

Specialty canola: high stability canola varieties

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ABSTRACT

Specialty canola often delineated as high oleic low linolenic acid oil (HOLL) canola, is the most promising new oilseed crop for Australia, and has the potential to become a major adjunct to canola production. Specialty canola oil with >65% oleic acid content (monounsaturated) and <3.5% alpha- linolenic acid content (ALA, an omega-3) has greatly enhanced stability and market applications over normal canola. It has excellent prospects for frying, including for food-service industry, restaurants, hospitals and other big commercial kitchens which are dominated by highly-saturated oils and fats such as palm and tallow. In Australia, only 5 cultivars, with these modified fatty acid profiles, are commercially available from companies including Nuseed and DPI-Victoria/Cargill. In 2005 NVT grain yield trials, Monola™ Cultivar NMC130, a non-herbicide tolerant HOLL cultivar developed by Nuseed, had a higher mean yield than leading check cultivars across several trials, including in all average to better rainfall zones of central and southern NSW and north central and western Victoria, the primary target regions for production of the specialty cultivars. This was the first time that an Australian-developed HOLL specialty canola out-yielded leading non-specialty check cultivars. NMC130 represents a fore-runner of a broad range of HOLL lines which display a break-down of the yield barrier between specialty and non-specialty canola cultivars. The success of Monola™ NMC130 has been followed by improved results for other non-herbicide resistant specialty cultivars from Nuseed including NMC131 in NVT and /or company trials. The development of herbicide tolerant and hybrid cultivars including triazine-tolerant cultivars such as NMT320 should enable a rapid uptake of the healthier HOLL canola in Australia. Ongoing improvements in HOLL triazine-tolerant cultivars over the next 2 to 3 years, followed by Roundup Ready cultivars, should be the basis for a rapid expansion of plantings and of commercial uptake in oil products.

INTRODUCTION

Canola has been an oilseeds success story in Australia, suitable for growing throughout the winter-cropping belt of southern Australia. With an average of 1.6m MT grain and 40% oil, canola represents >70% of domestic production of oilseeds. Canola oil is excellent for diets, with a low level of saturated fatty acids (7-8%) and good content of the main monounsaturated fatty acid, oleic acid (around 60%) and a good balance of the two main polyunsaturated fatty acids, with around 20% content of linoleic acid (C18:2) and 12% content of omega-3 alpha-linolenic acid (C18:3). Canola deservedly has become a market leader in margarines and bottled oils. However, canola oil has insufficient oxidative stability for many applications, including for frying and storage products; usage is ~120kt oil, which represents only 25% total domestic usage of 450kt of oils and fats. The alpha-linolenic acid content causes instability of the oil, leads to "fishy" odours and reduced sensory properties, reduces the shelf life of the oil and derived products and prevents the expanded usage of canola oil for commercial applications such as for frying, the largest market application for vegetable oil. An enhanced level of oleic acid and lower level of alpha-linolenic acid in canola oil, will also expand opportunities for usage of canola oil into non-food uses, such as vegetable oil lubricants, for printing inks, and as a petroleum substitute in cosmetics and colourings.

Monola™ Oil

Monola™ Oil is specialty oil often delineated as HOLL canola. It has been developed in a breeding program at Horsham, Victoria from normal canola modified by traditional plant breeding. It is the most promising new oilseed crop for Australia, and has the potential to become a major adjunct to canola production. Monola™ Oil is improved canola oil which retains its positive dietary features whilst having potential for expanded range of food and non-food products. Monola oil comprises approximately 6-7% saturated fatty acids, 70% monounsaturates and 23% polyunsaturates, with ~20% linolenic acid and 3 % linolenic acid (Table 1). The natural stability of Monola™ Oil reduces the need for hydrogenation, a

common process often used to enhance the stability of oils; but hydrogenated oils contain elevated levels of trans-fatty acids, linked with an increased risk of cardiovascular disease.

