How Green Is Green? Long-Term Relationships Between Green Seeds and Chlorophyll in Canola Grading

James K. Daun*

Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba R3C 3G8, Canada

ABSTRACT: Chlorophyll is undesirable in canola seeds because it is extracted into the oil resulting in problems during processing and utilization. In the Canadian grain grading system, and in similar systems in use in the United States and Australia, chlorophyll is estimated in canola seeds subjectively by crushing and counting the number of distinctly green seeds in a sample while simultaneously assessing the overall natural color of the crushed seeds. Chlorophyll contents of canola may be determined by extraction with solvent followed by spectrophotometric analysis or by using NIR instrumentation, capable of operating in the visible region and calibrated against samples with known amounts of chlorophyll. The relationship between the green seeds and chlorophyll content in canola export shipments from 1988 to 2001 was found to be linear. The intercept, referred to as the background chlorophyll, ranged from 6 to 16 mg/kg, and the slope ranged from 300 to 1000 mg/kg per green seed. In recent years, both the background chlorophyll and the slope have been increasing, resulting in an increase in the chlorophyll levels in top-grade canola exported from Canada. The increase may be partly a result of the change in proportion of species of canola grown in Canada, and also may result from changes in perception of what constitutes a green seed. The use of an objective measurement of chlorophyll is recommended to improve the consistency of the grading system.

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KEY WORDS: Canola, chlorophyll, grading, green seed, visual assessment.

The cotyledons in canola seeds contain chloroplasts and hence chlorophyll. As the seeds ripen in the pods, the chlorophyll content decreases (1), but even fully ripened seed contains some chlorophyll. The chlorophyll in canola seeds is extracted into the oil, from which it must be removed during the refining and bleaching process as it is undesirable not only from an aesthetic point of view but also because it promotes oxidation and inhibits hydrogenation (2).

In farmers' fields, the ripening process can be uneven for a variety of reasons. The resulting harvested material usually contains seeds with varying levels of chlorophyll. The visual grading system used in Canada, as well as in the United States and Australia, estimates the level of chlorophyll in a sample by counting the "distinctly green seeds" observed after crushing between 500 and 1000 seeds. An apparatus is used that allows individual seeds to be crushed, 100 at a time, onto masking tape. In a previous paper we examined this process and noted how the binomial distribution of the green seeds coupled with differences in perception of what constituted a distinctly green seed contributed to the error of the determination (3).

*Address correspondence at Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main St., Winnipeg, Manitoba, R3C 3G8, Canada. E-mail: jdaun@grainscanada.gc.ca

Despite its shortcomings, this method of determining distinctly green seeds has allowed a reasonably consistent segregation of canola according to the level of chlorophyll as long as large samples of seed of similar grade are bulked. In the past, studies have shown that a level of 2% green seed (the maximum allowed in top-grade canola) will, on average, give crude oil with chlorophyll levels less than the industry standard of 30 mg/kg (Fig. 1). This value has been shown to correspond to a level of about 25 mg/kg chlorophyll in the seed (Fig. 2). The effectiveness of the system can be seen in Figure 3, which shows the chlorophyll content of Canadian export shipments of canola from 1982 to present. Although the level of chlorophyll in No. 1 export shipments has, for the most part, remained less than 25 mg/kg, this level has increased in recent years, despite the absence of widespread severe weather conditions that might be expected to cause high levels of chlorophyll.

Examination of Figure 2 provides an interesting insight into the relationship between percentage of green seeds and chlorophyll. Over the region between 0 and 20% green, the relationship appears to be linear. The regression line does not pass through the origin but passes through a point on the *y* axis that represents the level of "background chlorophyll," i.e., the chlorophyll that is in the sample but not expressed as distinctly green seeds. This background chlorophyll is made up of a combination of the chlorophyll that is present, even in fully matured seed, and the chlorophyll in those seeds that have a green tint but are not so green as to be considered distinctly green.

