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Quality of genetically modified (GM) and conventional varieties of canola (spring oilseed rape) grown in western Canada, 1996–2001

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SUMMARY

The success of GM herbicide tolerant canola is demonstrated by its acceptance by the farm community in Canada. There have been continuing comments, however, including some from major customers, suggesting that GM canola has lower quality than conventional canola. Data drawn from both the Western Canada Canola/Rapeseed Co-operative Test data from 1998–2001 and from the Canadian Grain Commission's harvest surveys of canola from 1996–2001 were used to compare the quality of GM and conventional canola registered and grown. Weed seed contamination of harvest survey samples decreased significantly as the herbicide tolerant lines increased in production. While variety registration data suggested GM and conventional lines had no differences in oil content, data from harvest surveys suggested that GM lines tended to have slightly higher oil contents. Protein and oil contents remain inversely related with no differences in the inverse relationship due to GM. While registration requires that all lines have less than 12 micromoles per gram of glucosinolates, data from harvest surveys show GM lines to have significantly less glucosinolates than conventional lines, possibly due to decreased contamination with cruciferous weeds. A comparison of glucosinolate contents between non-GM herbicide tolerant canola and conventional non-herbicide tolerant canola showed similar differences. There were no significant differences in chlorophyll content, erucic acid levels or saturated fatty acids but harvest survey data showed GM lines were slightly more unsaturated than conventional lines. It would seem safe to conclude that differences in quality between GM and conventional canola are due to the functioning of the GM trait – herbicide tolerance – that allows the GM canola to perform to its potential in the field.

INTRODUCTION

The introduction of genetically modified (GM) seed into agricultural systems has been the source of considerable discussion and controversy, mostly over the perceived threat of genetically modified seeds causing damage to health or to the environment (Downey & Buth 2003). Even the meaning of the term 'GM' has become a source of confusion although in most places, including the current article, it refers to products of recombinant DNA technology.

In Canada, the first herbicide-tolerant GM canola lines were harvested in 1996. Since that time the proportion of GM canola grown in Canada has increased to the point where most of the seed planted is of that type (Downey & Buth 2003). Up to the present, with the exception of a short period of contract production of high lauric acid canola, the only GM canola varieties grown in Canada have been herbicide-tolerant types. Three different GM systems imparting tolerance to glyphosate, glufosinate and bromoxynil based herbicides have been incorporated into many Canadian varieties. Varieties incorporating a conventional herbicide-tolerant system based on mutation-derived resistance to imadazolinone herbicides (Pursuit or Clearfield™) have also been grown.

The success of these herbicide-tolerant seed types, despite the higher seed costs, is related to their large impact on the contribution to margin when compared with conventional types (Anon. 2002). Despite the success of GM canola in Canada, customers of Canadian canola have asked if the quality of the new GM seed is as good as the quality of conventional lines. The proponents of the herbicide-tolerant system had suggested that the trait would result in improved quality due to an increased ability to control weeds. Unfortunately, quality data gathered while testing varieties prior to registration does not give the full picture of the quality of a herbicide-tolerant variety since the plot testing used is not conducive to testing the effectiveness of the herbicide-tolerant trait in improving quality. To truly evaluate the quality of varieties, samples grown under commercial production should be evaluated.

An initial study (Daun 1999) compared the quality of GM canolas with that of conventional canola for the first 2 years of production. No significant differences were noted. The present work compares the quality of GM and conventional canola varieties from registration plot trials in Canada and also from the commercial crop grown in western Canada, including oil content, protein content, glucosinolate content, chlorophyll level and fatty acid composition over the first 5 years of production, 1996–2001.

MATERIALS AND METHODS

Data for registration plot trials were drawn from the Western Canada Canola/Rapeseed Co-operative Tests and Private Registration Data collected by the Western Canada Canola/Rapeseed Registration Committee Inc. between 1998 and 2001 (Gadoua 1996–2001). In these tests, each variety in the tests was compared against a uniform set of reference or check varieties at each of the locations across Western Canada according to a written set of procedures approved by the committee. A minimum of 10 station years of data was required for registration but usually as many as 25 sites, across three climatic zones, have been planted to the Co-operative Test and 13 sites across the same three zones have been planted to the private tests. In order to provide sufficient data for statistical analysis, data were drawn from test reports from the years 1998–2002, a total of 142 GM and 92 conventional lines of *B. napus* without special quality traits were recommended during that period.

