Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought-stressed Mediterranean-type environments

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Abstract

Seed fatty acid (FA) composition, oil and protein (meal) was assessed on five canola (*Brassica napus*) varieties in 14 cropping environments in southern Australia, including several low rainfall drought-stressed environments. We modelled the relationship between seed quality attributes and growing season rainfall and temperature using a linear mixed model. Variance components for variety and years within locations were relatively large, but variance components for variety × environment interaction were small or insignificant for most seed quality traits. Mean oleic acid content varied from 57% in 'Surpass 300TT' to 62% in 'ATR-Beacon'. As growing season rainfall decreased from 300 mm (moderate) to 150 mm (severe drought stress), mean oleic acid decreased by 3.8%, linoleic acid increased by 2.0%, linolenic acid increased by 1.7%, and saturated FA decreased by 0.4%. Seed oil (% dry weight) decreased by 3.2% and protein in meal (% dry weight) increased by 3.9% across the same rainfall range. High oleic acid composition was associated with higher rainfall and cooler average minimum and maximum temperatures during the growing season.

Keywords: oilseed rape; rapeseed; gas chromatography; specialty oil; genotype by environment interaction; G × E

Genotype and environment affect seed fatty acid (FA) profiles in canola (*Brassica napus* L.). A high level of natural genetic diversity is present in the *Brassica* C genome that could be used to alter the oil composition through pathway engineering (Barker et al. 2007), but knowledge is lacking on the impact of environment on FA composition. *Brassica napus* genetically modified for altered FA composition was subject to environmental influence (Scarth and Tang 2006). An improved understanding the effect of genotype and environment on an oil quality will be important for breeding and production of conventionally bred and genetically modified canola.

High temperature was reported to stimulate the oleic acid biosynthesis pathway, and under these conditions, rapeseed triglycerides were enriched with oleic acid (Trémolières et al. 1978). In low linolenic oilseed rape oil, the level of saturated and monounsaturated (C18:1) FA increased when developed under high average daily temperatures (30/25°C day/night) in growth room studies, while linolenic (C18:3) levels increased at lower average daily temperatures (Deng and Scarth 1998). In another study, Schierholt and Becker (2001) showed that oleic acid content in high oleic winter oilseed rape was environmentally stable under temperate regimes experienced in northern Europe.

In Mediterranean-type environments such as southern Australia, spring canola varieties are subject to high temperatures and drought stress at maturity. High spring temperatures and drought stress were associated with lower oil and higher protein content in canola (Si et al. 2003, Si and Walton 2004), while high oil contents were correlated with cooler spring temperatures (Pritchard et al. 2000). Similar results were obtained by Gunasekera et al. (2006) in *B. juncea* and *B. napus* in Mediterranean-type environments.

There is less information on the effect of genotypes and environment on FA composition in canola. In a number of canola variety trials in Victoria, Australia, growing season rainfall and annual rainfall at trial sites were not significantly associated with any seed quality component; however, there was a significant positive correlation between cumulative spring rainfall and linolenic acid content, and a significant negative correlation between maximum spring temperature and linolenic content (Pritchard et al. 2000). The content of saturated FA in canola oil was relatively stable in southern Australia (Gororo et al. 2003).

For breeders and marketers, it is important to understand the relative roles of genotype and environment on FA composition of canola across a range of rainfall environments and growing seasons. This study investigated seed oil FA composition of five canola varieties grown at 14 locations in southern Australia, and modelled the relationship between these seed quality attributes and seed oil (%) and protein in meal (%) with growing season rainfall and temperature. Several of the sites were affected by drought with <200 mm growing season rainfall, and this level of drought stress occurs periodically in Mediterranean-type environments.

Materials and Methods

Field experiments and meteorological recordings: Trials were conducted at Esperance, Miling, Mingenew, Nyabing and Tammin (Western Australia) and Kapunda (South Australia) in 2002 and 2003, and Ardlethan (New South Wales) and Elmore (Victoria) in 2003. Each trial was a randomized block design with three replicates and included five Australian canola varieties: ‘ATR-Beacon’, ‘ATR-Eyre’, ‘Karoo’, ‘Surpass 300TT’ and

'Surpass 501TT'. The pedigree relationships between these varieties (Cowling 2007) were used to calculate a coefficient of parentage (COP) between each pair of varieties (Table 1). In each year, rainfall and temperature records were taken from the closest Australian Bureau of Meteorology location for each field trial to calculate annual rainfall, growing season rainfall (May–November), and average minimum and maximum temperature for the growing season (May–November) (Table 2). The average growing season minimum and maximum temperatures were calculated by averaging the monthly average minimum and maximum temperatures between May and November.