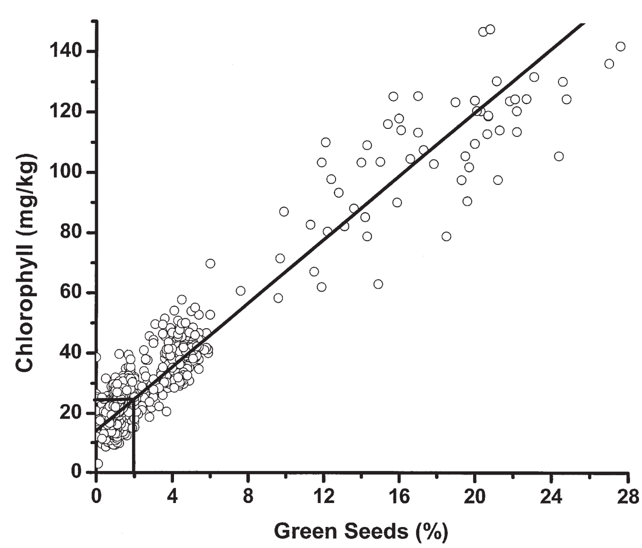

FIG. 1. Relationship between seed chlorophyll and green seeds for export shipments of Canadian canola seed, August 1992 to January 2002. Regression equation: chlorophyll = 5.3 × green + 14.2 (R = 0.949, n = 1719). At 2% green seeds, chlorophyll = 24.8 mg/kg (shown on graph).

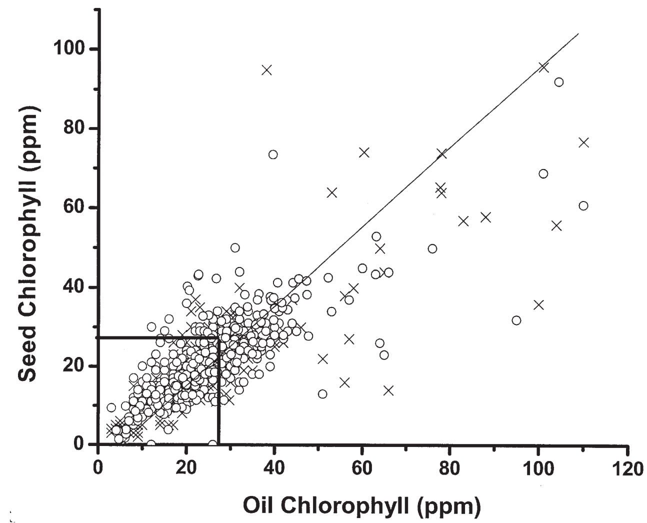

FIG. 2. Chlorophyll in seed and oil from Canadian crushing plants. Seed and oil samples were randomly taken ("grab samples") from six different plants during the period 1979 to 1994. Regression equation: seed chlorophyll = 0.65 × oil chlorophyll + 4.9 (R = 0.795, n = 735). At 30 mg/kg oil, Canadian General Standards Board standard for crude canola oil, seed chlorophyll = 24.3 mg/kg (shown on graph). ×, Crude oil chlorophyll; ○, degummed oil chlorophyll.

The slope of the regression line, multiplied by 100 to account for 100% of the seeds, can be equated to the concentration of chlorophyll in green seeds. The value from Figure 2 (530 mg/kg) is somewhat higher than the value of 200 to 400 mg/kg estimated from an analysis of individual seeds (3). The amount of chlorophyll in an individual green seed is not constant but will depend on the individual state of maturity of that seed. Studies have shown that chlorophyll content in immature samples of canola seed may exceed 700 mg/kg (4,5). The value of the chlorophyll concentration in green seeds, as determined by the slope, will range from a minimum point that triggers the response to any level above that point to the maximum level present in an immature seed.