In the Canadian variety registration system, varieties are evaluated for yield, oil content and protein content using the differences between the candidate cultivars and check cultivars. In the case of oil content, the check cultivars are selected to have a mean oil content of 45%. The summary data for yield, oil and protein provided a convenient measure by which to compare varieties. Data for varieties that were recommended for registration were used in the study. Data for erucic acid, saturated fatty acids and glucosinolates were drawn from the means for all varieties tested in the mid season growing zone (the largest canola growing area in Canada).

Data from the commercially grown canola crop was also drawn from the Canadian Grain Commission's harvest surveys of Canola. In these surveys, producers across western Canada submit 300 g samples of their harvested crop to the Canadian Grain Commission's Grain Research Laboratory. Approximately 1500 canola samples, each identified by variety grown and nearest delivery point were collected annually. The samples were cleaned and graded and then tested for oil, protein, glucosinolates and chlorophyll using Near Infrared Reflectance (NIR) technology. Composite samples by variety were also prepared annually and tested for fatty acid composition using gas liquid chromatography (DeClercq & Daun 1998).

Restriction of the data samples to *B. napus* canola without special oil quality traits and to samples where the variety name or at least a designation like 'Liberty Link' was provided gave a data set of 6243 samples representing 3790 conventional and 2452 GM for the years 1996–2001. For fatty acid composition data where composite samples by variety were tested, only those composite samples containing more than 15 crop survey samples were considered. Over the years studied, 129 composite samples representing 74 conventional and 55 GM varieties were tested.

Statistical analysis was carried out using the SAS System for Windows, V 8.02. (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414, USA).

RESULTS

Statistical analysis

Many of the data were found to be in non-normal distributions. In some cases, such as oil content, crude seed and meal protein content, the distribution was near normal but slightly skewed. In these cases, normal statistics were used since transformation to normalize of the data did not affect the results. The skew was very pronounced in data for such as glucosinolates, chlorophyll and erucic acid. In these cases, both transformations and graphical data were used in examining the results. Data for saturated fatty acids, linolenic acid and unsaturation index were normally distributed.

Oil content

Oil content has been the most important economic factor in canola seed. It has been estimated that canola oil has a value of up to eight times that of canola meal. Registration support data for the years 1998 to 2002 (Table 1) suggest that the oil content of GM varieties was not substantially different from that of non-GM cultivars supported for registration in the same period although the non-GM lines had significantly more oil than the GM lines in 2 of the 5 years. Data from commercial crop surveys (Table 2), on the other hand, showed that GM varieties of canola had substantially higher oil contents than non-GM varieties for the years 1996–1999 but

Table 1. *Comparison of oil, protein and maturity between non-GM and GM cultivars supported for registration in Canada between 1998 and 2002 (significant differences shown in bold type)*

	1998		1999		2000		2001		2002	
	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM
Number of samples	20	20	18	30	25	33	18	22	12	37
Oil content (% different from checks)										
Mean	0·98	0·43	1·17	1·04	1·43	0·64	1·35	1·04	0·32	0·35
Pooled S.E.	0·25		0·26		0·20		0·21		0·25	
Diff	**0·55**		0·13		**0·79**		0·31		−0·03	
t-value	2·20		0·48		3·97		1·43		−0·11	
Prob. >|t|	0·037		0·630		0·001		0·164		0·909	
Meal protein content (% different from checks)										
Mean	0·68	−0·06	0·52	0·18	0·67	0·14	0·94	0·74	0·63	0·41
Pooled S.E.	0·30		0·23		0·23		0·33		0·35	
Diff	**0·74**		0·34		**0·54**		0·20		0·22	
t-value	2·43		1·50		2·37		0·61		0·63	
Prob. >|t|	0·020		0·141		0·021		0·547		0·553	
Maturity (days different from checks)										
Mean	1·28	0·16	0·58	0·04	0·28	0·47	1·07	0·96	0·74	−0·36
Pooled S.E.	0·40		0·29		0·41		0·29		0·70	
Diff	**1·13**		0·54		−0·20		0·11		**1·10**	
t-value	2·81		1·84		−0·44		0·37		2·21	
Prob. >|t|	0·008		0·072		0·661		0·714		0·034	

that the oil contents were equivalent in the years 2000 and 2001.

Crude protein content

A strong inverse correlation exists between crude protein and oil content expressed either on a seed or meal basis (Daun & DeClercq 1995). The results for protein content thus mirror, in an inverse fashion, the results for oil content (Tables 1 and 2).