Plots were harvested by machine, and seed samples taken from each plot for seed quality analysis. Complete FA analysis was done by gas chromatography (GC), based on the average of three replicate samples (10 seeds each) from each plot, and two GC injections for each replicate (Aslam et al. 2008). Oil was extracted in 10% isopropanol in heptane, and converted to FA methyl esters using a trans-methylation process (Aslam et al. 2008). FA contents were expressed as percentage of total FA and were estimated on a GC-17A V3 (Shimadzu, Kyoto, Japan) equipped with flame-ionization detector and flow splitter. Results from GC were integrated by gc solution software (Shimadzu, Kyoto, Japan). Saturated FA content was taken as the sum of C14:0, C16:0, C18:0, C20:0 and C22:0 FA. Seed oil concentration (%) and protein concentration in meal (%) were measured on a dry seed basis in intact seed from each plot using NIR (Foss NIRS 5000).

Statistical analysis: GenStat Edition 8 (VSN International, Hemel Hempstead, UK) was used for all analyses. The REML procedure (Gilmour et al. 1995) was used to fit all linear

mixed models. Pearson's correlation coefficients were calculated between environmental variables and major FA, protein and oil contents.

For each FA, the variance components associated with variety and environment were calculated using a linear mixed model with no fixed effects. The random model included effects of variety, site and year within site and their interactions, as well as terms for variance between blocks, variance between plots, and variance between duplicates in each trial. The variance between duplicates was similar across trials, so a single variance was modelled.

Variety means and trial means for each FA were calculated from a linear mixed model fitted to data from all trials using the same fixed and random effects described above except that the variety effect was transferred to the fixed model.

The relationships between variety, environmental variables and seed quality attributes (oleic acid, linoleic, linolenic and saturated FAs, seed oil and protein in meal) were examined using a linear mixed model. The fixed model included linear and quadratic terms for growing season rainfall, growing season average minimum temperature and growing season average maximum temperature, as well as variety and interactions between variety and environmental variables. The quadratic response was used to represent a general curvilinear response as with only 14 trials it was not possible to differentiate between different curvilinear forms such as quadratic and exponential. Total year rainfall was excluded from the model as it did not explain as much variance between trials as growing season rainfall. The random model was the same as for the previous model. Year effects in this random model were those not

explained by the rainfall and temperature effects in the fixed model. This linear mixed model was fitted to data from all trials excluding the Esperance 2003 trial which had much higher growing season rainfall (636 mm) than any other trial (range 148–394 mm) and was considered an outlier to the model. Rainfall and temperature response lines were estimated from the model after excluding non-significant terms.

Results

Genotypes

The five canola varieties represented a broad cross-section of pedigrees in Australian canola germplasm (Cowling 2007). Some varieties, such as 'ATR-Beacon' and 'Karoo', were closely related with COP equal to 0.49, whereas others such as 'ATR-Beacon' and 'ATR-Eyre' were more distantly related (COP = 0.06) (Table 1). One of the parents of 'Surpass 501TT' is 'Surpass 400', which includes a resynthesized*B. napus* in its pedigree (Li and Cowling 2003).

Environmental variables

The highest growing season rainfall (636 mm) was recorded at Esperance during 2003, while the lowest growing season rainfall (148 mm) occurred at Tammin during 2002 (Table 2). The recorded rainfall was less than the long term average at each site during the drought year of 2002. In Esperance during 2003, rainfall was much higher than 2002 while temperature during October/November (seed maturation period) was lower than 2002, especially in October when a difference of 3°C was noticed. The results for Esperance in 2003 were

excluded from the rainfall and temperature models (Fig. 1) due to its very high rainfall compared with the other 13 locations (148–394 mm).

There were strong correlations between several of the climatic variables across sites in 2002 and 2003. As expected, average maximum temperature was highly correlated with the highest maximum temperature ($r = 0.943$; $P < 0.001$), and similarly average minimum temperature was highly correlated with the lowest minimum temperature ($r = 0.967$, $P < 0.001$) (Table 3). There was a moderate correlation between average maximum temperature and average minimum temperature ($r = 0.570$, $P = 0.041$) (Table 3). Growing season rainfall was highly correlated with total year rainfall ($r = 0.951$, $P < 0.001$) but not correlated with average maximum temperature or average minimum temperature (Table 3). On the basis of these correlations, total rainfall, growing season rainfall, average maximum temperature and average minimum temperature were used as independent variables to describe environment in the model. Later, total year rainfall was removed from the model for the reason described above.

Fatty acid composition

For all FA traits, variance component estimates were relatively large for variety and years within site (Table 4), indicating that varieties differed in quality attributes, and quality attributes of sites were not consistent from year to year. There were relatively small contributions from the interactions of variety with site and year for all quality attributes, from which it is concluded that varieties ranked consistently across locations and years for quality attributes (the exception being saturated FA). The percentage contribution to total variance

for variety was higher for oleic acid (49%) and linoleic acid (68%) than for linolenic acid (31%) and saturated FA (10%) (Table 4).