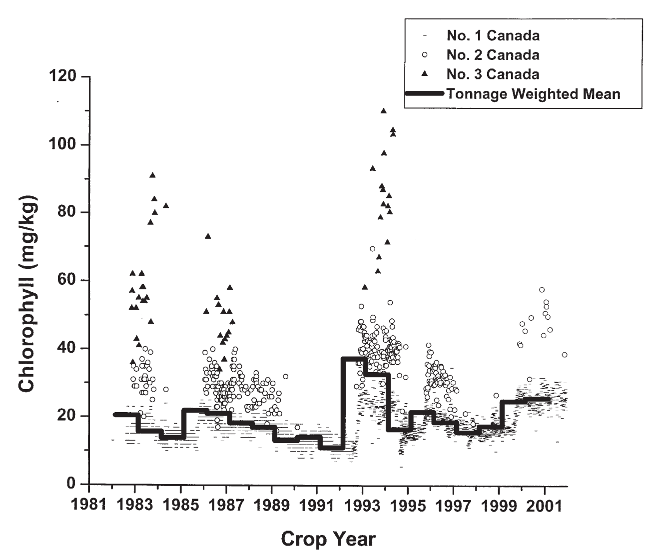

FIG. 3. Chlorophyll in statutory grades and the tonnage weighted mean chlorophyll by year for Canadian canola shipments. Even in years where frost severely damaged the crop, the relatively small number and tonnage of No. 2 Canada and No. 3 Canada shipments resulted in the overall average chlorophyll content in shipments remaining <30 mg/kg. The exception was the 1992 and 1993 crop years, where the crop was subjected to unusually severe frost resulting in a very low amount of top-grade seed and very small carryovers of high-quality seed from previous crops.

The objective of this study was to report on the background level and concentration of chlorophyll in green seeds in Canadian canola exports from 1988 to present and to relate variations in these values to possible contributing factors.

MATERIALS AND METHODS

The Canadian Grain Commission's surveys of exported seed were used as the basis for this study.

Export surveys for canola. These have been carried out since the 1960s. Since 1972, they have included assessment of each individual export shipment, and chlorophyll data have been available since 1982 (Fig. 3). In assessing the grade of export shipments, an estimate of the number of distinctly green seeds in a 1000-seed sample was assessed on a subsample drawn from a continuous automatic sampling apparatus for approximately each 1000 tonnes loaded. The average result was used in establishing the official grade of the sample. A composite sample made up of all the subsamples is forwarded to the laboratory in Winnipeg for testing.

Chlorophyll analysis. This was carried out using extraction with a heptane/ethanol mixture coupled with spectrophotometric estimation of chlorophyll according to Method Ak 2-92 (harmonized with ISO 10519) (7) or by rapid procedures based on that method. The rapid procedures included the use of a reflectance spectrometer (8), a modified ground-seed NIR analyzer (9), or a calibration on a whole-seed analyzer (10).

Statistical analysis. This was carried out using Microsoft Excel (graphical and simple statistics), Orign® 6.0 (Microcal Software Inc., Northampton, MA) (graphical statistics), InStat 3.05 (GraphPad Software Inc., San Diego, Ca) (t-tests), or the SAS System for Windows (SAS Institute Inc., Cary, NC) (linear regression analysis) as was most appropriate for the data being considered.

RESULTS AND DISCUSSION

There were significant variations in both the background chlorophyll (6 to 16 mg/kg) and the chlorophyll per green seed values (200 to 950 mg/kg) between and within the different years studied (Fig. 4). The background chlorophyll was particularly low in 1989 and 1991, years with particularly good quality and generally low levels of seed chlorophyll. In recent years, a general trend of increasing background chlorophyll parallels the trend toward an increasing proportion of *Brassica napus* canola in the Canadian canola crop (Fig. 4). *Brassica*