Chlorophyll (maturity)

In Canada, where there is a relatively short growing season, chlorophyll, measured in grading as per cent green seeds, has been an important quality factor (Daun & Symons 2000). It is likely that cultivars with later maturity will have higher levels of chlorophyll in a given growing environment, especially when the growing season is short and leads into fall frosts. Except for one year, there was no difference in the level of maturity between GM and non-GM varieties supported for registration between 1998 and 2002 (Table 1). Data from the commercial crop, however, showed that in the last 4 of the 6 years studied, GM varieties had significantly less average chlorophyll than non-GM varieties (Table 3). The reverse was true, however, for 1997, possibly due to GM varieties being concentrated in the eastern prairies, where chlorophyll levels are usually higher. Furthermore, analysis of the distribution of chlorophyll (Table 4) showed that, in the 5 years 1997–2001, a greater proportion of the non-GM lines was likely to grade No. 2 or lower (i.e. greater than 30 mg/kg chlorophyll) than the GM lines.

Glucosinolates

Canola is defined as seed with less than 18 μM/g glucosinolates. In the registration system, varieties must have less than 14 μM/g glucosinolates in order to be supported for registration. Thus it is not surprising that there was no difference in glucosinolate content between GM and non-GM lines in the variety registration tests (Table 5). In commercially grown canola, however, GM samples had significantly lower mean glucosinolates in all years (Table 3) and while only 2 GM samples (0·5% of 415 in 2001) were found with more than 18 μM/g glucosinolates in the 5 years of surveys, between 0·5 and 2% of the non-GM samples fell into that category (Table 4).

Erucic acid

Canola seed is expected to have less than 20 mg erucic acid/g oil by definition and the trade expects less than 10 mg/g oil in their standards. While there was a statistically significant difference in 2 of the 4 years, there

Table 2. *Comparison of oil, protein and meal protein between non-GM and GM cultivars grown in Western Canada, 1996–2001. Data from Canadian Grain Commission Harvest Surveys (significant differences shown in bold type)*

	1996		1997		1998		1999		2000		2001	
	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM
Number of samples	684	65	933	334	532	300	402	368	826	968	413	418
Oil content (g/kg)												
Mean	429·1	436·2	421·2	428·7	426·0	436·3	431·6	437·1	432·5	431·0	426·8	426·1
Pooled S.E.	2·7		1·5		1·6		1·5		0·9		1·5	
Diff	**7·2**		**7·5**		**10·3**		**5·5**		1·5		0·7	
t-value	−2·64		−4·89		−6·84		−3·76		1·62		0·50	
Prob. >|t|	0·009		<0·001		<0·001		<0·001		0·106		0·616	
Crude protein (g/kg, 85 g moisture/kg)												
Mean	207·4	198·2	218·2	213·3	218·1	208·1	210·1	203·6	212·3	210·3	223·2	224·9
Pooled S.E.	2·7		1·4		1·4		1·4		0·9		1·5	
Diff	**9·3**		**4·8**		**10·0**		**6·5**		**2·1**		1·7	
t-value	3·41		3·45		−7·14		4·71		2·38		−1·18	
Prob. >|t|	0·007		<0·001		<0·001		<0·001		0·017		0·238	
Crude protein meal (g/kg)												
Mean	389·9	378·0	403·5	400·8	407·4	397·0	396·9	389·1	402·0	397·0	417·8	420·1
Pooled S.E.	3·5		1·6		1·7		1·6		1·0		1·8	
Diff	**11·9**		**2·7**		**10·4**		**7·8**		**5·1**		2·4	
t-value	3·43		1·69		−6·11		4·85		1·93		−1·34	
Prob. >|t|	0·001		0·091		<0·001		<0·001		<0·001		0·181	

Table 3. *Comparison of chlorophyll content and glucosinolate content between non-GM and GM cultivars grown in Western Canada, 1996 to 2001*. Data from Canadian Grain Commission Harvest Surveys (significant differences shown in bold type)*