Sites with the highest oleic acid content were Elmore (2003), Ardlethan (2003), and Esperance (2002), with means of 63.2%, 62.8% and 62.6%, respectively, and sites with the lowest oleic acid were Tammin (2002) and Miling (2002) with means of 57.5% and 57.9%, respectively (Table 5). The variety with the highest oleic acid was 'ATR-Beacon' with an average value of 62.2%, while 'Surpass 300TT' had the lowest oleic acid average of 56.6% (Table 6).

While there were significant effects of variety on oleic acid content (P = 0.001; Table 7), there was no interaction between rainfall and varieties (P = 0.186). There was a quadratic response to growing season rainfall (P = 0.023; Fig 1a). As growing season rainfall decreased from 300 to 150 mm (drought stress conditions), the fitted means for oleic acid decreased by 3.8%. 'ATR-Eyre', 'ATR-Beacon' and 'Surpass 501TT' had very similar fitted values for oleic acid at all levels of rainfall. There was no evidence for a change in oleic acid above 350 mm, as indicated by the outlier site (Esperance 2003, 636 mm). A significant quadratic effect of average minimum temperature on oleic acid (P = 0.001) was the result of higher oleic acid values at the low (4.0 to 5.5°C) and high side of average minimum temperatures (9 to 10.5°C) during the growing season (Fig. 2a).

Linoleic acid was lowest at Mingenew 2003 (19.4%), Elmore 2003 (19.5%) and Ardlethan 2003 (19.9%) and highest at Miling 2002 (22.6%) and Kapunda 2002 (22.5%), while

linolenic acid was lowest at Elmore 2003 (8.6%) and Ardlethan 2003 (8.8%) and highest at Tammin 2002 (12.3%) (Table 5). 'ATR-Eyre' (18.8%) had the lowest level of linoleic acid and 'Surpass 300TT' (24.4%) had the highest level (Table 6). 'Surpass 501TT' (8.6%) had the lowest level of linolenic acid and 'Karoo' (11.0%) had the highest level. While there were significant effects of variety on linoleic and linolenic acid contents ($P = 0.001$; Table 7) the response to rainfall was similar across varieties ($P = 0.138$). There was a quadratic response to growing season rainfall for linoleic acid ($P = 0.001$; Fig. 1b) and a linear response for linolenic acid ($P = 0.036$; Fig. 1c). As growing season rainfall decreased from 300 to 150 mm, the fitted means for linoleic and linolenic acids increased by 2.0% and 1.8%, respectively. The effect of rainfall on linoleic acid was the inverse of oleic acid.

There was a quadratic relationship between linoleic acid content and average minimum temperature with the lowest levels of linoleic acid at the highest and lowest temperatures ($P = 0.001$; Fig. 2b). Linolenic acid increased significantly (by 0.6%) as maximum average temperature increased from 16 to 24°C (Fig. 3a).

For saturated FA, there were significant linear and quadratic responses to growing season rainfall which interacted with variety ($P < 0.001$). The overall quadratic effect was not significant ($P = 0.717$), therefore linear responses were fitted to the data. As growing season rainfall decreased from 300 to 150 mm, saturated FA decreased by 0.2% for 'Surpass 300TT' and 0.6% for 'ATR-Eyre' (Fig. 1d). Saturated FAs decreased by 0.2% as maximum average temperature increased from 16 to 24°C (Fig. 3b).

Variance between environments was reduced by adding explicit environmental terms to the model, with a range from 94% reduction for linoleic acid to 27% for saturated FA. This reflects the strong impact in the model of rainfall and average minimum temperature on linoleic acid, and relatively weak impact of explicit environmental terms on saturated FA (Table 7).

Discussion

Genotypes

The genotypes used in this study represented a broad cross-section of genetic diversity in Australian canola (Cowling 2007), with a wide range of COPs (Table 1). Varieties followed similar patterns of behaviour with respect to environmental variables, and G × E effects were small or insignificant for most variables (Table 4). For example, varieties with high COP, such as 'ATR-Beacon' and 'Karoo', differed in oleic acid content but had the same response to rainfall. Varieties with low COP, such as 'ATR-Beacon' and 'Surpass 501TT', had similar oleic acid content and the same response to rainfall (Fig. 1).

While coefficients of parentage can be incorporated into linear mixed models when genotype effects are random and estimated using best linear unbiased prediction (BLUP), Piepho et al. (2008) argue that the primary purpose of exploiting genetic correlation by this approach is to estimate breeding values (equivalent to additive genetic effects). 'Often the main focus is merely on the estimation of the whole genotypic value rather than on component genetic effects comprising the genotype, so BLUP not using the coefficient of coancestry is perfectly reasonable' (Piepho et al. 2008). Our purpose was to estimate genotypic values and their

relationship to environment, rather than breeding values, so we decided not to incorporate COP into the analysis.

Fatty acid composition

Our results show that drought stress is associated with lower oleic acid content and higher polyunsaturated FA content in canola seed oil. The total oil content and oleic acid content of *B. napus* seed increases during the period 15–30 days after flowering, while linoleic and linolenic acids decrease during this time (Rakow and McGregor 1975). Drought stress may cause premature seed maturity, or a reduction in the desaturation of stearic to oleic acid, thereby stopping the increase in oleic acid in developing seed as shown by Rakow and McGregor (1975).