Table 1. Fatty acid composition of Monola™ Oil vs. canola, palm and olive oil

Oil Type	Saturated	Mono- Unsaturated Oleic C18:1	Poly-Unsaturated	
	Palmitic+Stearic C16:0 + C18:0		Linoleic C18:2	Linolenic C18:3
Monola Oil	6-7%	70% (66-72%)	20% (17-22%)	2.9% (2.3-3.5%)
Canola Oil	7-8%	60% (57-62%)	20%	12-13%
Palm Olein	50%	45%	4%	
Olive Oil	16-24%	70-77%	6-8%	

MARKET AND INDUSTRY PERSPECTIVES FOR SPECIALTY CANOLA

Initial market perception suggested that >80% oleic and preferably >85% oleic was required for non-hydrogenated soft oils for frying. However, detailed studies by USDA Center for Crop Utilization Research, Peoria Illinois group headed by Dr Kathleen Warner demonstrated that the optimal vegetable oil for frying with regards to the following attributes:

- Frying performance/life of oil (restricted production of polar compounds);
- Shelf-life of end products (fries, potato chips, snacks)
- Dietary benefits of products (reduced saturates, derived fats);
- Sensory properties (re taste, flavour, odour)

is a vegetable oil with the following fatty acid profile

- 1) 5-7% saturates (C16:0 + C18:0 +C20:0);
- 2) 63-73 % oleic C18:1
- 3) 15-22% linoleic C18:2;
- 4) <=3% (3.5%, 4%) linolenic 18:3

A low level of linolenic acid is essential for frying stability, whilst a level of 15-20% linoleic acid enhances sensory properties; also a 6:1 ratio of polyunsaturates (C18:2; C18:3) is optimal for dietary intakes. Once oleic acid >75%, there was a significant reduction in sensory properties (flavour, taste) plus an associated increase in off-odours. For example, HO soybean oils with 85% oleic acid had poor sensory properties and gave unacceptable products. Food Science Australia played a key role in the research on Monola oil and also demonstrated the importance of low linolenic (18:3) in frying performance with 3% > 4% >>12%.

HOLL / LL Canola

The major direct market for specialty canola in Australia is for frying, for which the optimal fatty acid profile is 67-72% (64-75%) oleic, 2.5-3.0 (3.5%) C18:3, with no real benefit of higher oleic levels, and often with negative impact on sensory properties (taste, flavour). USA / Canada differentiate more than Australia between low linolenic acid (LL) canola and high oleic / low linolenic (HOLL) canola (Keith White pers. comm.). There are no precise definitions, but the following is indicative:

- LL Canola: 64-71% C18:1 oleic acid, <3.0 (3.5)% C18:3 linolenic acid
- HOLL Canola: >=70% (72%) oleic, <3.5% C18:3
- HO Canola: >~ 75% oleic

CHALLENGES WITH SPECIALTY CANOLA DEVELOPMENT

Breeding for HOLL canola has been difficult due to some negative correlations on changes in fatty acid profiles with maturities, plant type, blackleg disease resistance and ultimately grain yields. There are more constraints on plant type as both C18:1 level is increased and C18:3 levels reduced and such challenges are outlined below.

1. A reduction in the content of alpha-linolenic acid and an increase in the oleic acid content in the fatty acid profile of canola oil, both appear to impact negatively on the blackleg

resistance of canola plants, as well as on other agronomic parameters such as maturity (lead to later-maturing plants) and height (taller and less-determinate plants). The reasons for this impact of fatty acid profiles on agronomic performance are uncertain. As alpha-linolenic acid content is reduced firstly from around 12% in canola to below 5.0%, then progressively lower to 2%, or the oleic acid content is increased from around 60% in canola to above 65% the progressively higher (70%, 75%, 80%), the blackleg resistance of lines significantly decreases; and even more so for enhanced combinations of reduced C18:3% and enhanced C18:1%. Early LL, HO and HOLL canola lines introduced into Australia from breeding programs in North America and Europe exhibited poor resistance to blackleg disease. It is perhaps easier to develop HOLL lines in Canada, due to "reduced" concerns of blackleg disease in Canada compared to Australia.