	1996		1997		1998		1999		2000		2001	
	non-GM	GM	Non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM
Number of samples	684	65	933	334	532	300	402	368	826	968	413	418
Chlorophyll (mg/kg)*												
Mean*	18·5	18·6	11·7	13·2	14·8	12·5	20·9	16·0	20·7	15·8	23·4	19·5
Pooled S.E.*	2·2		2·0		1·7		2·2		2·7		2·0	
Diff.	−0·2		**−1·5**		**2·3**		**4·9**		**5·0**		**3·8**	
t-value	−0·13		−3·41		4·8		6·58		0·504		5·46	
Prob. >|t|	0·901		0·007		0·004		<0·001		<0·001		<0·001	
Glucosinolates (micromoles per gram seed)†												
Mean*	10·2	7·8	11·8	9·5	11·5	8·9	10·0	8·3	10·1	9·3	11·0	10·1
Pooled S.E.*	1·0		1·0		1·0		1·0		1·0		1·0	
Diff	**2·4**		**2·3**		**2·5**		**1·6**		**0·8**		**0·9**	
t-value	10·94		18·61		16·07		10·38		8·01		5·31	
Prob. >|t|	<0·001		<0·001		<0·001		<0·001		<0·001		<0·001	

* Detransformed after transformation (exponential 0·47) to produce a normal distribution for analysis.
† Detransformed after transformation (logarithm base 10) to produce a normal distribution for analysis.

was no substantial difference in average erucic acid content of the oil between GM and non-GM lines tested in the mid season zone in the years 1997, 1999, 2000 and 2001 (Table 5). Similarly, there was no difference in the average erucic acid content of the oil between GM and non-GM varieties in the composite

Table 4. *Comparison of the proportion of non-GM and GM canola samples falling into critical ranges for chlorophyll and glucosinolates. Samples with chlorophyll greater than 30 mg/kg are unlikely to grade in the top grade and samples with glucosinolates greater than 18 µM/gram will not qualify as canola*

	Number of samples		Percentage of class (GM or non-GM)			
			Chlorophyll >30 mg/kg		Glucosinolates >18 µM/gram	
Year	Non-GM	GM	Non-GM	GM	Non-GM	GM
1996	684	65	0%	11%	0·7%	0·0%
1997	933	334	3%	2%	2·1%	0·0%
1998	532	300	5%	3%	1·5%	0·0%
1999	402	368	21%	11%	0·5%	0·0%
2000	826	968	23%	11%	1·2%	0·0%
2001	413	418	22%	16%	2·2%	0·5%

Table 5. *Comparison of saturated fatty acids, erucic acid and glucosinolates between non-GM and GM cultivars entered into the Western Canada Co-operative Rapeseed test, Mid-Season Zone Between 1997 and 2001* (*significant differences shown in bold type*)

	1997		1998		1999		2000		2001	
	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM
Glucosinolates (micromoles per gram)*										
Number of samples	52	22	39	32	28	35	36	33	31	42
Mean*	11·6	12·7	11·5	11·9	10·2	9·5	8·1	7·9	11·2	11·4
Pooled S.E.*	1·1		1·1		1·0		1·0		1·0	
Diff	−1·1		−0·4		0·8		0·3		−0·2	
t-value	−1·79		−0·64		1·81		0·82		−0·45	
Prob. >|t|	0·078		0·522		0·076		0·415		0·653	
Erucic acid (mg/g oil)										
Number of samples	37	22	nd†	nd	32	35	36	33	31	45
Mean*	00·8	00·6			1·1	0·6	0·4	0·3	0·3	0·2
Pooled S.E.*	12				11		12		13	
Diff	**0·3**				**0·5**		0·1		0·1	
t-value	2·49				3·75		0·087		1·2	
Prob. >|t|	0·016				0·001		0·39		0·235	
Saturated fatty acids (mg/g oil)										
Number of samples	49	22	32	32	32	36	36	33	31	45
Mean§	67·5	67·6	67·8	67·4	67·8	68·1	65·2	66·9	66·0	66·5
Pooled S.E.§	27·8		21·6		22·2		20·4		20·8	
Diff	−0·1		0·4		−0·3		−1·7		−0·5	
t-value	−0·12		0·69		−0·44		−3·28		−0·96	
Prob. >|t|	0·907		0·493		0·66		0·002		0·342	

* Detransformed after transformation (logarithm base 10) to produce a normal distribution for analysis.
† nd – No data available for that year.
§ Detransformed after transformation (exponential 3·144) to produce a normal distribution for analysis.

samples from commercial production for the years 1996 to 2001 (Table 6). The distribution of erucic acid is strongly skewed, however, and inspection of the distributions of data (Fig. 1) suggested that there might be a tendency for non-GM canola to have higher levels of erucic acid.