Contrasting results have been reported on the effect of high temperature or water deficit on oleic acid in oilseed rape. Under high temperatures, rapeseed triglycerides were enriched with oleic acid (Trémolières et al. 1978). In low linolenic oilseed rape oil, the level of saturated and monounsaturated (C18:1) FA increased when developed under high average daily temperatures (30/25°C day/night) in growth room studies (Deng and Scarth 1998). In contrast, water deficit after flowering reduced oleic acid content by up to 7% in the *B. napus* cv. ‘Cérès’ (Champolivier and Merrien 1996), and a decrease in oleic acid in seed was associated with water deficiency during vegetative growth and flowering in *B. napus* cv. ‘Drakkar’ (Bouchereau et al. 1996).

The biochemical pathway of desaturation moves from stearic, oleic, linoleic, with final desaturation to linolenic acid (Ohlrogge and Browse 1995, Harwood 1996, Dyer and Mullen 2005). The desaturation steps from stearic to oleic acid and from linoleic to linolenic acid were the steps in FA synthesis with greatest sensitivity to heat stress in some Australian canola cultivars (Aksouh et al. 2001). Oleic acid decreased when a maximum temperature of 40°C (for 4 h in middle of the day) was used under controlled conditions (Aksouh et al. 2001). Green (1986) reported higher oleic acid and lower linoleic and linolenic acids in flax at 27/22°C (day/night) temperature, compared with 30/25°C (day/night) temperature. Heat stress may reduce the efficiency of the first desaturation step (C18:0–C18:1), and increase efficiency of the second or third desaturation steps (C18:1–C18:2 and C18:3). Further work is needed to determine the cause of the reduction in oleic, and increase in polyunsaturated FA, under drought stress in canola.

In a previous study in Australia, linolenic acid content of canola was positively correlated with higher cumulative spring rainfall and negatively correlated with higher maximum temperatures in spring (Pritchard et al. 2000). Actual values for cumulative spring rainfall were not provided in the paper, so it is difficult to compare with our results, but there were no significant correlations between FA composition and growing season rainfall or temperature (Pritchard et al. 2000). The highest content of oleic acid in canola occurred in regions with warm and wetter springs (Wimmera and central Victoria) while lowest occurred in cool moist southern Victoria.

The data in the current study were modelled against climatic variables, and there was a significant negative relationship between oleic acid and growing season rainfall (below

350 mm). Above 400 mm growing season rainfall, there was no evidence for increased oleic acid.

Elevated levels of linolenic acid occurred at low rainfall sites in all genotypes (Fig 1c) and at higher average maximum temperatures (Fig 3a). It is difficult to separate the effect of rainfall and temperature on canola grown under field conditions, due to the negative correlation between these two parameters (Table 3), and because the data averaged over a daily or monthly basis are a broad approximation of the conditions that directly affect seed growth and quality. Trémolières et al. (1982) reported a decrease in the relative contents of linolenic acid-containing triacylglycerols and an increase in oleic acid-containing triacylglycerols in rapeseed varieties, when temperature increased from 12 to 27°C (under controlled conditions). Controlled conditions inevitably differ from field conditions, as sudden changes in environmental conditions cannot be prevented in field conditions while controlled conditions remain constant. It is not surprising therefore, that our results differ from some controlled environment experiments.

There is interest in some consumer markets for reduced saturated FA content in canola. In this study, canola varieties showed a small decrease in saturated FAs under drought stress environments, in tandem with an increase in oleic acid and oil content (Table 7). However, there was not a significant correlation between saturated FA and oil content or oleic acid across environments (Table 3), probably because the genotypic effects were relatively weak compared to error variance for saturated FA (Table 4). In soybean, saturated FA (palmitic, stearic) and oleic acid increased while polyunsaturated FA (linoleic and linolenic) decreased with high temperatures during seed development under growth chamber conditions (Sato and

Ikeda 1979). The changes in saturated FA we observed in the field in canola are not large and should not be considered important from a consumer point of view.

Seed oil and protein

Oil and protein content of canola seed was greatly influenced by growing season rainfall and average minimum temperature. In previous studies (Pritchard et al. 2000, Si et al. 2003, Si and Walton 2004), there was a decline in seed oil and increase in seed protein as growing season rainfall decreased below 350 mm. The results of this study support the accepted view that high oil contents are correlated with cool and wet springs; a long, cool pod ripening period increases the time in which oil may accumulate and increases oil content of seed (Mailer and Cornish 1987, Mailer and Pratley 1990, Pritchard et al. 2000). Mendham et al. (1990) also observed in *B. napus* that at maturity a cool environment and adequate water supply enhanced oil content, while drought stress reduced oil content at both high and low temperature. In the present study, sites with >350 mm growing season rainfall gave the highest oil content, which is consistent with earlier findings where higher rainfall and cooler temperatures (after flowering) produced higher seed oil content in canola (Walton et al. 1999). Under the growing conditions of the southern Australian wheatbelt, crop maturity coincides with a decrease in rainfall and an increase in maximum temperature, so if cooler and moister conditions prevail during crop maturity then high yield and high seed oil contents can be anticipated.