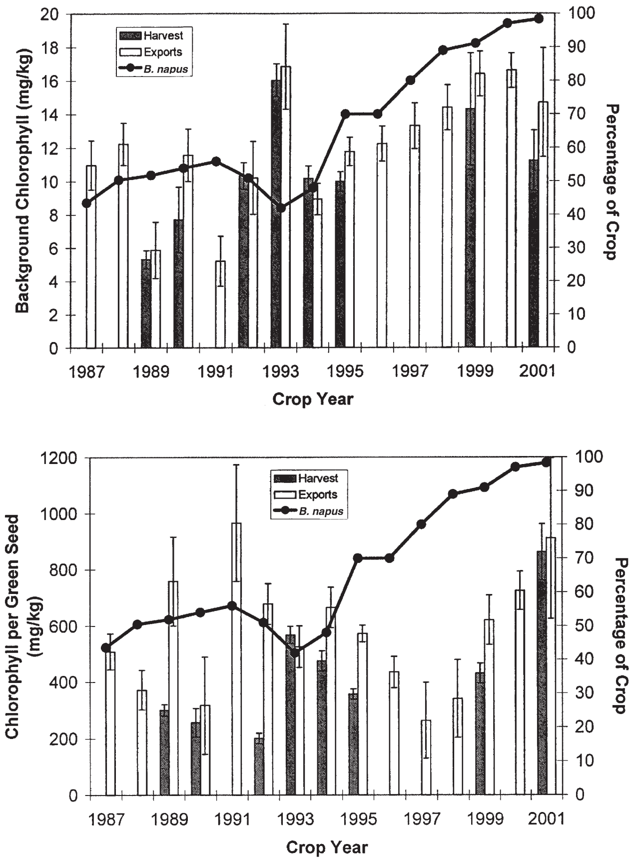

FIG. 4. Annual variation in background chlorophyll (top) and chlorophyll per green seed (bottom) for canola export surveys. Error bars reflect 95% confidence intervals for the means. The *Brassica napus* line reflects the estimated percentage of the crop made up of varieties from that species.

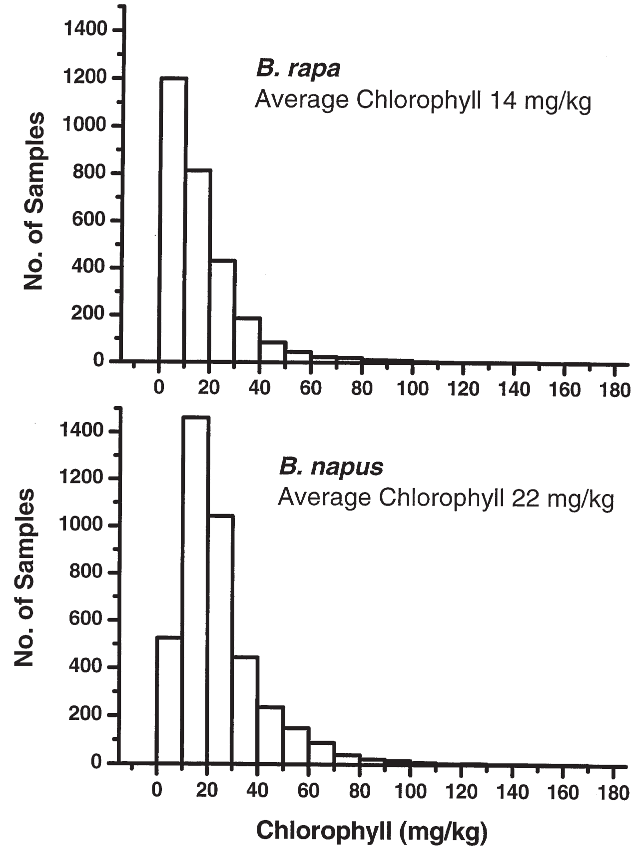

FIG. 5. Distribution of chlorophyll in canola by species; bulked data from 1989, 1990, and 1992–1995 harvest surveys. Since the distributions were not normal, it was not possible to compare means directly. The distributions were normalized using a Box–Cox conversion, and a *t*-comparison of the converted data showed a significant difference in the mean chlorophyll content between *Brassica napus* and *B. rapa* at a high degree of probability.

napus canola matures approximately 20 d later than *B. rapa* canola. The *B. rapa* canola also tends to be more determinant in its flowering pattern than *B. napus* canola. As a result, *B. rapa* canola is usually harvested when it is well matured and the maturation tends to be more even. The *B. rapa* canola often may be straight-combined, whereas it is usually necessary to allow *B. napus* canola to lie in swath for up to 2 wk to achieve maturation. Evidence suggests that, under certain conditions, the chlorophyll level in canola may become fixed so that even swathing will not result in a significant decrease (11).

The differences in agronomic features mean that *B. rapa* canola usually has less chlorophyll than *B. napus* canola (Fig. 5). Seed from *B. rapa* canola has lower levels of background chlorophyll than seed from *B. napus* canola (12). The shift in production to less *B. rapa* means less seed with lower background chlorophyll is available to dilute the background chlorophyll of the *B. napus* seed. Since the growing area for canola has not changed, a great deal of *B. napus* canola is currently being grown in areas more suited to the earlier-maturing *B. rapa* type. It might be expected that the *B. napus* canola grown in these areas would have even higher levels of background chlorophyll and chlorophyll per green seed than *B. napus* canola grown in areas with longer growing periods.