Saturated fatty acids

Much Canadian canola is marketed in both Canada and the USA as being low in saturated fatty acids. In order to meet the labelling requirements for this claim, the oil must contain less than 70 mg saturated

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Table 6. *Comparison of fatty acid composition between non-GM and GM cultivars grown in Western Canada, 1996 to 2001. Data from Canadian Grain Commission Harvest Surveys (significant differences shown in bold type)*

	1996		1997		1998		1999		2000		2001	
Number of samples	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM	non-GM	GM
	10	1	18	8	17	9	10	12	13	13	6	12
Saturated fatty acids (mg/g oil)												
Mean	73·2	71·1	72·4	73·6	72·3	72·2	71·8	71·8	71·8	71·6	70·8	71·7
Pooled S.E.	2·4		0·6		1·1		1·2		1·1		0·9	
Diff	2·1		−1·2		0·1		0·0		0·2		−0·9	
t-value	0·88		−1·84		0·10		0·04		0·19		−1·06	
Prob. >|t|	0·404		0·078		0·924		0·971		0·852		0·306	
Linolenic acid (mg/g oil)												
Mean	92·2	91·0	92·3	90·3	82·7	82·7	92·9	96·7	96·3	100·5	90·4	96·6
Pooled S.E.	6·8		3·4		2·5		3·5		3·6		3·6	
Diff	1·2		2·0		0·0		−3·8		−4·2		−6·2	
t-value	0·18		0·59		0·00		−1·08		−1·19		−1·73	
Prob. >|t|	0·860		0·558		0·996		0·285		0·246		0·104	
Unsaturation index (Average double bonds per fatty acid)												
Mean	1·295	1·300	1·295	1·294	1·273	1·284	1·295	1·312	1·301	1·318	1·286	1·314
Pooled S.E.	0·02		0·01		0·01		0·01		0·01		0·01	
Diff	−0·005		0·001		−0·011		**−0·017**		**−0·017**		**−0·027**	
t-value	−0·22		0·11		−1·58		−2·16		−2·24		−3·61	
Prob. >|t|	0·830		0·915		0·127		0·043		0·035		0·002	
Erucic acid (mg/g oil)												
Mean*	1·6	1·2	0·6	0·2	1·7	1·3	0·8	0·7	0·8	0·6	0·3	0·2
Pooled S.E.*	58·3		20·4		14·2		16·7		14·6		16·0	
Diff	0·4		0·4		0·4		0·1		0·2		0·1	
t-value	0·16		1·54		0·7		0·34		0·7		0·73	
Prob. >|t|	0·878		0·138		0·492		0·737		0·488		0·474	

* Detransformed after transformation by logarithm base 10 to produce normal distribution for analysis.

fatty acids/g. Achieving this level has become difficult in Western Canada as the proportion of low saturated *B. rapa* has decreased (Daun & DeClercq 1998). In only one year was there a significant difference between the saturated fatty acid composition of GM and non-GM canola for samples from the cooperative trials (Table 5) and there were no differences in saturated fatty acid content between GM and non-GM canola in samples from the commercial crop (Table 6).

Unsaturated fatty acids

Linolenic acid is the key polyunsaturated fatty acid in canola oil. Low levels of this are desired in order to improve oxidative stability of the oil. There was no difference in linolenic acid contents between GM and non-GM canola oils from the commercial crop (Table 6). The total degree of unsaturation in a triglyceride can be given as a single number unsaturation index – the average number of double bonds in a fatty acid. It was interesting that in the 3 years 1999–2001, GM canola had a higher degree of unsaturation than non-GM canola.

Table 7. *Total weed seeds and inconspicuous seeds (wild mustard or charlock) in cleaned samples of canola from harvest surveys. Results means of grade and crop district composites as determined by registered seed analysts*

Year	Other weed seeds (%)	Wild mustard (Charlock) (%)	Non-GM canola in crop (%)
1995	0·48	0·35	100
1996	0·33	0·24	91
1997	0·42	0·24	70
1998	0·27	0·23	50
1999	0·21	0·15	38
2000	0·24	0·23	32
2001	0·08	0·20	23

DISCUSSION

The results showed that there were significant quality differences between GM and non-GM canola grown in Canada between 1996 and 2001. Differences in oil

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Table 8. *Comparison of samples of non-GM herbicide tolerant (HT) and non-GM conventional canola (non-HT) for glucosinolate content (micromoles per gram)*. Data from Canadian Grain Commission Harvest Surveys (significant differences shown in bold type)*