Conversely, a decrease in protein content of meal was observed in this study in higher rainfall conditions. A negative correlation between seed oil and protein is a common observation, and

has been reported previously in rape (Bouchereau et al. 1996, Triboi-Blondel and Renard 1999, Pritchard et al. 2000, Aksouh et al. 2001). In the Mediterranean-type environment of Western Australia (similar to the present conditions) there was a strong negative correlation across genotypes between oil and protein content in seed of *B. napus* (Gunasekera et al. 2006) and in narrow-leafed lupin (Cowling and Tarr 2004).

In the present study, genotypes ranked relatively consistently across environments for oil and protein content. This is similar to the results of Si et al. (2003) and Gunasekera et al. (2006) where they found stability of genotypes for oil and protein across environments in canola and mustard. We found a non-significant effect of genotype × growing season rainfall interaction for seed oil but a significant G × growing season rainfall effect for protein (Table 7). The range of seed oil content across environments was similar to the range of genotypic means for seed oil contents (Fig. 1). An earlier study found that environmental variation exceeded cultivar variation for oil and protein contents in canola in Victoria, Australia (Pritchard et al. 2000).

Potential importance of FA results in high oleic low linolenic varieties

In this study, where 'normal' canola varieties were used, drought stress in field trials was associated with a decrease in oleic acid, a slight decrease in saturated FA, and an increase in linoleic and linolenic acids. All genotypes reacted similarly in FA composition to the reduction in growing season rainfall from 350 mm down to 150 mm, except for saturated FA, where there was a significant genotype × rainfall interaction. These changes in FA

composition with drought stress were lower in magnitude than the variation among genotypes in the same study.

In this experiment, the choice of genotype was of greater importance than environment to achieve a desired FA composition. However, FA composition demands of the high oleic low linolenic (HOLL) market may be more stringent than the canola market. If the results of this research are confirmed in HOLL varieties, drought stress may prevent some HOLL varieties from reaching the HOLL grade by reducing oleic acid and increasing linolenic acid. In any case, it is likely that economic demands for production of HOLL varieties will restrict them to more secure rainfall environments (or irrigated sites), with average maximum temperatures in the growing season at the cooler end of the values recorded in this study. Also, it is likely that the environment will have an impact on FA profiles in genetically modified specialty oil *B. napus* (Scarth and Tang 2006).

An earlier study of effect of G × E on narrow-leafed lupin seed quality under Western Australian conditions (Cowling and Tarr 2004) found that genotypic variance was relatively large compared with G × E variance. Our study also found relatively low variance for G × E for canola seed quality attributes. There was relatively constant ranking of genotypes across sites, for most FA. It can be concluded that relatively few trials will be sufficient to identify with confidence the ranking of genotypes for FA composition of canola in southern Australia.

Acknowledgements

This research was supported by Australian Research Council project LP0210571, with industry partners Council of Grain Grower Organizations Ltd and Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ). NPZ carried out NIR oil and protein analysis of canola seeds. Canola Breeders Western Australia Pty Ltd provided the field trial data and plot harvest seed samples. We thank Dr Cameron Beeck for assistance with analysis of coefficient of parentage values.

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Table 1. Coefficients of parentage for pairs of canola varieties included in the study

	Karoo	**Surpass 300TT**	**Surpass 501TT**	**ATR-Eyre**
Surpass 300TT	0.016			
Surpass 501TT	0.016	0.250		
ATR-Eyre	0.145	0.009	0.009	
ATR-Beacon	0.492	0.016	0.016	0.061

Table 2. Average minimum and average maximum temperature during the growing season and rainfall (growing season and total year) at different experimental field sites across Southern Australia

Experimental sites	**Average temperature (°C) during the growing season (May–November)**		**Rainfall (mm)**	
	Average minimum (AvMinT)	**Average maximum (AvMaxT)**	**Growing season (RainGS)**	**Total year (RainTY)**
Year 2002				
Miling	8.9	23.0	181.6	246.8
Esperance	10.1	20.4	342.8	448.2
Kapunda	7.1	18.4	259.8	344.8
Nyabing	7.1	19.2	231.0	299.6
Tammin	6.2	22.7	147.6	225.1
Mingenew	9.6	24.8	191.6	244.9
Year 2003				
Miling	8.4	21.6	255.8	281.6
Esperance	10.0	19.0	636.2	749.0
Kapunda	6.9	17.0	394.0	536.4
Nyabing	7.4	18.5	327.8	445.4
Tammin	6.6	21.0	253.4	384.6
Mingenew	9.5	23.8	363.6	403.6
Ardlethan	6.3	19.0	288.6	371.2
Elmore	4.7	16.2	332.9	543.2