2. The agronomic parameters associated with reduced levels of alpha-linolenic acid and higher oleic acid contents in the oil fraction, also impact negatively on grain yield; both potential grain yields under optimal conditions and realized grain yields under sub-optimal conditions in the field. Grain yields of specialty canola lines have been less than 85% of yield of leading non-specialty cultivars in field trials prior to 2003, and lower than this under conditions of significant moisture stress or blackleg disease. It is unknown whether the yield reduction observed in LL and HOLL cultivars is a pleiotropic effect of the fatty acid modification or whether could be due to linked genes associated with yield reductions. Also, it could be because less selection pressure has been put on yield because of the added selection put on fatty acid content. It may be worthwhile to evaluate near isogenic LL/HOLL lines to determine possible linkage drag or pleiotropism.
3. In Australia, approximately 70% plantings of canola are to "triazine-tolerant" (TT) canola cultivars since the control of broadleaf weeds, especially crucifers, has been a problem with conventional cultivars and also because of the absence of GM herbicide options available in other international markets. Development of TT cultivars in the mid-1990's significantly enhanced weed control options for canola growers and led to adoption of TT cultivars in over 95% canola plantings in Western Australia, whilst they have a significant role in South Australia and eastern States. Triazine-tolerance RNA is closely linked to mitochondrial RNA associated with chloroplast proteins; as a result, TT cultivars exhibit an inherently lower level of photosynthetic activity (reduced CO₂ fixation), which in turn leads to reduced early vigour and plant growth, and subsequently reduced grain yields (85% to 90%) compared with non-TT cultivars. The development of HOLL TT canola lines has been even more difficult, best described as a "double-whammy," due to the combination of reduced photosynthetic activity from triazine tolerance and the negative impacts on agronomic parameters in canola plants associated with low levels of alpha-linolenic acid and/or high levels of oleic acid in the oil fraction of the seeds. Initial HOLL TT lines had very poor agronomic type, poor resistance to blackleg disease and seed yields well below 70% of leading non-specialty TT cultivars.

These three challenges have been addressed in our breeding program. Initial Monola™ cultivars NMC201 and NMC202 were released in 2003 and the second wave of Monola cultivars namely Monola NMC130 and NMC131 have improved blackleg resistance and enhanced yield potential, with NMC130 and 131 yielding 100-110% Sapphire in average to better rainfall regions and improved more stable fatty acid profiles. Promising newer cultivars such as NMC116 and NMT320, NMT323 have the potential to extend planting zones and fatty acid profiles over the next 3-4 years.

HOLL CULTIVARS

In 2005 National Variety Trials (NVT), Monola NMC130, a non-herbicide tolerant HOLL cultivar developed by Nutrihealth (now a subsidiary of Nuseed) group led by Dr Keith White, was higher yielding than the check cultivar across several trials, including in all average to better rainfall zones of central and southern NSW and north central and western Victoria, the primary target regions for production of the specialty cultivars (Table 2). This was the first time that an Australian-developed HOLL specialty canola had out-yielded leading non-specialty check cultivars. NMC130 effectively represents the fore-runner of a broad range of HOLL lines which display a break-down of the yield barrier between specialty and non-specialty canola cultivars.

Table 2. Grain yield (t ha⁻¹) of Monola™ NMC130 vs. AV-Sapphire in NSW and Victoria in 2005