	1996		1997		1998		1999		2000		2001	
	non-HT	HT	non-HT	HT	non-HT	HT	non-HT	HT	non-HT	HT	non-HT	HT
Number of samples	647	37	813	120	405	127	310	92	514	312	263	150
Mean	10·4	8·6	12·2	9·8	11·8	10·9	10·3	9·0	10·3	9·7	11·3	11·2
Pooled S.E.	1·0		1·0		1·0		1·0		1·0		1·0	
Diff	**1·8**		**2·4**		**0·9**		**1·3**		**0·6**		0·1	
t-value	4·96		11·48		5·15		4·87		4·00		0·64	
Prob. >|t|	<0·001		<0·001		<0·001		<0·001		<0·001		0·521	

* Detransformed after transformation by logarithm base 10 to give a normal distribution for analysis.

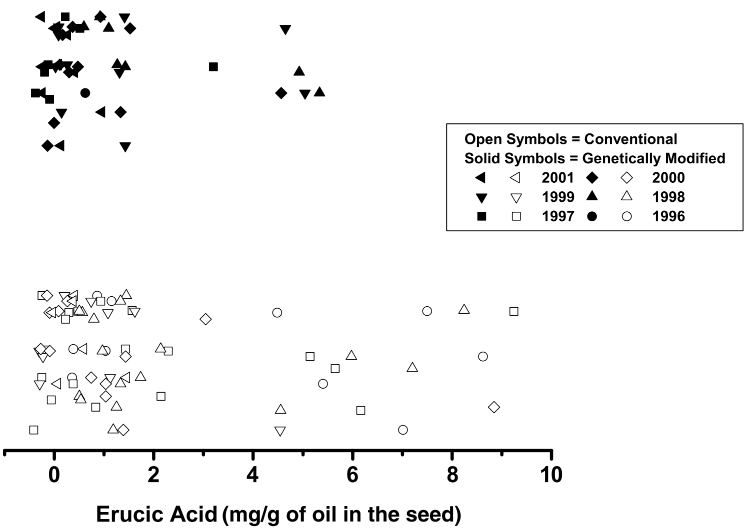

Fig. 1. One dimensional jittered scattergram showing distribution of erucic acid levels in GM and non-GM variety composites prepared from commercially harvested canola between 1996 and 2001. Inspection of the data showed that the non-GM points with erucic acid greater than 6 mg/g all came from a single variety that was noted in the cooperative variety trials to have a level of erucic acid greater than 6 mg/g.

and protein content, noted for several years in the study, may be due, at least in part, to the popularity of some high-yielding hybrid canola varieties (non-GM) that have a lower oil content and higher protein content than many of the more conventional lines. Other differences in quality might be linked back to the GM trait. For example, differences in chlorophyll content, with GM lines tending to lower chlorophyll, could be due to improved field management from the use of the herbicide-tolerant characteristic resulting in more even and perhaps earlier maturation. Similarly, the significantly lower glucosinolate content in GM lines grown commercially is probably due to the ability to clean up brassicaceous weeds (Table 7) as well as older volunteer non-GM canola. Wild mustard (charlock) has been a major weed problem in Canadian canola and the seed is difficult to remove (Daun *et al.* 1983). This hypothesis is supported by similar differences found in glucosinolate content between non-GM herbicide tolerant (Pursuit Smart) and non-GM conventional types of canola (Table 8).

This work also points out the difficulty in evaluating the effects of the herbicide-tolerant trait based on plot trials alone. While the data from the registration support documents, which are based on results from standardized plot trials, showed little or no consistent and substantial differences in quality between GM and non-GM lines, significant differences were noted when data from the commercial crop were analysed. Even yield comparisons using plot studies may not show the same results. Herbicide-tolerant canola had only a mixed yield advantage to a commercial line (Harker *et al*. 2000). This is in sharp contrast to the consistent economic advantages found in the canola field trials (Anon. 2002).

CONCLUSIONS

GM canola was found to have more oil content, less protein content, less glucosinolates and less chlorophyll than non-GM canola. The oil from GM canola also tended to be slightly more unsaturated than the oil from GM canola but there was no difference in erucic acid, unsaturated fatty acids or linolenic acid between the two types.

The most significant differences were in chlorophyll content and in glucosinolate content and these differences are likely due to the GM-endowed characteristic herbicide tolerance rather than the genetic modification itself.

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