Table 3. Correlation coefficients between seed quality attributes, rainfall (total year and growing season) and average temperature regimes in different *Brassica napus* varieties grown at different experimental sites across southern Australia in 2002 and 2003

	Oleic acid	Linoleic acid	Linolenic acid	Saturated FA	Seed oil (%)	Protein meal (%)	RainGS (mm)	RainTY (mm)	AvMinT	AvMaxT	Highest MaxT	Lowest MinT
Oleic acid	1											
Linoleic acid	−0.79**	1										
Linolenic acid	−0.59**	0.12*	1									
Saturated FA	0.08^{NS}	0.04^{NS}	−0.42**	1								
Seed oil (%)	0.38**	−0.22**	−0.37**	0.06^{NS}	1							
Protein meal (%)	−0.22**	0.22**	0.32**	−0.44**	−0.51**	1						
RainGS (mm)	0.17*	−0.14*	−0.13*	0.11*	0.52**	−0.55**	1					
RainTY (mm)	0.21**	−0.14*	−0.23**	0.23**	0.39**	−0.53**	0.95**	1				
AvMinT	−0.07^{NS}	−0.02^{NS}	0.24**	−0.38**	0.42**	−0.13*	0.29**	0.05^{NS}	1			
AvMaxT	−0.12*	−0.03^{NS}	0.31**	−0.35**	0.15*	0.26**	−0.45**	−0.63**	0.57**	1		
Highest MaxT	−0.15*	0.02^{NS}	0.36**	−0.46**	0.20**	0.23**	−0.29**	−0.50**	0.72**	0.95**	1	
Lowest MinT	−0.07^{NS}	−0.03^{NS}	0.23**	−0.32**	0.45**	−0.20**	0.45**	0.22**	0.97**	0.42**	0.59**	1

NS, non-significant; *significant at P=0.05; **significant at P=0.01.

AvMinT, average minimum temperature; AvMaxT, average maximum temperature; Highest MaxT, highest maximum temperature during growing season (May–November); Lowest MinT, lowest minimum temperature during growing season (May–November); RainTY, total year rainfall; RainGS, growing season rainfall (May–November); FA, fatty acid.

Table 4. Variance components (vc), standard errors (se) and percentage contribution to total variance (%) from random model which included variety, site, and interactions between site, year and variety, for fatty acid (FA) components in five canola varieties tested across six sites in 2002 and seven sites in 2003

	Oleic acid			**Linoleic acid**			**Linolenic acid**			**Saturated FA**		
	vc	**se**	**%**	**vc**	**se**	**%**	**vc**	**se**	**%**	**vc**	**se**	**%**
Variety (V)	5.89	4.24	49	5.25	3.74	68	0.95	0.69	31	0.02	0.02	10
Site (S)	0.00	na	0	0.58	0.68	8	0.00	na	0	0.00	na	0
Years within site (SY)	3.60	1.54	30	0.84	0.54	11	1.45	0.60	48	0.13	0.05	62
S × V	0.00	na	0	0.02	0.10	0	0.00	na	0	0.00	na	0
SY × V	0.91	0.29	8	0.16	0.12	2	0.28	0.08	9	0.03	0.01	12
Trial.Block	0.09			0.09			0.00			0.00		
V.Trial.Block	1.50			0.73			0.34			0.03		
Residual	0.01			0.00			0.00			0.00		
Total	11.99			7.68			3.03			0.21		

Experimental variance included trial.block, variety.trial.block and residual.

na, standard error is not available when variance component is set to 0 as a boundary value.

Table 5. Site means for canola seed fatty acid (FA) composition, seed oil, and protein in meal, and average 5% least significant difference (5%LSD), across six trial sites in 2002 and eight sites in 2003

Site-year	Site means for seed quality attributes					
	Oleic acid (%)	**Linoleic acid (%)**	**Linolenic acid (%)**	**Saturated FA (%)**	**Seed oil (%)[1]**	**Protein meal (%)[1]**
Esperance-2002	62.61	20.21	9.31	6.83	43.68	42.59
Kapunda-2002	58.22	22.54	11.52	6.49	41.48	45.52
Miling-2002	57.87	22.57	11.99	6.47	39.34	48.57
Mingnew-2002	60.04	20.53	10.52	6.50	46.11	43.99
Nyabing-2002	58.99	21.62	10.59	6.50	40.49	46.88
Tammin-2002	57.48	22.14	12.29	6.66	40.25	49.08
Ardethan-2003	62.81	19.88	8.81	7.00	45.15	42.59
Elmore-2003	63.20	19.51	8.55	7.34	41.84	44.13
Esperance-2003	60.06	20.26	11.50	6.51	48.64	42.06
Kapunda-2003	59.64	22.46	9.03	7.33	43.78	40.94
Miling-2003	60.15	20.68	10.27	7.22	44.98	42.75
Mingnew-2003	61.65	19.41	10.15	6.89	47.88	42.81
Nyabing-2003	58.74	21.53	10.62	7.32	41.79	44.33
Tammin-2003	61.84	20.07	9.10	7.35	44.40	41.87
5%LSD	1.02	0.74	0.47	0.14	1.06	0.78

[1]Seed oil (%) and protein meal (%) are on dry weight basis.