Cultivar	New South Wales								Victoria					%AV-SAPPHIRE	
	North East		N/W	South East			South West		North East		South West	Wimmera			
	Cobbora	Wellington	Parkes	Wagga	Albury	Cowra	Harden	Deniliquin	Lockhart	Katamatite	Wilby	Hamilton	Gerang		Minyip
AV-Sapphire	2.4	1.9	2.6	2.3	3.2	2.9	2.4	1.5	2.9	1.6	1.1	3.1	1.3	1.2	100
Monola NMC130	2.1	2.0	2.6	2.9	3.3	3.0	2.8	2.0	2.7	1.6	1.4	2.7	1.7	1.6	113
03N733l	2.4	2.0	2.6	2.5	3.4	3.9	2.8	1.9	3.1	1.5	0.9	3.0	1.9	1.7	110
AG Spectrum	2.4	2.1	2.5	2.2	3.3	2.4	2.4	1.5	2.9	1.6	1.3	2.3	1.6	1.1	103
AV Jade	2.6	2.0	2.4	2.5	3.4	3.1	2.9	1.5	3.0	1.7	1.1	2.1	1.6	1.1	105
AV Ruby	2.5	2.1	2.8	3.1	3.7	3.2	2.9	1.6	3.1	1.8	1.3	2.4	1.6	1.1	114
CBI4401	2.6	2.2	3.1	2.9	4.0	3.6	3.1	2.1	3.0	1.9	1.2	3.3	1.4	1.8	121
Hyola 61	2.2	2.1	2.7	1.9	3.1	2.7	2.6	1.4	2.5	1.6	0.9	2.9	1.3	1.1	95
Hyola 75	2.8	2.2	3.2	3.1	3.8	3.9	3.1	1.8	3.9	2.1	1.6	2.8	1.6	1.6	129
Pioneer 46C04	2.4	2.4	2.6	2.5	3.1	2.3	2.5	1.6	2.7	1.7	1.2	2.7	1.5	1.3	106
Pioneer 46C76	2.3	1.9	2.5	1.8	2.9	2.5	2.2	1.1	2.5	1.7	0.9	2.5	1.7	1.2	92
Skipton	2.5	2.0	2.8	2.0	3.2	3.0	2.3	1.7	2.6	1.5	1.0	2.3	1.6	1.3	100
Warrior	2.5	1.9	2.1	1.8	2.8	2.5	2.2	1.5	2.5	1.4	1.0	1.8	1.4	0.8	88
Site Mean (t/ha)	2.3	2.0	2.5	2.3	3.1	2.8	2.5	1.6	2.7	1.6	1.1	2.5	1.5	1.2	
CV (%)	6.0	9.5	6.3	8.3	9.7	6.9	6.7	6.2	8.9	6.8	13.8	12.6	8.9	12.0	
LSD (t/ha)	0.23	0.31	0.26	0.31	0.48	0.31	0.26	0.15	0.40	0.18	0.24	0.55	0.29	0.25	

In 2005, Nutrihealth also included a specialty TT cultivar in Victorian NVT trials. NMT311 averaged 107% ATR-Beacon across 7 sites with CV<15%, with oil content similar to ATR-Beacon. NMT311 had an acceptable provisional NBSR blackleg rating of 6.5 but a slightly sub-optimal for fatty acid profile for frying; NMT311 was released for restricted contract production in 2006. Nuseed has new specialty TT's with elevated yield potentials and improved fatty acid profiles in NVT Trials and advanced internal trials in 2007, including NMT320 and NMT323, with several anticipated for restricted release under contract production in 2008-09. Growers are showing strong interest in new specialty TT canola cultivars and this should enable a rapid increase in the production of specialty canola and the commercial uptake into end-products. Monola crops are grown under contract to CropNetwork with a premium paid to compensate growers for the additional requirements necessary to identity preserve the crop and the harvested grain. Identity- Preservation (IP) is critical to ensure the quality of the end product is not compromised.

The Department of Primary Industries, Victoria, in collaboration with Cargill Specialty Canola Oils, also has been developing HOLL cultivars for Australia with the first two cultivars, Cargill100 and Cargill101 released in 2006. Two replacement cultivars Cargill 102 and Cargill 103 were released in 2007 and have yields competitive with other conventional canola cultivars combined with high provisional blackleg ratings (Maher *et al.* 2007). DPI-Victoria/ Cargill expect to release a hybrid non-herbicide tolerant hybrid cultivar, 06H932 (Table 3) in 2008 and herbicide-tolerant cultivars in the following years (Wayne Burton pers. comm.). The development of herbicide tolerant and hybrid cultivars should enable a rapid uptake of the healthier HOLL canola. HOLL canola could replace palm olein, together with tallow, which are popular frying oils in the commercial market due to frying stability, taste and relatively low price. It has been estimated that in the domestic market more than 50,000 tonnes of oil could replace hard fats that are currently imported and this represent an extra \$25 million annually to the oil seed industry in Australia. Current HOLL production ~25kt of grain and the increase is likely to be steady, rather than spectacular, with perhaps 10-20% of Australian crop being HOLL cultivars within 5-7 years (Keith White pers. comm.).