Our previous work (3) also indicated that individuals perceived "distinctly green seeds" at different levels of chlorophyll. In our long-term study, results for samples tested at Vancouver, on the West Coast of Canada, were compared to results for those tested at Thunder Bay, at the head of Lake Superior in eastern Canada (Table 1). There were no significant differences in background chlorophyll between the two ports, but chlorophyll per green seed was significantly different between the two ports over all the periods compared. This result is a little puzzling, because the majority of the low-background chlorophyll *B. rapa* canola has been grown in the Western prairies and it would be expected that, especially in the earlier years examined, exports from Vancouver would have significantly lower background chlorophyll than would exports from Thunder Bay. The early frosts that affected much of the canola crop in 1992 and 1993, especially in the western prairies, might account for this difference—or it might be a function of different groups of people assessing the seeds. Similarly, the difference in chlorophyll per green seed between Vancouver and Thunder Bay might be a difference of perception or it might be because of a higher amount of chlorophyll in the green seeds in shipments originating in

TABLE 1
Chlorophyll and Regression Statistics for Chlorophyll and Green Seeds for Samples of Canola from Canadian Grain Commission Export Surveys[a]

Period	Port	No. samples	Mean chlorophyll (mg/kg)	CV	R^2	Background chlorophyll (mg/kg)[b] Mean	Background chlorophyll (mg/kg)[b] SE	Chlorophyll content of green seeds (mg/kg)[c] Mean	Chlorophyll content of green seeds (mg/kg)[c] SE
1992–2001	Thunder Bay	175	22.5	20.1	0.641	12.68	0.66	811	46
	Vancouver	1454	22.6	20.2	0.684	12.82	0.21	589	11
1997–2001	Thunder Bay	57	21.1	15.7	0.008	21.83	1.27	−93	142
	Vancouver	662	21.7	20.3	0.560	11.53	0.39	851	30
1994–1996	Thunder Bay	87	20.1	18.5	0.604	11.01	0.88	772	68
	Vancouver	457	19.6	15.3	0.776	10.57	0.27	540	14
1992–1993	Thunder Bay	29	32.7	12.2	0.825	15.77	1.64	821	71
	Vancouver	333	28.4	13.5	0.847	13.10	0.42	600	14

[a]Years were grouped to provide sufficient samples from Thunder Bay to provide a reasonable regression analysis. The 57 samples from Thunder Bay during the period of 1997 to 2001 were very uniform in quality and there was no significant regression between green seeds and chlorophyll for that period.
[b]Unpaired *t*-test with Welch correction showed no differences in the background chlorophyll between the two ports for any of the periods tested.
[c]Unpaired *t*-test with Welch correction showed significant differences in the chlorophyll per green seeds between the two ports for all of the periods tested.

Thunder Bay. The eastern prairies, which are the source of most of the canola exported through Thunder Bay, have been noted for producing seed with chlorophyll problems (13).

This study showed that, whereas the assessment of green seeds in the canola grading system has operated, in general, to segregate canola shipments according to levels of chlorophyll, there have been variations in the consistency of this assessment from year to year and from location to location. In recent years, the levels of both the background chlorophyll and the chlorophyll per green seed have increased, resulting in many shipments of canola leaving with chlorophyll levels in excess of the 25 mg/kg expected in top-grade canola. Interestingly, however, this increase has not resulted in any significant level of complaints from customers compared with, for example, the complaints from the Japanese processors in 1992 and 1993 (14) when the crop was severely damaged. Perhaps technological advances have allowed processors of canola seed to handle higher levels of chlorophyll. If this is the case, it would be useful to reassess the maximum levels of chlorophyll allowed in top grades of both seed and oil.

Variability in green seed assessment will remain, however, as long as this measurement is carried out by human beings. Technology has existed since the late 1980s that would allow this measurement to be replaced by a direct measurement of chlorophyll (9). It remains for the grain industry to accept adoption of this technology as a more objective way of measuring the chlorophyll levels in canola. Until this happens, the target will continue to move as the personnel and the types of seed measured continue to change.

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