Table 6. Variety means for canola seed fatty acid (FA) composition, seed oil, and protein in meal, and average 5% least significant difference (5%LSD), across 14 trial sites

Variety	Site averages for quality attributes					
	Oleic acid (%)	**Linoleic acid (%)**	**Linolenic acid (%)**	**Saturated FA (%)**	**Seed oil (%)[1]**	**Protein meal (%)[1]**
ATR-Beacon	62.17	18.97	10.47	6.77	42.94	45.00
ATR-Eyre	61.87	18.82	10.86	7.00	44.21	43.55
Karoo	58.86	20.57	11.02	6.79	41.20	42.89
Surpass 300TT	56.55	24.42	10.58	7.10	43.71	44.01
Surpass 501TT	61.63	21.67	8.58	6.77	45.70	45.31
5%LSD	0.20	0.12	0.11	0.04	0.25	0.24

[1]Seed oil (%) and protein meal (%) are on dry weight basis.

Table 7. Probability values obtained by sequentially adding fixed terms to the linear mixed models for seed quality attributes of *Brassica napus* varieties grown in a range of experimental sites (excluding Esperance in 2003)

Fixed term	**df**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**	**Saturated FA**	**Seed oil (%)**	**Protein meal (%)**
AvMaxT	1	0.199	0.501	**0.030**	**0.044**	0.101	0.247
AvMinT	1	0.553	0.740	0.580	0.694	0.200	0.116
$AvMaxT^2$	1	0.380	0.123	0.420	0.920	0.204	0.573
$AvMinT^2$	1	**<0.001**	**<0.001**	0.137	0.778	0.843	0.922
RainGS	1	**0.004**	**<0.001**	**0.036**	**0.012**	**<0.001**	**0.005**
$RainGS^2$	1	**0.023**	**<0.001**	0.228	0.717	**0.041**	0.197
Variety	4	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Variety x AvMaxT	4	0.220	0.190	0.782	0.216	0.195	0.391
Variety x AvMinT	4	0.996	0.842	0.986	0.379	0.549	**0.034**
Variety x $AvMaxT^2$	4	0.736	0.699	0.789	0.522	0.981	0.145
Variety x $AvMinT^2$	4	0.879	0.383	0.795	0.438	**0.043**	0.957
Variety x RainGS	4	0.186	0.607	0.138	**<0.001**	0.209	**<0.001**
Variety x $RainGS^2$	4	0.792	0.453	0.179	**<0.001**	0.278	0.686
Reduction in variance between environments due to adding explicit environmental terms (%)		74	94	43	27	65	39

Each effect is adjusted (i.e. tested after removing effects of these terms) for all terms above it in the table.

AvMaxT, average maximum temperature ($AvMaxT^2$ is the square of the values); AvMinT, average minimum temperature ($AvMinT^2$ is the square of the values); RainGS, growing season rainfall ($RainGS^2$ is the square of the values); df, degrees of freedom; Values in bold are significant at P=0.05.

Figure 1. Linear mixed model of effect of growing season rainfall on seed quality attributes of five *Brassica napus* varieties grown at 13 sites in southern Australia. Esperance 2003 (636 mm growing season rainfall) was excluded from the models. The bar represents the 5% least significant difference among variety means at 300 mm growing season rainfall