Table 3. HOLL canola cultivars released/ targeted for release in Australia

YEAR	CULTIVAR	TYPE	MATURITY	BREEDER
2003	Monola NMC201	Non-herbicide Tolerant	Medium	Nutrihealth
2003	Monola NMC202	Non-herbicide Tolerant	Medium	Nutrihealth
2006	Monola NMC130	Non-herbicide Tolerant	Medium	Nutrihealth
2006	Cargill 100	Non-herbicide Tolerant	Medium	DPI-Vic/Cargill
2006	Cargill 101	Non-herbicide Tolerant	Medium	DPI-Vic/Cargill
2006	Monola NMT311	Triazine Tolerant	Medium	Nutrihealth
2007	Cargill 102	Non-herbicide Tolerant	Medium	DPI-Vic/Cargill
2007	Cargill 103	Non-herbicide Tolerant	Medium	DPI-Vic/Cargill
2008	Monola NMT370	Triazine Tolerant	Medium-Late	Nutrihealth
2008	Monola NMT320	Triazine Tolerant	Medium	Nutrihealth
2008	Monola NMC131	Non-herbicide Tolerant	Medium	Nutrihealth

2008	06H932	Non-herbicide Tolerant Hybrid	Medium	DPI-Vic/Cargill
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In Canada, specialty canola is primarily with conventional (non-herbicide), Roundup Ready and Imi-Resistant types. There is a rapid swing to F1 hybrids in Canada, across all these types, as for non-specialty. Cargill is also developing specialty In-Vigor F1 hybrids, under license from Bayer Crop-Science.

TEMPERATURE STRESS EFFECTS ON LOW LINOLENIC CANOLA

In Australia, the main interest in the canola industry is for specialty canola with lower levels of low-linolenic acid (C18:3). Cultivars with C18:3 content < 3% have been selected but at times moderate variability has been observed across locations and years, to around 0.5-1.0% C18:3, indicating some sensitivity of this character to different environmental conditions. Planting areas typically have a Mediterranean-type climate with canola sown in the late autumn or early winter (Connor, 1993) and with plants then often experiencing moderate drought and heat stress during seed-filling. Post-flowering high temperatures hasten crop maturity, with a lowering of the oil content and degree of unsaturation of oils (Green 1986). Conversely, when seed-filling occurs at low temperatures, more polyunsaturated fatty acids are observed. Similarly, linolenic acid (C18:3) increases during exposure to low temperature during seed-filling.

Cultivar, temperature and irradiance have been reported to have a significant effect on the fatty acid composition of oilseeds, with temperature having the greatest effect (Baux *et al.* 2007; Pritchard *et al.* 2000; Deng and Scarth 1998; Green 1986). The biochemical pathway of desaturation of oleic acid to form linoleic (C18:2), by the action of oleoyl desaturase enzyme (*FAD 2* genes), and further desaturation for the synthesis of linolenic acid (C18:3) by linoleoyl desaturase enzyme (*FAD 3* genes) is well documented (Lehninger 1982). Baux *et al.* (2007) observed increases in linolenic acid during cold acclimation, suggesting that the desaturase enzymes and/or the genes that encode them may be regulated in response to low temperature. In particular, this implies that the gene for the linoleoyl desaturase, the last step in the biosynthetic pathway to linolenic acid, may be cold-temperature regulated correlating with the documented increase in linolenic acid at low temperatures from flowering.

Information on the effect of temperature on final linolenic acid content is useful in determining areas most reliable for producing low linolenic canola allowing new LL and HOLL Australian cultivars to meet optimal standards for both domestic markets and export. Within Nuseed, by analysing climatic data, we have established "more reliable" production regions, particularly the better rainfall regions, less prone to severe frosts during seed-fill, and which regions produce the higher oil content and most consistent fatty acid profiles.

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