites.

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