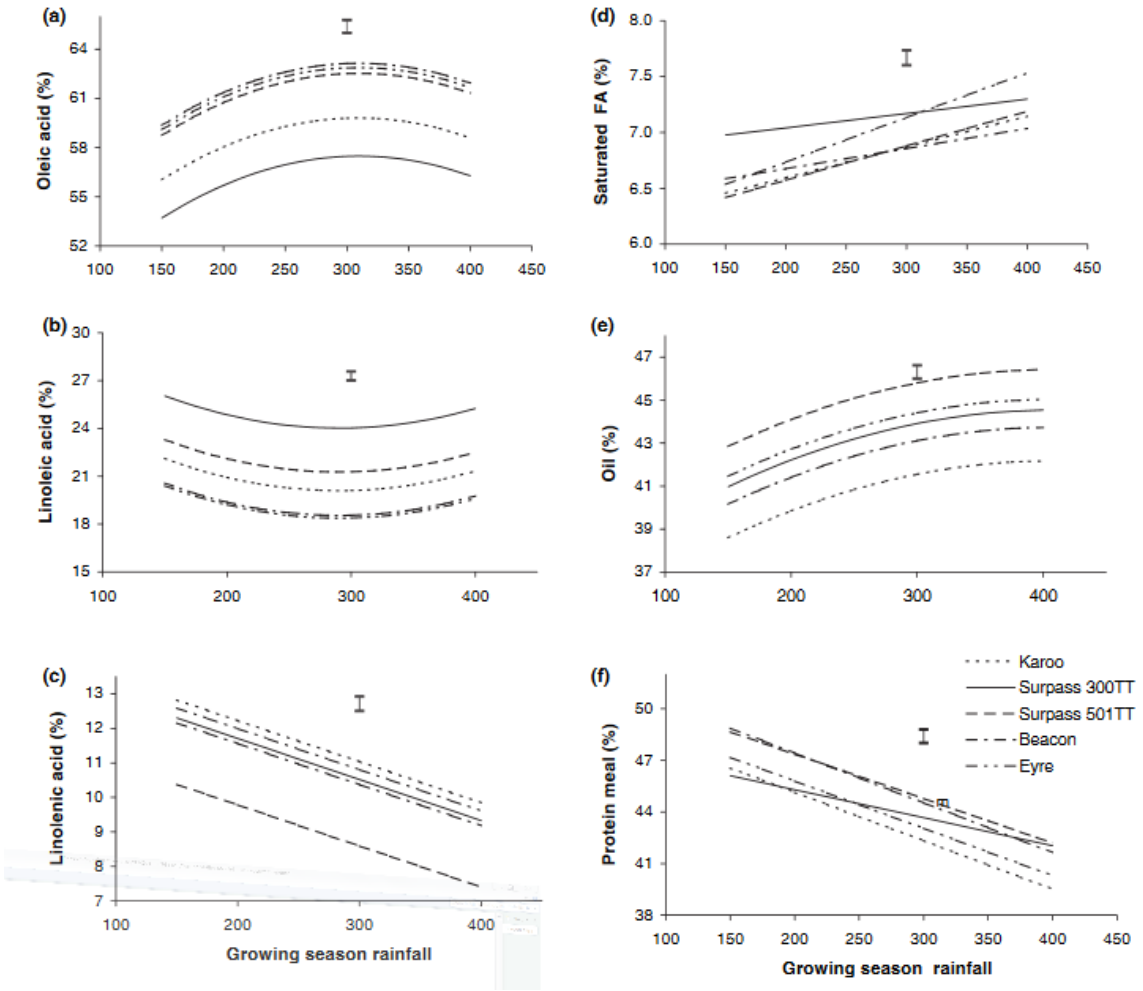

Figure 2. Linear mixed model of effect of average minimum temperature in the growing season on oleic and linolenic acid contents of five Brassica napus varieties grown at 13 sites in southern Australia. The bar represents the 5% least significant difference among variety means at 8°C average minimum temperature

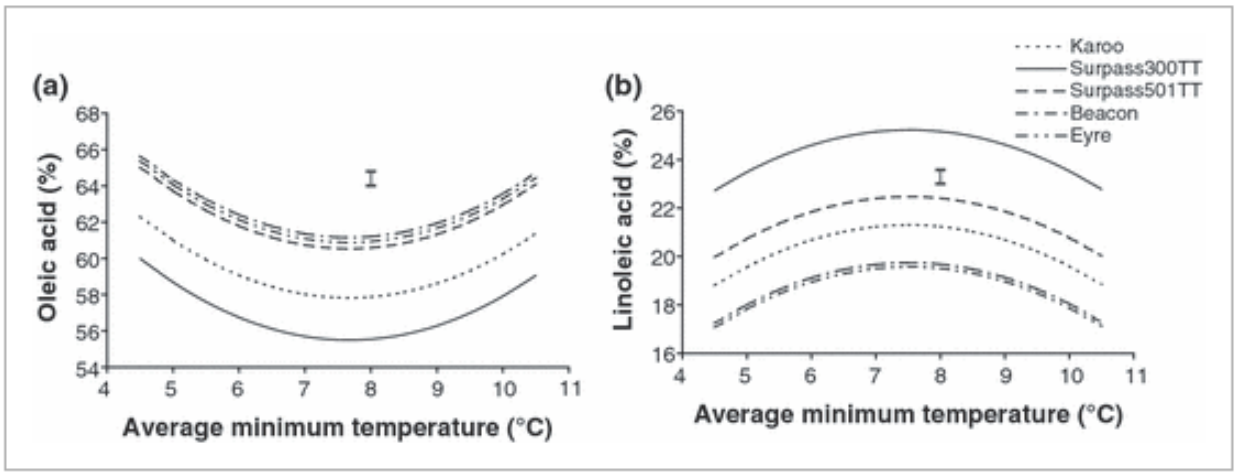

Figure 3. Linear mixed model of effect of average maximum temperature in the growing season on linolenic acid and saturated fatty acid contents of five *Brassica napus* varieties grown at 13 sites in southern Australia. The bar represents the 5% least significant difference among variety means at 20°C average maximum temperature